International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

FORMULATION, DEVELOPMENT AND EVALUATION OF NANO STRUCTURED LIPID CAREERS OF ISOCONAZOLE INCORPORATED GEL FOR EFFECTIVE FUNGAL TREATMENT

Mohit*, Eisha Ganju, Bhaskar Kumar Gupta
School of Pharmacy and Research, Peoples University, Bhopal (M.P.)

***Correspondence Info:**
Mohit
School of pharmacy and research, Peoples University, Bhopal (M.P.)
*Email:*dubeymohit019@gmail.com

***Article History:**

Received: 20/04/2023
Revised: 10/05/2023
Accepted: 15/05/2023

ABSTRACT

Fungal infections are opportunistic, which means they infect persons with highly damaged immune systems. The effectiveness of topical antifungal treatment is dependent on medication penetration through the targeted tissue. NLCs (nanostructured lipid carriers) are innovative pharmaceutical formulations. Also, gel combined with lipid nanoparticles was used to reduce skin irritation and to transfer medications into deeper layers of skin. This study deals with formulation, development and evaluation of nano structured lipid careers incorporated gel for effective fungal treatment (isoconazole). The formulation & evaluation of NLC gel was done according to standard methods available. The particle size ranged from 215.25 to 278.85. The drug content was found to be maximum in F14 formulation which is 99.45 %. The entrapment efficiency of F14 was noted to be 83.32. The % Cumulative Drug Release for F14 was estimated to be 99.12 % in 12 hours. Further by using F14 formulation the gel was made. The viscosity of gels ranged from 3110±7 to 3542±10cps while pH spanned between 6.81±0.02 to 6.98±0.01. The percentage of drug was observed to be maximum in G-2 which is 99.12±0.15. The extrudability extends from 159±3 to 175±5g. While the spreadibility was seen to range from 7.45±0.13 to 10.21±0.12. The *in vitro* drug release study of optimized gel formulation G-2 suggested that about 98.74% drug release is achieved in 12 hours. The mean particle size & zeta potential was observed to be 214.45nm & -36.48mV respectively. The Cumulative % Drug Release for optimized formulation of gel G2 estimated to be 98.74% in 12 hours. The R^2 value 0.955 indicated that it follows first order kinetics. It can be concluded that the isoconazole-loaded NLCs gel have a prolonged release profile and have the potential to treat fungal topical infections**.**

Keywords: Isoconazole, Nano structured lipid careers, Gel, Fungal infections, Topical drug delivery.

INTRODUCTION

Fungal infections are opportunistic, which means they infect persons with highly damaged immune systems. Symptoms of such illnesses include fever, coughing, and shortness of breath. Superficial fungal infections of the hair, skin, and nails are a major cause of morbidity around the world, especially in the tropics, where heat and humidity foster the growth of fungi that cause cutaneous infections. Direct touch is all that is required to spread the virus from one contaminated surface or host to another. The most common forms of fungal infection in

males are dermatophytoses, while candidiasis and pityriasis versicolor are other instances of important superficial mycoses (Richardson and Warnock, 2012; Romani *et al.,* 2011).

The best test for dermal delivery is stratum corneum, and innovative ways have been investigated to increase its permeability. There have been a few improvements in the treatment of fungal infections, such as focusing on the site of disease, lowering the risk of adverse responses, increasing treatment viability, and improving patient compliance. A variety of topical antifungal agents have been utilised in the treatment of dermatological skin infections (Ma *et al.,* 2020).

The effectiveness of topical antifungal treatment is dependent on medication penetration through the targeted tissue. As a result, effective medication concentration levels in the skin should be achieved. When antifungals are applied topically, the drug components must penetrate through the stratum corneum, the skin's external layer, to reach lower layers of the skin, particularly the viable epidermis. Antifungal drugs can be delivered into the skin more effectively using carriers such as colloidal systems, vesicular carriers, and nanoparticles. After dermal delivery, antifungal medicines should reach an effective therapeutic level in viable epidermis (Akhtar *et al.,* 2015).

