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FORMULATION DEVELOPMENT AND EVALUATION OF LULICONAZOLE NANOCRYSTALS LOADED ALOEGEL FOR ENHANCEMENT OF SOLUBILITY AND ANTIFUNGAL ACTIVITY

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ABSTRACT

Superficial zymosis in disease patients will cause several disorders and complications. Currently, new topical treatment choices are critically required to treat these flora infections. Luliconazole (LZL) may be a topical antifungal medication used for zymosis treatment. The aim of this paper was to develop a brand new topical luliconazole nanocrystal (LNC) incorporated Aloegel. This study advised the potential use of LNC embedded in an exceedingly aloe gel as a drug delivery system for topical antifungal treatments. Luliconazole is a promising therapeutic candidate for the topical treatment of fungal infections. However, it has limited water solubility and skin permeability. To get around these problems, a luliconazole-loaded aloegel was made by mixing Vit E, tpgs, and methanol. When compared to other antifungal creams, LNC Aloegel demonstrated superior spreadability, viscocity, and ease of application, as well as a better in vitro drug release profile. Gel formulation stability was also discovered to be stable at both low and high temperatures up to 400 degrees Celsius. A topical medication delivery formulation incorporating LNC including aloe vera proved to be a potential nano carrier for the delivery of pharmaceuticals targeting infectious skin disorders. The formulations have been found to be efficacious, as evidenced by enhanced medication penetration through the skin.

Keywords *– Antifungal,Luliconazole(LCZ), Nanocrystals, Aloe vera gel, Modified nanoprecipitation method.*

1. INTRODUCTION

Fungal infections are the most prominent cause of skin illness. The global prevalence of parasitic illnesses is increasing. Oral therapy for skin infections has been linked to deadly consequences, extended treatment times, and narrow-mindedness, but topical treatment for superficial parasitic illnesses has been linked to poor drug dissolvability[1-4]. skin disturbance, and decreased skin porosity. Based on the features of the living things and the host, transmissible illnesses are classified into three types: superficial, subcutaneous, and fundamental. Dermatophytosis is a shallow dermatophyte-induced illness that affects the stratum corneum, hair, and nails. Dermatophytosis affects about 1% of the population. Contagious infections are becoming more common in patients using antimicrobials, corticosteroids, immunosuppressive medicines, and contraception. The frequency of superficial parasite contamination rises with age, the atmosphere, and disease changes. Luliconazole (LCZ) is an antifungal imidazole that is used to treat fungal skin infections [1]. It is accessible in the form of a bioavailable topical cream.Luliconazole reduces ergosterol binding via a 14-alpha demethylase P-450 correlation[6]. The lack of the main structural components of

ergosterol permits the substance of the cells to be pierced and released. In recent years, nano-carrier-based topical formulations such as nanoparticles, nanoemulsions, lipid nanoparticles, nanocrystals, and so on have grown in popularity as possible drug carriers for topical administration due to their distinct benefits and adaptability over conventional formulations. Nanocrystals are now gaining popularity and have been proven to be superior to other nano-particulate systems due to their high drug payload capacity, usage of minute amounts of excipients, greater chemical stability, less toxicity, and ease of scale up and manufacture. Because of enhanced solubility and extended retention at the site of infection, drug formulation as nanocrystals improves bioavailability and skin penetration[7,8]. Dermal administration with nanocrystals had a synergistic effect on depot development in hair follicles and improved penetration[8]. The nanocrystals' greater penetration will be owing to improved saturation solubility. In the first stage, this led to a higher concentration gradient between the skin and the formulation. In the second stage, the concentration gradient between the follicle region and nearby cells stayed the same. Aloe vera may be able to treat skin problems. Aloe Barbados has a common name and belongs to the Xanthorrhoeaceae family. Aloe Vera promotes wound healing by increasing blood flow throughout the area and preventing cell death in the lesion[10]. According to the World Health Organization Portal of Health Products and Critical Medicine, aloe vera gel has some effective medicinal properties and an efficient cure, which means that it activates the activity of macrophages and fibroblasts with an increase in collagen synthesis and proteoglycan. Aloe wound healing activity is based on the above mechanism[11-33].

