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Research Article

Design And Evaluation of Topical Gel Containing Solid-Lipid Nanoparticles Loaded with Luliconazole

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ABSTRACT
New topical pharmaceutical options are critically needed to obviate fungal infections. Luliconazole is a potent antifungal drug for the treatment of fungal infection. Due to bioavailability barriers of luliconazole, the current study is associated to develop an optimized topical luliconazole solid lipid nanoparticles (SLN) gel formulation against tropical fungal infection with prolonged therapeutic potential. Luliconazole loaded SLN were prepared through the solvent diffusion method using stearic acid & poloxamer 188 and optimized as per their entrapment efficacy. Thereafter, the optimized SLN wes subjected to physicochemical evaluation, followed by the preparation of different gel formulation. The physicochemical parameters of the optimized gel formulation (G3) were evaluated. Further anti-fungal activity of the G3 was determined against the growth of Candida albicans by TLC-Bioautography assay. The results reveal that SLN F6 shows a significant entrapment with 92.13%±0.975 entrapment efficacy. In particle size, size distribution and zeta potential analysis, SLN exhibit a mean particle diameter of ~344.3 nm, PDI of 0.168, intercept value 0.98 and zeta potential ~18.8 mV. The G3 shows a higher entrapment with 91.39%±0.187 entrapment efficacy and in-vitro drug release profile of the G3 with 1.5 % carbopol 934 w/v shown a sustained release profile with 79.57%±0.213 desolvation rate even after 24 hrs. The anti-fungal activity of SLN G3 gel showed a strong zone of inhibition of the growth of C. albicans. Hence, the study concludes that luliconazole loaded SLN G3 gel formulation containing 1.5% w/v carbopol 934 is suitable for topical application and having strong anti-fungal activity.

Keywords: Solid lipid nanoparticles, Luliconazole, Tropical gel formulation, Anti-fungal activity

INTRODUCTION
Fungal infection is generally characterized by the progressive onsets of species of fungi and causes severe health problems in immune-restricted individuals with high morbidity and mortality. It is greatly associated with the patients having hematologic, allogeneic, prolonged leukopenia and autologous grafts disorders. Fungal infections generally curve the whole body's system and lead the serious lethality to the body's cellular system1. The subcutaneous mycosis and is caused by the chronic fungal infection which targets dermis and the subcutaneous tissue and it is then termed as subcutaneous mycosis2. Sporotrichosis is one of the most common types of tropical infection caused by progressive onsets of the fungus Sporothrix schenckii3. For the inhibition of subsequent progression of any fungal infection, a drug should be much effective without having liabilities to produce any serious harm. The obvious and palliative choice for patients is the only way to cure the progressive prevalence of fungal infection. Although, the large number of pharmaceuticals are available in the market which are conventionally utilized as tropical medicaments for the treatment of cutaneous and subcutaneous fungal infections. The pharmaceuticals are available in form of creams, lotions, gels, etc. Due to bioavailability barriers or lack of availability of the drug to the therapeutic site is a major concern of patient compliance. Therefore, in light of the therapeutic concern of a topical anti-fungal drug, the release rate of the drug should be controlled by the type of formulation to achieve a sufficient therapeutic value and can provide an extended pharmacological effect.
Solid lipid nanoparticles (SLN) are an advanced pharmaceutical novel drug delivery system (NDDS) in the modern era of pharmaceuticals. SLN was discovered in 1991, which represents traditional colloidal carriers such as polymeric and micro, liposomes emulsions, and nanoparticles The conventional approach of SLN is associated to enhance drug permeation ability, good release profile and targeted drug delivery

with excellent physical stability and low degradability etc[6, 7] .Nanoparticles ranging from 10 to 1000 nm in size have promising effects in enhancing the bioavailability of the drug. The formulations hyphenated with SLN is a major consideration in the era of colloidal drug carrier system which generates an alternative particulate in the field of NDDS[8].

Luliconazole is a contemporary and wide-spectrum anti-fungal agent that is approved by the FDA (USA). Due to the bioavailability barrier of luliconazole, it does not encompass the topical delivery system9. In fungal infection, cutaneous and subcutaneous encompassment is required to customize the drug permeation ability which can situate high drug concentrations at the site of therapeutic action. However, many topical pharmaceuticals of luliconazole are present in market that have minor skin permeability with shorter skin retention and it leads major patient compliance10. In current time, nanoformulation have gained exponential growth in field of pharmaceuticals due to high complexity in drug load capacity, limitized excipients quantity, steadiness in drug stability, lesser harmfulness, and easy scale-up and processing. SLNs have strangely wide-ranging properties which mark them beneficial for high permeation ability in topical delivery of drugs and potentiate extended retention at the site of contagion[11].

Apart from these, TLC bioautography is approached to direct coupling of the active compounds on the TLC plate with visualizable enzyme reactions can be used for direct screening of activity or activity-oriented constituents. Such TLC-based bioautography through enzymatic reaction provides us the initial stage to develop activity-based screening of active compounds[12].