NLCs (nanostructured lipid carriers) are innovative pharmaceutical formulations made up of physiological and biocompatible lipids, surfactants, and co-surfactants. NLC has developed as a second generation lipid nanocarrier and a viable alternative to first generation nanoparticles throughout time. In recent years, researchers have focused on NLCs as an alternative to SLNs, polymeric nanoparticles, emulsions, microparticles, liposomes, and other nanoparticles. These nanocarriers can be used to transport both hydrophilic and lipophilic medicines. NLCs have emerged as a viable pharmacological delivery technology via oral, parenteral, ophthalmic, pulmonary, topical, and transdermal routes (Beloqui *et al.,* 2016; Iqbal *et al.,* 2012).

Synthetic polymer gels are popular formulations used to improve viscosity and residence time during cutaneous treatment by combining with medications or drug carriers. They are usually clear or semi-transparent and do not have the oily, unpleasant sensation that most oil-based ointments or lotions do. Some polymer gels may promote cell proliferation and migration, which aids in wound healing. Previously, gel combined with lipid nanoparticles was used to reduce skin irritation and to transfer medications into deeper layers of skin. It can improve lipid nanoparticle dispersion and stability, making them easier to apply to the skin for psoriasis treatment (Joshi and Patravale, 2006; Han *et al.,* 2012).

Isoconazole is an antifungal imidazole that is used to treat superficial skin and vaginal infections. Isoconazole is an azole antifungal medication that works similarly to clotrimazole in treating foot and vaginal infections (Czaika *et al.,* 2013). So, this study deals with formulation, development and evaluation of Isoconazole of nano structured lipid careers incorporated gel for effective fungal treatment.

MATERIALS & METHODS

Chemicals and reagents

Carbopol 934, Propylene glycol, Water (ml) Lipid, Soy lecithin, Stearyl amine Pluronic F-68 (1% w/v) were obtained from S.D. Fine chemicals Mumbai. All chemicals and reagents use were of standard analytical grade.

Preparation of Isoconazole loaded Nanostructured lipid carriers

Nanostructured lipid carriers were prepared by using microemulsion technique (Muller *et al.,* 2007) and o/w microemulsions were initially prepared. The oil phase, lipophilic surfactant and continuous phase used are glyceryl tripalmitate, soy lecithin and pluronic F-68 (hydrophilic surfactant) respectively. The lipid and soy lecithin were melted at 70oC and the drug was added with constant stirring. 10 ml of aqueous surfactant solution containing pluronic F-68 heated at the same temperature was added to the melted lipid with mechanical stirring for 15 min. A clear microemulsion was obtained at a temperature close to the melting point of the lipid used. Stearyl amine was used as a positive charge inducer and added to melted lipid. Nanostructured lipid carrierswere obtained by dispersing the warm o/w microemulsion which is added drop wise into ice cold water in a beaker under continuous stirring. After completion of stirring, the Nanostructured lipid carriersdispersion was subjected to ultrasonication for 15 min.

Table 1: Composition of nanostructured lipid carriers by varying amount of Lipid

Components	Formulation code		
	F1	**F2**	**F3**
Lipid	**50**	100	200
Soy lecithin	**1**	1	1
Stearyl amine	**1**	1	1
Pluronic F-68 (1% w/v)	**1**	1	1
Stirring speed (rpm)	**1500**	1500	1500
Stirring time (hrs)	**3**	3	3

Table 2: Composition of Nanostructured lipid carriers by varying Stirring time

Components	Formulation code				
	F4	**F5**	**F6**	**F7**	**F8**
Lipid	50	50	50	**50**	50
Soy lecithin	1	1	1	**1**	1
Stearyl amine	1	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	1	**1**	1
Stirring speed (rpm)	2000	2000	2000	**2000**	2000
Stirring time (hrs)	1	2	3	**4**	5