As a result, the study's goal was to develop and characterise LNC-loaded Aloegel as a viable carrier for topical distribution. First, LNCs were synthesised using a modified nanoprecipitation process, and then they were incorporated into the manufactured aloegel. The prepared formulation was tested for particle size distributions, in vitro drug release by snake shed, and antifungal activity. The study's findings revealed that LNC-loaded Aloegel had higher drug retention in the skin than commercial cream and a larger zone of inhibition.

2. MATERIAL AND METHODS

2.1. Materials

Luliconazole was obtained from Arti Pharmaceuticals, Drug Bhandup, Mumbai; Vit E TPGS was a gift from Matrix Life Science, Aurngabad, Maharashtra; and HPMC K100 and carbopol 934P were obtained from Balaji Drugs, Nashik, and Maharashtra.

2.2. Preformulation studies

In the preformulation characterization, the physicochemical parameters of the drug substance are characterised with the goal of designing a drug delivery system.

2.3. Organoleptic properties

The pure drug substance was studied for its organoleptic properties such as color, odor, and appearance.

2.4. Determination of aqueous solubility

The determination of the aqueous solubility of luliconazole was estimated through the shake-flask method. After 1 gm of luliconazole was dissolved in distilled water and acetate buffer PH 5.5, followed by centrifugation at 50 rpm and 37 C for 2 hours, the resulting solution was filtered and analysed spectrophotometrically at 299nm.

2.5. Partition Coefficient

The shake flask method was used to determine the partition coefficient of luliconazole. To a 10 ml solution of n-octanol and water (1:1), 100 mg of luliconazole were added. After that, the water and luliconazole mixture were shaken for 7 hours, separated in a separating funnel for 24 hours. The organic and aqueous phases were separated and a UV spectrophotometer was used to determine the concentration of luliconazole.

Absorbance were taken at 293 nm in both phases.

$$\text{Partition coefficient} = \frac{\textit{Concentration of drug in the organic phase}}{\textit{Concentration of drug in tne aqueous phase}}$$

2.6. Determination of λmax by UV Spectrophotometer

The wavelength at which the medicine absorbs the most is known as the maximal wavelength. λmax is a feature of the drug. Cannot be readily modified. The substance's maximum can be found out by scanning the material in the 200-400 nanometer range.

2.7. Methanol Calibration Curve Construction

By dissolving precisely weighed 10mg of Luliconazole in 10ml of Methanol, a stock solution of a pure medication, Luliconazole, with a concentration of 1000g/ml was generated. 1 mL of this solution was taken and diluted up to 10 mL with the appropriate solvent (100 g/ml). Appropriate quantities of the aforementioned solution (0.2, 0.4, 0.8, and 1ml) were diluted further to achieve a final concentration of 2 to 10g/ml. The spectra of this solution was measured in the region of 200–400 nm using a UV–Visible Spectrophotometer against a blank (Methanol).

3. Preparation of Nanocrystals

The improved nanoprecipitation process was used to make luliconazole nanocrystals. Initially, the medication was dissolved in 10 mL of methanol to generate an organic solution, which was then added to 50 mL of Vit E tpgs aqueous solution using a 22-gauge syringe with stirring at 2000rpm with a mechanical stirrer. The aquatic solution was kept at 2 °C with an ice bath for fast precipitation, avoiding crystal development and obtaining a uniform size distribution. After a rapid addition of organic phase to the aqueous phase kept at 2 °C, antisolvent precipitated quickly.For a further half hour, the formed dispersion was agitated at 2000rpm and probe sonicated for 30 min, and was obtained using a fast cooling centrifuge for 10 minutes at 4°C and 10000rpm. LNC were re-dispersed in 1% HPMC for a duration of time while swirling to produce modified nanocrystals, which were collected by centrifugation at 10000 rpm at 4 °C, filtered by Whatmann filter paper 0.2 m, and allowed to dry.