Hence, the present study is associated with the optimization and evaluation of topical gel containing SLN loaded with luliconazole. Further, the prepared optimized formulation is evaluated for the anti-fungal activity against C. albicans by TLC-Bioautography assay.

MATERIALS AND METHODS

Reagents and chemicals

Luliconazole was procured as a gift sample from SMS Pharmaceuticals, INDIA, Carbopol 934 purchased from sigma Aldrich, Italy, Steric acid purchased from Fisher Scientific India Pvt. Ltd, Ethanol purchased from Merck, India, n-octanol purchased from SD Fine-chem. Ltd, Mumbai, Methanol purchased from Fisher Scientific India Pvt. Ltd, MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide} obtained from Jamia Hamdard, New Delhi, Poloxomer 188 purchased from Central Drug House (P) Ltd, Sodium Hydroxide, Potassium Dihydrogen orthophosphate, and Disodium hydrogen orthophosphate purchased from Thomas Baker, New Delhi.

Preformulation studies

Determination of the absorption maximum of luliconazole in ethanol

The absorption maximum of luliconazole was determined as per the standard protocol with some modification. In brief, the stock solution of luliconazole was prepared at the concentration of 1 mg/ml in methanol. Further, it was followed by serial dilution to get the concentration of luliconazole as 2, 4, 6, 8, 10 µg/ml, and then it proceeded to UV spectrophotometric analysis at the λmax of 299 nm. The measurement was taken in triplicate and obtained data were analyzed statistically[13].

Determination of Aqueous Solubility

The determination of aqueous solubility of luliconazole was estimated through the Saturation shake - flask method. An optimum amount of luliconazole was dissolved in distilled water and acetate buffer pH 5.5 then followed by vortex and centrifugation at 50 rpm and 37 °C for 48 hrs, the resulting solution was filtered and analyzed spectrophotometrically at 299 nm. The measurement was taken in triplicate[14].

Determination of lipophilicity

Lipophilicity of luliconazole was determined through the traditional shake flask method as described in protocol with some modification. In brief, an optimum uniform amount of luliconazole was poured into three different volumetric flasks and then the measured quantity of lipids such as stearic acid, prectrol, dynasan 114 placed to each flask simultaneously. The resulting heterogeneous mixture proceeded to the vortex and then centrifugation at 50 rpm at 37 °C for 48 hrs. The supernatant was collected and filtered using a syringe filter of 0.22 µm. The filtrate was then analyzed spectrophotometrically at 299 nm[14].

The further partition coefficient of the luliconazole was determined using n-octanol and water partition system. The measured amount of luliconazole was placed into a conical flask containing measured volumes of an n-octanol and aqueous buffer solution. The flask was shaken with a uniform time interval for 48h to attain equilibrium and then the resulting mixture placed to a separating flask with a final shaking and kept remains undisturbed to be separated into two layers. The targeted measurement was

proceeded to be analyzed spectrophotometrically at 299 nm. The resulting values of both the phases were determined in the form of the log10P of the ratio was calculated. All the measurement was taken in triplicate[15].

Infrared spectroscopy (FTIR)

The spectral analysis for luliconazole and stearic acid was performed by a Win-IR, Bio-Rad FTS spectrophotometer. The individual sample was assorted with potassium bromide and later proceed for spectroscopical observation under the range of 4000 to 400 cm^{-1}[16].

Preparations of SLN

The SLN was prepared using a referenced protocol of the solvent diffusion method with some modification9. Briefly, a known amount of luliconazole and stearic acid was placed into 5 ml of ethanol and heated at 60±3.0℃ on a water bath. The obtained solution was placed into 5 ml of aqueous poloxomer 188 solutions at 4 – 8℃ under magnetic stirring at 2000 rpm with the help of a syringe. The SLN formed instantly and recovered by centrifugation at 2000 rpm for 30 min at 4℃. The obtained heterogeneous mixture further proceeded to high- pressure homogenization via APV 2000 homogenizer at 1200 bars. The obtained mixture was placed to be stable at room temperature, which turns to clear nanocrystals by recrystallization of the dispersed lipid[9].

Evaluation of SLN

Evaluation of entrapment efficacy (EE)

The EE of SLN loaded with luliconazole was estimated through the described method with some modification. In brief, the prepared SLN was dried at room temperature then 5 mg of dried SLN were dissolved in 10 ml HPLC grade ethanol and further proceeds by filtration through a syringe filter of 0.22 µm capacity. The concentration of luliconazole was determined spectrophotometrically at 299 nm9. The measurement was taken in triplicate and based on percentage entrapment, the best one was selected for further evaluation. The EE has been determined according to the following equation:

% EE = W(initial drug) −W(free drug) / W(initial drug) x 100 Where, W(initial drug) is the mass of drug added initially, W(free drug) is the mass of free drug detected in the supernatant after centrifugation.

Physicochemical property: Physicochemical Properties of the SLN dispersions were characterized as color, odor, pH, and the solubility of SLN F6 in the aqueous medium[9,17].