Table 3: Composition of Nanostructured lipid carriers by varying Stirring speed

Components	Formulation code			
	F9	**F10**	**F11**	**F12**
Lipid	50	50	**50**	50
Soy lecithin	1	1	**1**	1
Stearyl amine	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	**1**	1
Stirring speed	1000	1500	**2000**	2500
Stirring time	4	4	**4**	4

Table 4: Composition of Nanostructured lipid carriers by varying amount Surfactant

Components	Formulation code			
	F13	**F14**	**F15**	**F16**
Lipid	50	**50**	50	50
Soy lecithin	1	**1**	1	1
Stearyl amine	1	**1**	1	1
Pluronic F-68 (1% w/v)	0.5	**1**	1.5	2
Stirring speed	2000	**2000**	2000	2000
Stirring time	4	**4**	4	4

Preparation of gel base

Carbopol 934 (1-3% w/v - Nanostructured lipid carriers based gel formulation i.e. G-1 of 1% w/v, G-2 of 2% w/v, G-3 of 3% w/v) was accurately weighed and dispersed into double (distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate Nanostructured lipid carriers containing gel in which previously prepared Nanostructured lipid carriers was added. Nanostructured lipid carriers preparation corresponding to 5% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Table 5: Formulation optimization of gel base

Ingredient (%)	G-1	G-2	G-3
Drug (Invasomes equivalent to 0.1%)	0.1	0.1	0.1
Carbopol 934	1	2	3
Propylene glycol	0.2	0.2	0.2
Water (ml)	100	100	100

Evaluation of nanoparticles

Particle size and zeta potential

Particle size and zeta potential of the Nanostructured lipid carriers were measured by photon correlation spectroscopy using a Malvern Zetasizer the results shown in table below (Ricci *et al.*, 2005).

Entrapment efficiency

Entrapment efficiency was determined by dialysis method. Nanostructured lipid carriers entrapped Isoconazole were separated from the free drug by dialysis method. The above said formulations were filled into dialysis bags and the free Isoconazole dialyzed for 24 hours into 50 ml of phosphate buffer 7.4 saline. The absorbance of the dialysate was measured at 272 nm against blank phosphate

buffer 7.4 saline and the absorbance of the corresponding blank phosphate buffer 7.4 saline was measured under the same condition. The concentration of free Isoconazole could be obtained from the absorbance difference based on standard curve. Standard curve was made by measuring the absorbance at 272.0 nm for known concentrations of Isoconazole solution. The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug.

Total drug content:

From the prepared nanostructured lipid carriers formulation 1ml of suspension is dissolved in the 10 ml of 7.4 PBS buffer and ethanol mixture. The amount of Isoconazole was determined using UV spectrophotometer at 272nm.The placebo formulation prepared similarly to drug loaded nanostructured lipid carriers is used as blank. The total drug content was calculated.

***In vitro* drug release in gastrointestinal fluids of different pH**

The prepared Nanostructured lipid carriersdelivery system was evaluated for in vitro drug release. The drug release studies were carried out using USP XXII paddle type Dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. A weighed quantity of formulation (100 mg) was spread over the surface of dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 272.0 nm for Isoconazole and using UV visible spectrophotometer. The release of Isoconazole was calculated with the help of Standard curve of Isoconazole.

Evaluation of gel

Measurement of viscosity

Viscosity measurements of prepared topical Invasomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted.

Drug content

Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol (Agrawal *et al.*, 2010). This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 272 nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube (Lacerda *et al.*, 2011).

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer et al. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan.To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80 g of weight was noted. Good spreadibility show lesser time to spread.

***In-vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell.

During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of 272 nm.

Stability studies

Stability study was carried out for drug loaded gel at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25-28 ± 2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any viscosity and % assay.