As a result, the study's goal was to develop and characterise LNC-loaded Aloe gel as a viable carrier for topical distribution. First, LNCs were synthesised using a modified nanoprecipitation process, and then they were incorporated into the manufactured aloegel. The prepared formulation was tested for particle size distributions, in vitro drug release by snake shed, and antifungal activity. The study's findings revealed that LNC-loaded Aloegel had higher drug retention in the skin than commercial cream and a larger zone of inhibition.

Table 1: Formulation of Luliconazole Nanosuspension

Sr. No.	Formulation code	Drug (mg)	Conc^{n} of stabilizer (Vit E Tpgs)	Methanol (ml)	Water (ml)	Stirring speed (R.P.M.)
1	**NS17**	10	0.25	10	50	2000
2	**NS18**	10	0.1	10	50	2000
3	**NS19**	10	0.5	10	50	2000
4	**NS20**	10	1	10	50	2000

3.1 Evaluation of Nanocrystals

3.1.1 Particle size and size distributions

The particle size of nanosuspension in distilled water was determined using dynamic light scattering using Zetasizer Model ZEN3690 (Malvern Instruments, UK). The average particle size diameter (Ave) and the polydispersity index (PDI) were determined as parameters. These indices indicate the mean particle diameter and the width of the size distribution.

3.1.2 Determination of Drug Content

The UV-visible Spectrophotometer was utilised in order to ascertain the drug content of the produced LNC after it had been allowed to dry at room temperature. After dissolving 5 mg of nanocrystals in 10 ml of methanol inside of a 10 ml volumetric flask, the mixture was filtered using Whatmann filter paper with a 0.2 m poresize. At a maximum wavelength of 299 nm, spectrophotometry was used to measure the concentration of the filtrate.

3.1.3 Zeta Potential

The particle size of a nanosuspension in distilled water was determined using dynamic light scattering using Zetasizer Model ZEN3690 (Malvern Instruments, UK).Zeta potential were measured parameter

3.1.4 Entrapment Efficiency

Determination of entrapment efficiency is appropriate for estimating the free concentration of drug in the supernatant following centrifugation. For the assessment of entrapment efficiency, 10ml of freshly created nanosuspension was centrifuged for 10 minutes at 1000 rpm. The supernatant was collected, and a UV-Visible spectrophotometer was used to measure the absorbance of the supernatant solution at 275 nm.

3.1.5 Saturation Solubility

LNC in large amounts was dispersed in distilled water and acetate buffer with a pH of 5.5, then rotated at 50 rpm and 37℃ for 3 hours in order to achieve equilibrium. The mixture was then filtered through Whatmann filter paper with a pore size of 0.2 m and measured with an UV spectrophotometer at 299 nm.

3.1.6 Scanning Electron Microscopy

The morphology of nanocrystals was examined by SEM (Carl Zeiss SMT Ltd, Zeiss EVO 18), Sophisticated Analytical Instrument Facility (SAIF) IIT Bombay.

3.1.7 Differential Scanning Calorimetry

The DSC was conducted using an instrument (Mettler DSC 1 star system, Mettler Toledo Switzerland) RCPIPER, SHIRPUR. In DSC analysis, 10 mg of sample was put in an aluminium pan and scanned at 10 C/min over the temperature range of 0–800 °C in an inert environment maintained with nitrogen.

3.1.8 Powder X-Ray Diffraction

PXRD analysis was carried out on the nanocrystal utilising a D8 Advanced Diffractometer (Bruker AXS D8 Advance, Serial Nr-D8-03/202035, D76181 Karlsruhe, Germany) at RCPIPER, Shirpur at a scanning speed of 2° C/min across a 2° range of 5–40° C.

3.1.9 FTIR Study

FTIR analysis for Luliconazole, Vit. E TPGS, Physical Mixture (LZL: Vit. E TPGSin 1:1) and LNC was performed by Perkin Elmer two-spectrum USA SHIMADZU FTIR 8400S) RCPIPER, Shirpur. Each sample was mixed with potassium bromide in a 1:100 ratio and then compressed into pellets that were observed from 4000 to 400 cm^{-1}.