Particle size analysis and zeta potential measurement

The average particle size and zeta potential were determined as per the described protocol with some modification. The analysis was performed at room temperature by zeta potential/ particle size analyzer. SLN F6 was diluted with phosphate- buffered saline and the pH of the solution was stabilized at 7.4 and then the sample proceeded for analysis[18].

Optical microscopic analysis

Optical microscopic analysis of optimized formulation SLN F6 was analyzed with the help of digital light optical microscope equipped with a fluorescent lamp (Labomed LX-400) at 100x magnification. it was aimed to determine whether the luliconazole SLN is effectively localized with homogenous and uniform texture within the SLN dispersion19.

FTIR of SLN F6

The spectral analysis for SLN F6 was performed by a Win-IR, Bio-Rad FTS spectrophotometer. The individual sample was assorted with potassium bromide and later proceed for spectroscopical observation under the range of 4000 to 400 cm^{-1}[16].

Development and evaluation of gel

Development of gel

The gel was developed as per the referenced protocol with slight modification. Briefly, Carbopol 934P was placed in a defined quantity of distilled water while constant stirring at 600 rpm and followed by the adding of methylparaben sodium (0.02% w/v) and propylparaben sodium (0.1% w/v) and remained undisturbed with continuous stirring for a 30 min. Prepared gel base was set aside for 24 hrs. Next, SLN F6 was disseminated with the measured quantity of propylene glycol (5% w/w) and 1% ethanol (20% w/w) and far ahead it was added to carbopol gel bases with continuous shaking at 1000 rpm and followed by churning for 30min. Tri-ethanol amine (TEA) was subjected to the final stage to maintain pH (5.5 - 6.5) for drug stabilization and stirred thoroughly to obtain a clear gel9.

The same procedure was applied to get four formulations having a varying amount of carbopol and the aim is associated to prepare different forms of gel is to obtain the best homogeneous and uniform texture with stable physicochemical reliability in respect of %age release of the leading moiety. Different formulations of SLN gel are enlisted as in Table 1;

Table 1: Different prepared gel formulations containing solid lipid nanoparticles

Formulation code	Carbopol 934 % (w/v)
G1	0.5
G2	1
G3	1.5
G4	2

Determination of the % EE

The % EE of different prepared batches of the gel was estimated by quantitating the free mass drug in the diffused phase of the gel solution after centrifugation. In brief, 1g of the gel was diffused with ethanol and vortexed for 5 minutes to ensure the proper extraction of drugs in ethanol. Then, the obtained mixture was proceeded for centrifugation at 15000 rpm for 60 minutes at 4°C temperature. The supernatant was collected from the centrifuged mixture and allowed to analyze for quantitative analysis spectrophotometrically at 299 nm20. The EE percentage was calculated from the equation as follows; % EE = W(initial drug) −W(free drug) / W(initial drug) x 100 Where, W(initial drug) is the mass of drug added initially, W(free drug) is the mass of free drug detected in the supernatant after centrifugation.

Determination of pH

The pH of the gel was evaluated as per the standard protocol with the help of a digital pH meter. The glass electrode of the pH meter was immersed in the optimized SLN gel formulation and revolved to determine the pH of gel[9].

Determination of viscosity

The viscosity of gel was evaluated as per the standard protocol with some modification as follows. In brief, the obtained SLN gel was evaluated based on physical appearance and then the viscosity of SLN gel was evaluated through Brookfield Viscometer[21].

Spreadability

The spreadability of the gel was determined as per the described protocol with some modification22. In brief, 500 mg of optimized formulation was put on the acrylic plate at the middle center and the second plate was concentrically situated above it. The width of the circle in which the gel was spread was measured as the primary width. An approx 500 g weight was applied on the above plate for few mins. The spreadability of the gel was estimated as per the rises in the diameter due to the dissemination of the gels and the obtained diameter of the disseminated gel was noted.Drug release and kinetics profiling The drug release and kinetics profiling of optimized formulation (SLN G3) gel were evaluated by in-vitro drug release profiling methods using the dialysis bag technique17. 1g of gel sample was accurately weighed and placed to cellulose dialysis membrane. The membrane was tied with a thread and placed to a flask containing 50 ml ethanol and phosphate buffer solution. The container was placed to a magnetic stirrer at 37 °C with the constant stirring at 50 rpm. Thereafter, 1 ml of sample was withdrawn at regular intervals of 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24, and the withdrawn amount was replenished with dissolution media at the same time withdrawn. The released mass of SLN entrapped luliconazole was quantitated spectrophotometrically at 299 nm in respect of blank. Each measurement was taken in triplicate. In-vitro drug release profiles of prepared luliconazole loaded SLN gel formulation was evaluated statistically by various kinetic models named as zero-order, first-order, Higuchi and Korsemeyer–Peppas model. The kinetics models were determined statistically to enlighten the mechanism of drug release profiling. The high regression coefficient value was considered to be much effective for the initialization and acceptance of kinetics orders.

FTIR analysis of SLN G3

The spectral analysis for SLN G3 was performed by a Win-IR, Bio-Rad FTS spectrophotometer. The individual sample was assorted with potassium bromide and later proceed for spectroscopical observation under the range of 4000 to 400 cm^{-1}[23].