Table 6: Result for Particle size, Entrapment efficiency and drug content of drug loaded Nanostructured lipid carriers

Formulation Code	Particle size	Entrapment Efficiency	Drug Content
F1	**245.65**	**83.32**	**98.78**
F2	269.98	78.85	98.85
F3	265.58	73.32	98.12
F4	278.85	69.98	97.85
F5	258.98	81.12	98.85
F6	254.45	77.78	96.65
F7	**233.36**	**85.45**	**99.12**
F8	245.65	73.15	99.45
F9	269.98	71.15	99.05
F10	258.74	68.85	99.45
F11	**220.25**	**83.32**	**97.78**
F12	274.45	73.32	96.65
F13	263.32	63.32	98.74
F14	**215.45**	**83.32**	**99.45**
F15	236.65	81.12	98.78
F16	245.78	87.74	98.78

Table 7: Particle size and Entrapment efficiency of Optimized Nanostructured lipid carriers

Formulation Code	Particle size (nm)	Entrapment Efficiency	Zeta potential
F14	215.45	83.32	-36.48

Table 8: Cumulative % drug release

S. No.	Time (hrs)	% Cumulative Drug Release
1	1	5.25
2	2	11.12
3	3	15.65
4	4	22.23
5	5	36.65
6	6	45.65
7	7	65.58
8	8	73.32
9	9	88.58
10	10	92.23
11	12	99.12

Table 9: Characterization of gel based formulation

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
G-1	3542±10	6.85±0.01	97.85±0.12	175±5	10.21±0.12
G-2	3256±8	6.81±0.02	99.12±0.15	165±6	8.95±0.15
G-3	3110±7	6.98±0.01	97.65±0.14	159±3	7.45±0.13

Table 10: *In vitro* drug release study of optimized gel formulation G-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	18.15
2	1	32.25
3	2	41.15
4	4	63.32
5	6	70.25
6	8	87.78
7	10	95.65
8	12	98.74

Table 11: Result of mean particle size & Zeta potential

Formulation	Mean particle size (nm)	Zeta potential (mV)
F14	215.45nm	-36.48mv

Table 12: *In-vitro* drug release data for optimized formulation G-2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	18.15	1.306	79.77	1.902
1	1.000	0	32.25	1.552	64.35	1.809
2	1.414	0.301	41.15	1.661	54.15	1.734
4	2.000	0.602	63.32	1.817	34.44	1.537
6	2.449	0.778	70.25	1.865	26.68	1.426
8	2.828	0.903	87.78	1.949	11.11	1.046
10	3.162	1	95.65	1.985	3.35	0.525
12	3.464	1.079	98.74	1.993	1.55	0.19

Table 13: Regression analysis data of optimized gel formulation G-2

Batch	Zero Order	First Order
	R^2	R^2
G-2	0.941	0.955

RESULTS AND DISCUSSION

In total 16 formulations of Nanostructured lipid carrier were made by changing the ratio of chemicals used for its manufacturing. The particle size ranged from 215.25 to 278.85. The drug content was found to be maximum in F14 formulation which is 99.45 %. The entrapment efficiency of F14 was noted to be 83.32. The % Cumulative Drug Release for F14 was estimated to be 99.12 % in 12 hours.

Further by using F14 formulation the gel was made. The viscosity of gels ranged from 3110±7 to 3542±10cps while pH spanned between 6.81±0.02 to 6.98±0.01. The percentage of drug was observed to be maximum in G-2 which is 99.12±0.15. The Extrudability extends from 159±3 to 175±5g. While the spreadibility was seen to range from 7.45±0.13 to 10.21±0.12.

The *in vitro* drug release study of optimized gel formulation G-2 suggested that about 98.74% drug release is achieved in 12 hours. Controlled drug release from Nano lipid carriers can result in a longer half-life and delayed enzymatic attack in systematic flow. The drug release behaviour of Nano lipid carriers is affected by the manufacturing temperature, emulsifier composition, and oil content in the lipid matrix. The drug amount on the nanoparticles' outer shells and on the particulate surface is released in a burst

manner, whilst the drug absorbed into the particulate core is released over time.