4. Preparation of Aloe Gel

Depending upon the trial batch, the final batches of Aloe gel are formulated. From the trial batches, 1.5 % concentration were selected and placed in a sufficient quantity of water at constant stirring at 500-600 rpm, followed by the addition of methylparaben sodium and propyl paraben sodium, stirred for 30 Min. added to pure extracted aloe vera gel and stirred continuously for 30 minutes at 2000rpm. Added drug nanocrystals and stirred at 2000. Triethanolamine was added slowly for pH adjustment and to get a stiff gel. Volume was made up with water and stirred continuously till a uniform gel was formed.

4.1Evaluation of Aloe gel

4.1.1Determination of pH

The use of a digital pH metre allowed for the accurate measurement of the pH of the gels. In order to determine the pH of the aloe gel, the glass electrode of the pH metre was dipped into the prepared gel and then rotated.

4.1.2 Determination of viscosity

A Brookfield Viscometer was used to determine the gel's viscosity. At 50 and 100 rpm, the viscosity of various samples of gel was measured and compared to determine the desired viscosity range.

4.1.3 Determination of Uniformity

A gel content uniformity study was conducted to assess the system's homogeneity. To determine system homogeneity, the lowest layers of the gel (1 g) were removed and dissolved in methanol. To remove drugs from the matrix, the gel was vortexed (5 minutes) and centrifuged (10 minutes), and the drug content was measured using a UV Visible Spectrophotometer.

4.1.4 Entrapment Efficiency

1g of gel was diffused with ethanol and vortexed for 10 minutes to achieve effective drug extraction in ethanol, then centrifuged for 40 minutes, and the supernatant was decanted and spectrophotometrically assessed at 299nm.

% EE= W (Initial drug) –W (free drug)/×100

4.1.5 Spreadability

The spreadability of a sample was evaluated using glass plates. After applying 0.5 g of gel in a 1 cm diameter circle on glass plates, a second glass plate was placed on top. A 500g weight was allowed to rest on the upper glass plate for 5 minutes. The diameter of the gel grew as a result of the gel spreading.

4.1.6 Antifungal study

Using Candida albicans (MTCC No 183), an in vitro inhibition zone assay was conducted. After solidification, 100 L wells were punched into the agar plate by (KHEDKAR BAC TEST LABORATORY, NASHIK).Plates were sterilised at 1210C for 15 minutes before use to prevent the growth of other undesirable microbes except *Candida albicans,* followed by a uniform spread of 100 µL Candida albicans (4 \106 cfu/ml) across the entire agar surface using a sterilised spreader rod. After 1 h of rest, 100µL Aloe gel were filled, plate was incubated for 72 h at 300C. The inhibition zone assay samples were examined.

4.1.7 In-Vitro drug release profile of Aloe gel and marketed formulation cream

In vitro release tests were conducted to compare the improved formulation (nanocrystal loaded aloegel) to the commercial version of luliconazole cream (Luliford cream 1% w/w). Using the Franz-diffusion cell, the comparative version of the Nanocrystal aloe gel and Luliford commercial cream were tested,Snake shed was used for the study. The skin was placed in Franz diffusion vertical cells between the receptor and donor compartments, with the receptor compartment facing the stratum corneum. The receptor was filled with physiological saline solution (pH 7.4) and agitated continuously at 50 rpm and 37 0.5 °C. Each 1 gramme of drug-loaded aloe gel and commercial cream was applied to the skin area, and the receiving solution was drained at specified

intervals and replaced with fresh solution after each sampling. Aliquots were subsequently filtered and examined at 299 nm for drug entrapment.

5. RESULTS AND DISCUSSION

5.1 Preformulations studies

Preformulation studies have been conducted to determine the physicochemical properties of the drug. The colour and appearance of the drug were white and crystalline in nature and odourless.