Scanning Electron Microscopy (SEM)

The morphological analysis of SLN G3 was examined by SEM using the standard protocol with some modification9. The little sample of SLN gel was placed on a glass stub and vacuum dried. After that, the stub having the sample was sited to the SEM chamber coated with gold-palladium and then the sample was observed microscopically at an accelerating voltage of 10 kV.

Anti-fungal activity of SLN G3 gel against Candida albicans by TLC-Bioautography assay. 10 mg of SLN G3 gel was dissolved in methanol and vortexed for 5 min for the proper dissolution.

6 µL of the resulting solution was applied manually on a 3 x10 TLC plate in the form of a spot using the syringe of 100 µL capacity. The solution was applied to the TLC plate thrice for the optimization of drug activity. Thereafter, the prepared TLC plate was dipped smoothly in a suspension of freshly cultured C. albicans was made in molten Mueller Hinton agar (50°C) medium at a final concentration of approx. 2×107 cfu/mL. After that, the plate was incubated at 37°C for 24 hrs. After the incubation period, the plate was dipped in the aqueous solution of MTT (2 mg/mL) for visualization of the zone of inhibition SLN G3 gel against C. albicans growth24.

RESULTS AND DISCUSSION

Preformulation study of drug

Determination of the absorption maximum of luliconazole in ethanol

The potential drug absorption was calculated as per standard protocol through the absorption maximum of luliconazole at 299 nm λmax against the concentration 2-10 µg/ml. The regression equation and coefficient were found to be 0.0664x - 0.0478 and 0.998 respectively. The associated aim to determine the luliconazole absorption maxima and method validation is for qualitative and quantitative analysis 13.

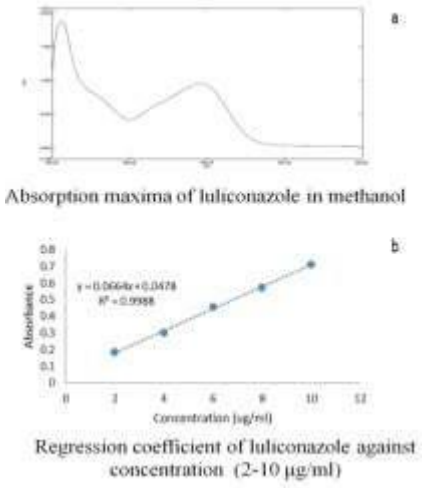

Fig.1: Absorption maxima of luliconazole (a) and regression coefficient against the different concentration of luliconazole (µg/ml) (b)

Physicochemical studies of luliconazole were conducted to evaluate the physicochemical properties of the drug. The studies conducted to evaluate luliconazole hydrophilic and lipophilic compatibility. The result shows that luliconazole having poor solubility potential with water which was found to be 0.00585 ± 0.293 mg/ml, and the solubility of luliconazole in stearic acid, prectrol, dynasan 114 was obtained 23.754±0.47, 18.314± 0.85, 22.875±0.32 mg/ml. Besides, the non-aqueous solubility was obtained 17.984 ± 0.52 mg/ml for luliconazole in n-octanol. The log10P value of luliconazole in

Method development for the preparation of SLN

The method deals with various modified stearic acid, prectrol, dynasan 114, and n- octanol was obtained as 3.98, 3.30, 3.87, and 3.65 simultaneously.

The FTIR analysis of luliconazole and stearic acid was performed to compare better compatibility analysis before and after formulation. In spectral analysis, the spectral data of luliconazole reveal the principal absorption peaks at 2979.43 cm-1 for C-H stretching, 2515 & 2608 cm-1 for S-H stretching, 2198.82 cm-1 for C≡N stretching, 1728, 1812, 1887 for C=C alkene Stretching, 1554.35 cm-1 for C=N stretching, 1471.35 cm-1 for C=C aromatic ring stretching, 820.41 cm-1 for para C–H distribution stretching and 759.46 and 1098.45 cm-1 for C-Cl stretching were detected. These detected principal peaks confirmed the purity and authenticity of the luliconazole as similar to the referenced report 9. The spectra data of stearic acid reveals the principal absorption peaks at 2915.20 cm-1 & 2848.02 cm-1 in the high-frequency region recognized with -CH2- band asymmetric and symmetric stretching vibrations, whereas and 1700.89 cm-1 for -COOH stretching is attributed in the low-frequency region. The identified

adsorption peaks are the characteristic peaks of stearic acid. Moreover, the spectral data was confirmed with the referenced report and all the detected principal peaks confirmed as the principle adsorption peaks of stearic acid [25].