The mean particle size & zeta potential was observed to be 214.45nm & -36.48mV respectively. Because the physical strength of vesicle dispersion is dependent on particle size, and as particle size falls, surface area increases, particle size is a significant parameter in producing control and quality. The electric potential of a particle in suspension is denoted by ZP. It is a parameter that can be used to analyse the physical stability of colloidal dispersions. The surface charge generates a potential surrounding the particle that is greatest near the surface and decays with particle dispersion into the medium. The ZP can be calculated by measuring the speed of particles in an electrical field.

The Cumulative % Drug Release for optimized formulation of gel G2 estimated to be 98.74% in 12 hours. To obtain the release constant and regression coefficients, the release data were fitted to various kinetic models. The R^2 value for zero order & first order evaluated as 0.941 & 0.955 respectively.

CONCLUSION

NLCs-containing Gel formulation of isoconazole indicated optimal particle size, entrapment efficiency, and in-vitro release kinetics. The final NLC encapsulated Gel formulation demonstrated adequate spreadability, viscosity, and permeation characteristics, demonstrating the ability of NLCs containing gel to treat deep skin fungal infections as indicated and demonstrated by ex-vivo permeation investigations. And it may be stated that using NLCs incorporated gel is considerably superior over conventional dosage form.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Richardson, M.D. & Warnock, D.W. (2012). *Fungal Infection: Diagnosis and Management*. John Wiley & Sons: Chichester, UK.
- Romani, L. (2011) Immunity to fungal infections. *Nature Reviews. Immunology*, 11, 275–288.
- Ma, Z., Wang, X. & Li, C. (2020) Strategies of drug delivery for deep fungal infection: A review. *Pharmaceutical Nanotechnology*, 8, 372–390
- Akhtar, N., Verma, A. & Pathak, K. (2015) Topical delivery of drugs for the effective treatment of fungal infections of skin. *Current Pharmaceutical Design*, 21, 2892–2913
- Beloqui, A., Solinís, M.Á., Rodríguez-Gascón, A., Almeida, A.J. & Préat, V. (2016) Nanostructured lipid carriers: Promising drug delivery systems for future clinics. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 12, 143–161
- Iqbal, M.A. MD S, Sahni JK, Baboota S, Dang S, Ali J. Nanostructured lipid carriers system: recent advances in

drug delivery. Journal of drug targeting. 2012 Dec 1;20(10):813-30.

- Joshi, M. & Patravale, V. (2006) Formulation and evaluation of nanostructured lipid carrier (NLC)-based gel of valdecoxib. *Drug Development and Industrial Pharmacy*, 32, 911–918
- Han, F., Yin, R., Che, X., Yuan, J., Cui, Y., Yin, H. & Li, S. (2012) Nanostructured lipid carriers (NLC) based topical gel of flurbiprofen: Design, characterization and in vivo evaluation. *International Journal of Pharmaceutics*, 439, 349–357 [DOI:
- Czaika, V.A., Siebenbrock, J., Czekalla, F., Zuberbier, T. & Sieber, M.A. (2013) Reactive oxygen species and the bacteriostatic and bactericidal effects of isoconazole nitrate. *Mycoses*, 56 (Supplement 1), 16–22
- Müller, R.H., Petersen, R.D., Hommoss, A. & Pardeike, J. (2007) Nanostructured lipid carriers (NLC) in cosmetic dermal products. *Advanced Drug Delivery Reviews*, 59, 522–530
- Ricci, M., Puglia, C., Bonina, F., Di Giovanni, C., Giovagnoli, S. & Rossi, C. (2005) Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): In vitro and in vivo studies. *Journal of Pharmaceutical Sciences*, 94, 1149–1159
- Agrawal, Y., Petkar, K.C. & Sawant, K.K. (2010) Development, evaluation and clinical studies of acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. *International Journal of Pharmaceutics*, 401, 93–102
- Lacerda, S.P., Cerize, N.N. & Ré, M.I. (2011) Preparation and characterization of carnauba wax nanostructured lipid carriers containing benzophenone-3. *International Journal of Cosmetic Science*, 33, 312–321