5.2 Aqueous solubility

LZL solubility has been obtained as 0.003533 ±0.00524mg/ml.

5.3 Partition coefficient

The partition coefficient (log10 P) of luliconazole was 11.166± 0.008, and it showed that the drug was lipophilic.

5.4 Determination of λ max by UV-Spectrophotometer

The middle dilution, i.e. 8, may be used to determine the maximum absorbance using a UV-visible spectrophotometer in the range of 200-400 nm, from the number of dilutions of stock solution. For the estimate of Luliconazole, the absorption maximum was employed as a max. The λ max of the substance was found to be 297nm.

5.5 Construction of a Calibration Curve in Methanol

The absorption maxima of luliconazole in methanol were found at 297nm, which was used as the analytical wavelength. The concentration was plotted against the absorbance.In the range of 2 to 10g/mL, Beer's rule was followed. The experimental data was subjected to regression analysis.The experimental curve's regression equation was y=0.094x+0.500. The correlation value was determined to be 0.983, indicating that the medication concentration an absorbance had a linear relationship. Figure 1 shows a calibration curve of the drug in methanol.

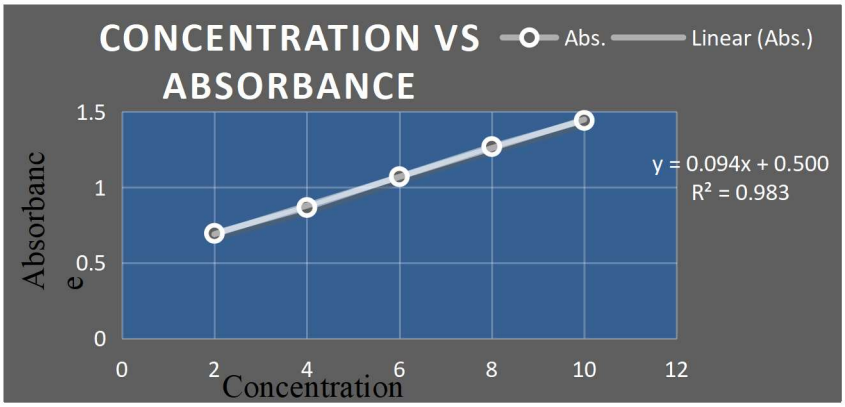

Fig. 1: Calibration plot of Luliconazole in methanol

5.6 Particle Size and Size Distributions

The nanocrystals were successfully synthesised, and the particle size and PDI value of Vit E TPGS Batch NS1 Nanocrystals showed good particle size and PDI as compared to other batches of 0.389m, which are ideal for rapid dissolution and hair depot development. It is possible for particles between 0.2 and 0.8 microns to penetrate deeply into hair follicles.

Table 2: Particle Size and size distribution Analysis

Sr. No.	Formulation Code	Particle size	PDI
1	NS17	0.389	0.259
2	NS18	0.487	0.372
3	NS19	0.638	0.499
4	NS20	0.899	0.697

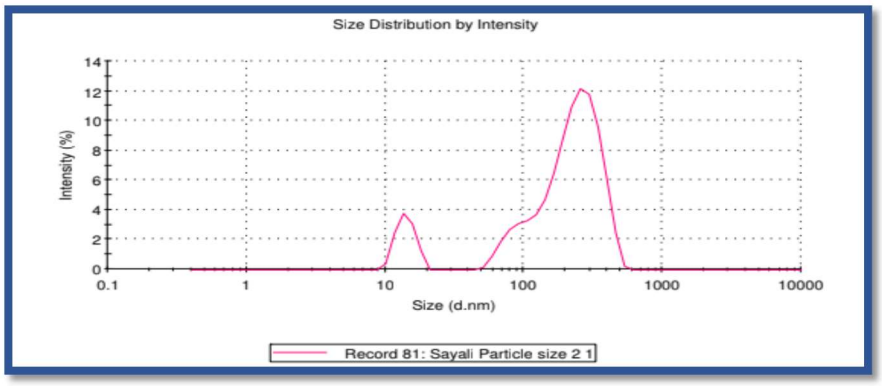

Fig. 2: Particle size and size distribution

5.7 Determination of Drug Content

The drug content of the nanocrystals was in the range of 86.40 to 93.39%, respectively. The percent drug content was shown in table 3. The total drug content of the entire nanocrystal batch was found to be greater than 90%. The batch SN19 shows the maximum total drug content of 93.308% and the batch NS17 shows a minimum drug content of 86.40. This can be shown in figure 3.