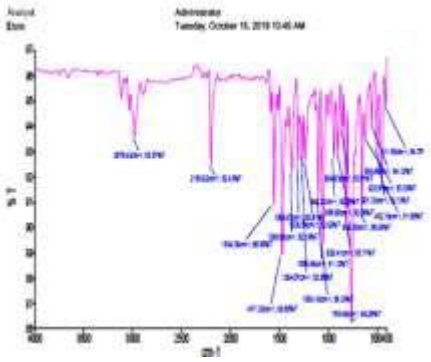

Fig.2: FTIR spectrum of luliconazole

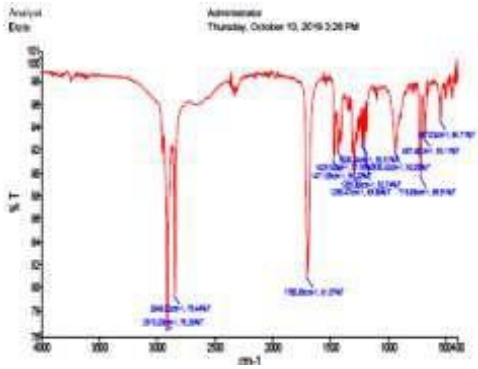

Fig.3: FTIR spectrum of stearic acid

nano-precipitation methods for the optimization of SLN in respect of EE of luliconazole at both treatment segments, i.e. nano-precipitation and cooling sonication probe. The temperature was controlled by 4°C and 25°C in both segments. The instant adding of the organic phase to the aqueous phase was conserved at 4°C which gives immediate precipitation due to hyphenation with anti-solvent. The temperature was controlled at the initial phase of nano-precipitation which helped to achieve homogeneity. High-pressure homogenization supports to get uniform homogeneity by decreasing larger crystals size and bead milling aggregation26. Further, in the optimization of SLN, the method was archived step by step with alternate changes in the concentration of stearic acid and poloxomer 188 (w/v) ranging from 0.5-2 %. All the prepared groups of SLN were coded successfully and proceed to quantitate percent entrapment of active moiety spectrophotometrically at 299 nm. The obtained data were evaluated statistically. The SLN which deal with the high entrapment of luliconazole was chosen as the optimized SLN and proceed for further evaluation.

Table 2: Preparation of different solid lipid nanoparticles loaded with luliconazole

SLN code	Luliconazole % (w/v)	Concentration of bases for the formation of SLN	
		Stearic acid % (w/v)	Poloxomer 188 % (w/v)
F1	1	0.5	1
F2	1	0.7	1
F3	1	1	1
F4	1	2	1
F5	1	1	0.5
F6	1	1	0.7
F7	1	1	1.5
F8	1	1	2

Evaluation of SLN Evaluation of EE

Initially, in the pre-formulation studies, luliconazole was characterized physicochemically and spectroscopically. After the successful formation of different batches of nanoparticles, the percentage EE of luliconazole was determined. The percentage of EE was evaluated spectrophotometrically at 299 nm. Thereafter the results reveal that SLN F6 and SLN F1 have the highest and lowest % EE of luliconazole loaded SLN by 92.13%±0.975 and 53.78%±1.052 w/w respectively. Similarly, a study cited by Ige et al. reported a maximum % EE by 90–95% w/w27. Therefore, based on percent drug entrapment, SLN F6 was selected as an optimized SLN and was proceed for further evaluation includes physicochemical properties and gel formation. The percent drug entrapment of all SLN groups have been shown graphically in Figure 4.

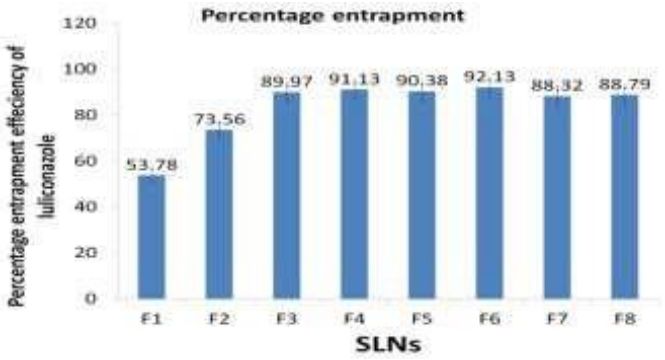

Fig.4: Percentage entrapment efficiency of luliconazole in SLN

Physicochemical property

The SLN F6 was evaluated based on their physicochemical characteristics such as color, odor, pH stability, and aqueous solubility. The physicochemical results reveal that SLN has a white transparent color with homogeneous and uniform texture, aromatic odor, better stability at 7.4 pH, and water solubility was found 0.01819± 0.035 mg/ml, i.e. much enough than luliconazole solubility.

Particle size analysis and zeta potential measurement

The particle size analysis and zeta potential measurement of luliconazole SLN were identified successfully using a Nano ZS90 Zetasizer system. Zeta potential is one of the important parameters used to forecast the physical stability of nanoparticles. The stability of the nanoparticle system depends on the high zeta potential value which points toward better stability of the nanosystem since it could deliver a deterring force between the nanoparticles28. As shown in Fig. 5, SLN shows a quite high value of zeta potential by ~18.8 mV and states to the high stability of the nanosystem. In particle size analysis, SLN unveiled with the mean particle diameter by ~344.3 nm, unimodal size distribution, a polydispersity index (PDI) by 0.168, intercept value 0.98 and 92% peak intensity. The PDI is a parameter that represents the dissemination factor with the low aggregation of nanoparticles when PDI value would be < 0.529.

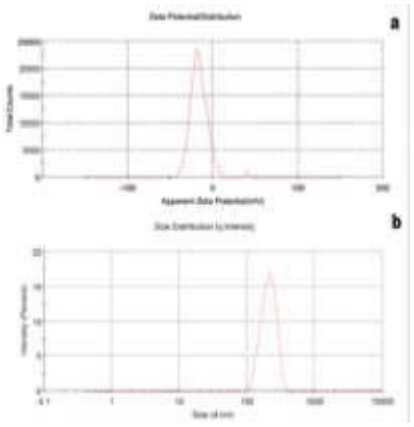

Fig.5: Zeta potential (a), particle size and size distribution (b) of luliconazole SLN F6

Optical microscopy

Optical microscopy of optimized preparation i.e. SLN F6 was defined with the help of digital light optical microscope at 100x magnification and the observation shows that the luliconazole SLN is effectively localized with homogenous and uniform texture within the SLN dispersion. It states that only particles with a mean diameter higher than 2.5 µm which were visualized clearly against microscopy resolution power. Moreover, SLN preparation has even no self-assembled structures was observed. The micellar structures were not observed during the observation of optical microscopy. Optical microscopy images of luliconazole loaded SLN F6 shown in figure 6.

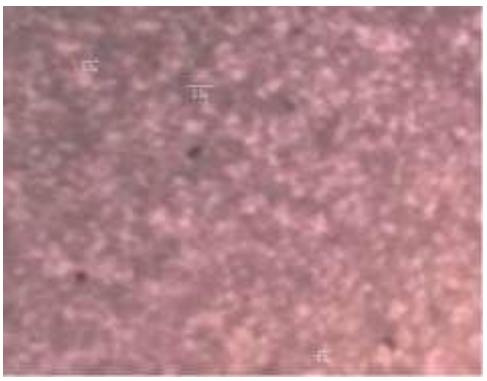

Fig.6: Optical microscopy images of luliconazole loaded SLN F6

FTIR analysis of SLN F6

FTIR analysis of SLN F6 was performed to determine the possible interaction between the drug and drug additives. The spectral data reveal the principal absorption peaks of luliconazole at 2955.75 cm-1 for C-H stretching, 2523 & 2647 cm-1 for S-H stretching, 2201.52 cm-1 for C≡N stretching, 1556.90 cm-1 for C=N stretching, 1471.88 cm-1 for C=C aromatic ring stretching and 720.33 and 1101.29 cm-1 for C-Cl stretching. Whereas, the principal absorption peaks of stearic acid were found at 2914.97 cm-1 & 2848.05 cm-1 in the high-frequency region attributed to -CH2- band asymmetric and symmetric stretching vibrations, whereas and 1698.03 cm-1 for -COOH stretching is attributed in the low-frequency region. The spectral analysis of the optimized SLN confirmed that there are no more changes in the luliconazole after the successful formation of SLN. The spectral data strongly supports referenced values as reported 25.

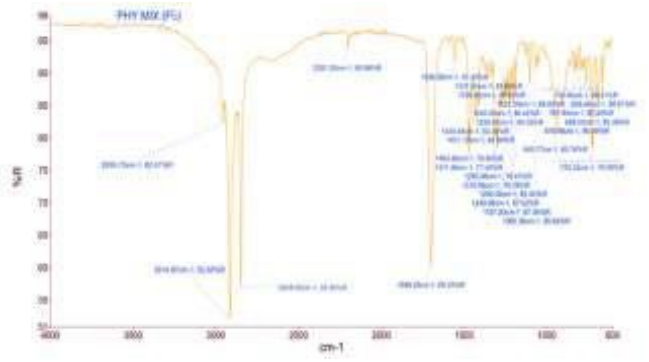

Fig.7: FTIR spectra of SLN F6

Optimization and evaluation of SLN gel

The topical gel containing SLN loaded with luliconazole was prepared successfully by the stirring method using carbopol 934 as a gelling agent. The method of preparation of different SLN gel was found unpretentious and robust. Initially, all the four different preparations of SLN gel coded as G1, G2, G3, and G4, were evaluated to quantitate the percent entrapment of luliconazole spectrophotometrically at 299 nm. The resulting data shows that SLN G3 with 1.5% carbopol w/w showing the highest percentage of drug entrapment with 91.39% ± 0.187. Thereafter the optimized formulation is further evaluated to access physiochemical parameters includes visual appearance, pH, viscosity and spreadability. The resulting data reveals that the viscosity of G3 gel as found to be 369cP, similar

to the gel viscosity as reported by Jana et al and the pH was found to be 6.12±0.25530. Further in spreadability evaluation, the spreadability factor of the prepared SLN gel was found to be 4.5 and it states that the prepared gel produces excellent spreadability as an ideal topical formulation. Spreadability is one of the important physical properties of any topical formulation from a patient's compliance point of view[17].

Fig.8: Visual appearance of prepared SLN G3 gel

In vitro drug release and kinetics study Statistical models are commonly used to forecast the release mechanism and compare the release profile. The in-vitro release profile of the drug was performed in a prepared buffer system using the dialysis bag technique for 24 hrs. The desolvation percentages of luliconazole from SLN are increased in the proportion of time as illustrated in Figure 9 and Table 5. The pieces of evidence of release profiles show that the developed SLN is proficient to release the drug in a regulated manner. The slow release of the leading moiety from the most SLN form is based on homogeneous drug entrapment throughout the systems31. Ekambaram et al. state the same concept and claimed that a controlled drug desolvation profile can be attained when the drug is uniformly distributed in the lipid matrix. Poloxamer 407 having immense efficacy against the drug release rate from SLN then Cremophor RH40 because of its higher HLB value than cremophor RH 4032. Besides, the Poloxamer 407 has high exterior spreadability so that it eases the effects of interfacial tension between the SLN and dissolution medium. It also reduces the accumulation of drug particles and increases the drug dissolution rate. Moreover, lipid mass in the SLN can control the size of nanoparticle and increase drug desolvation strength. The thickness of the lipid surrounded nanoparticle increases the length of drug disassociation resulting prolonged effect of drug release[33].

Table 3: Percentage drug release profile of G3 and control gel

Sr. no.	Time in hours	Percentage drug release of G3	Percentage drug release of control gel
1	0	0	0
2	0.25	7.375±0.153	1.923± 0.011
3	0.5	14.002±0.185	2.052± 0.155
4	1	22.064±0.102	3.042± 0.158
5	2	32.289±0.173	3.182± 0.162
6	3	40.622±0.165	5.094± 0.122
7	4	47.048±0.151	7.815± 0.205
8	6	55.582±0.163	8.706± 0.215
9	8	62.309±0.134	9.387± 0.118
10	12	69.939±0.115	9.035± 0.205
11	24	79.578±0.213	9.773± 0.158

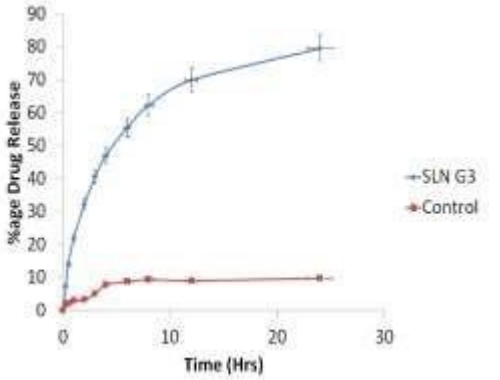

Fig.9: In-vitro drug release profile of SLN gel and control gel

Furthermore, an in-vitro drug release profile for the optimized formulation was applied to various kinetic models (zero-order, first-order, Higuchi, and Krosmayer Peppas model). To state kinetics profiling of drug release, the obtained data were analyzed statistically in respect of the rate constant and highest correlation. The best-fitted line was found in all the models except a little suitability in the zero-order equation. The resulting data describe the dissemination of the drug in a controlled or regular manner from homogenous matrix systems and it states why the drug disseminates at a slower rate. The observations concluded that SLN G3 is far efficient as a potential topical formulation for sustained drug delivery. This finding is almost similar to a virtuous covenant as per the previous shreds of evidence. The graphical representation of the kinetics order of SLN G3 gel shown in Figure 10

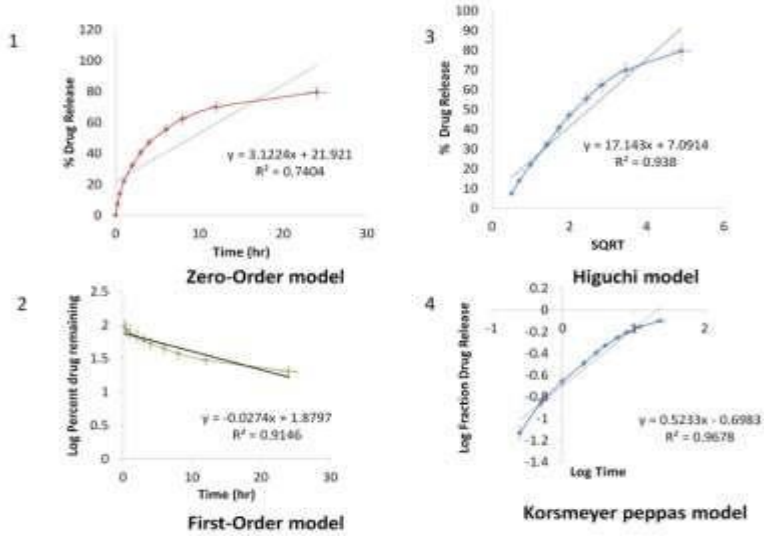

Fig.10: Kinetics order of SLN G3 gel

FTIR spectral analysis of SLN G3 gel

The FTIR spectral analysis of SLN gel G3 was performed successfully to determine the possible interaction between the drug and drug additives and the obtained spectral data was matched with the spectral data of luliconazole and stearic acid. The findings of the spectral analysis show the principal absorption peaks at 3331.36 cm-1 for N–H stretching, 2961.88 cm-1 for C-H stretching, 2193.49 cm-1 for C≡N stretching, 609.26 & 1044.56 cm-1 for C-Cl stretching for luliconazole. Whereas, the principal absorption peaks of stearic acid were ascribed to 2932.49 cm-1 & 2863.16 cm-1 in the high-frequency region attributed to -CH2- band asymmetric and symmetric stretching vibrations, and 1639.31 cm-1 for -COOH stretching is attributed in the low- frequency region. The spectral analysis of the optimized formulation G3 reveals that no more possible interaction between the drug and drug additives even after the successive formation of topical gel. Hence it can be said that the spectra show the purity and authenticity of the SLN G3 gel.

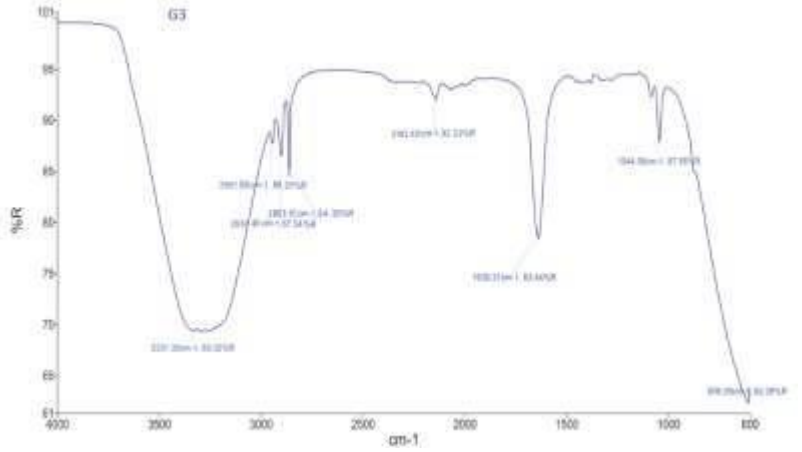

Fig.11: FTIR spectra of SLN gel G3

Scanning electron microscopy

The shape of the enhanced formulation was confirmed through the SEM study and is shown in Figure 12. Most of the vesicles are well specified, spherical, and discreet having large internal aqueous space. The low density of nanoparticles is shown in the SEM analysis which may lead due to the factor of dilution of nanosuspension before preparing the SEM photographs. SEM studies reveal that luliconazole loaded SLN in gel had a spherical shape with a smooth surface.

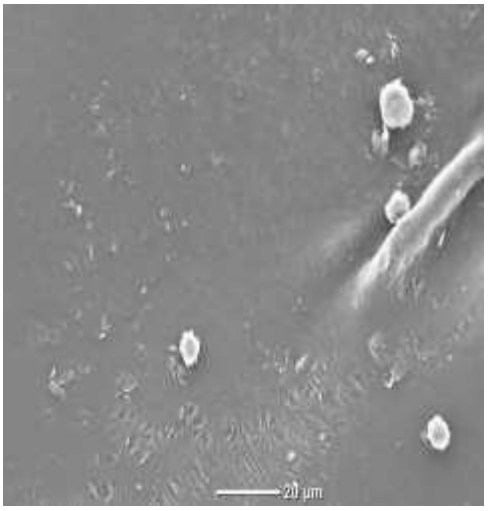

Fig.12: SEM analysis of SLN G3 gel

Anti-fungal activity of SLN G3 gel against Candida albicans by TLC-Bioautography assay The anti-fungal activity of SLN G3 gel against C. albicans by the TLC-Bioautography assay was conducted successfully. The TLC-Bioautography results reveal that 6µL solution of SLN G3 gel having enough zone of inhibition against the growth of C. albicans. The resulting zone of inhibition on the TLC plate was scaled out using a stationary scale. The measurement of each zone was found 1.25, 1.24, 1.25 cm with an average inhibition of 1.2466±0.0056 respectively as mentioned in figure 13. Although, so many researches have been conducted to evaluate the anti-fungal activity of luliconazole and reveal it as a potent anti-fungal agent. In a study conducted by Hiroyasu Koga et al., claimed that 0.031-0.25 µg/ml luliconazole having immense potential against C. albicans 34. Anti-fungal activity of SLN G3 gel against C. albicans by TLC-Bioautography assay is shown in figure 13

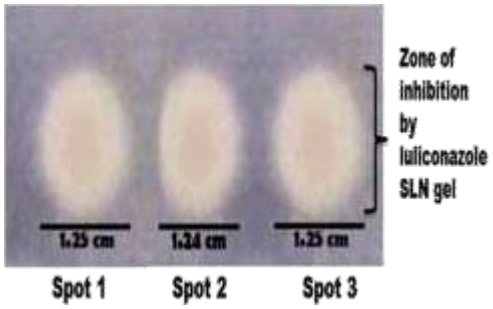

Fig.13: Zone of inhibition by luliconazole SLN gel against C. albicans.

CONCLUSION

The present study concludes that the luliconazole loaded SLN G3 gel formulation having carbopol 934 (1.5% w/v) is optimized as an ideal formulation and suitable for topical application. Further, the TLC-Bioautography assay reveals it to have strong anti-fungal activity.

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