Table 3:% Drug content and formulation code

Sr no	Formulation Code	% Drug Content
1	NS17	86.40
2	NS18	98.39
3	NS19	93.308
4	NS20	91.80

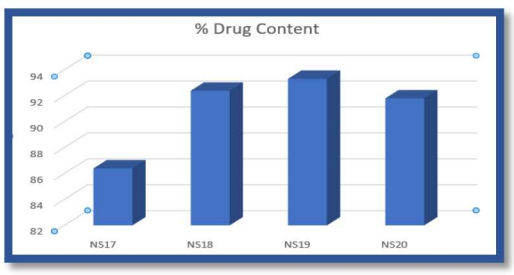

Figure 3:3:% Drug content and formulation code

5.8 Zeta potential

Zeta potential and standard deviation values were directly measured. Zeta potential was investigated to understand the stability of vesicles and colloidal properties Batch NS4 showed good good result (-25.5mV)

Table 4: Zeta potential Analysis

Sr. No.	Formulation Code	Zeta potential
1	NS17	-22.1
2	NS18	-19.5
3	NS19	-25.5
4	NS20	-23.9

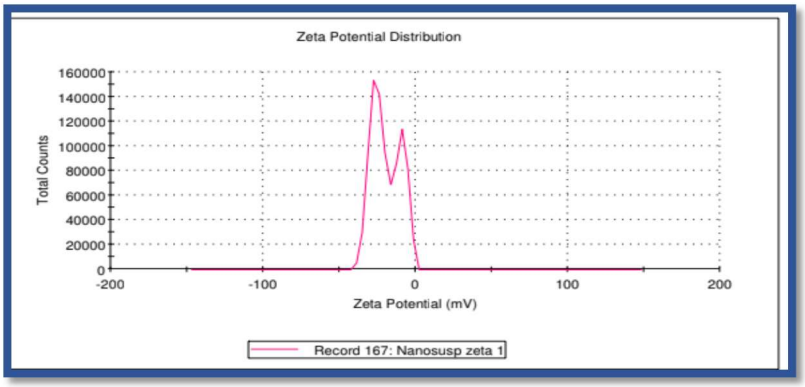

Figure 4: Zeta Analysis

5.9 Entrapment Efficiency

The entrapment efficiency of the nanocrystal batches is highlighted in fig.4. It is observed that the formulation batch NS17 shows a minimum entrapment efficiency of 82% and the formulation batch NS20 shows a maximum entrapment efficiency of 93.2%. The entrapment efficiency of the nanocrystals was found to be in the range of 82-93%, respectively.

Table 5: Entrapment Efficiency of the nanocrystals

Sr no	Formulation Code	% Entrapment Efficiency
1	NS17	82.80
2	NS18	90.29
3	NS19	93.20
4	NS20	92.31

Fig. 5:% Entrapment efficiency

5.10 Saturation Solubility

The batch SN19 had a saturation solubility of 0.0927 g/ml, which was greater than the bulk drug. In comparison to the pure medication, the nanocrystal's saturation solubility was enhanced.

Table 6: Saturation solubility

Sr no	Formulation Code	solubility
1	NS17	0.0503µg/m
2	NS18	0.0761µg/m
3	NS19	0.0927µg/m
4	NS20	0.0677µg/m

5.11 Scanning Electron Microscopy

Luliconazole nanocrystals (Batch NS18) morphology has been successfully identified, LNC was a narrow size plate shaped and found be regular.

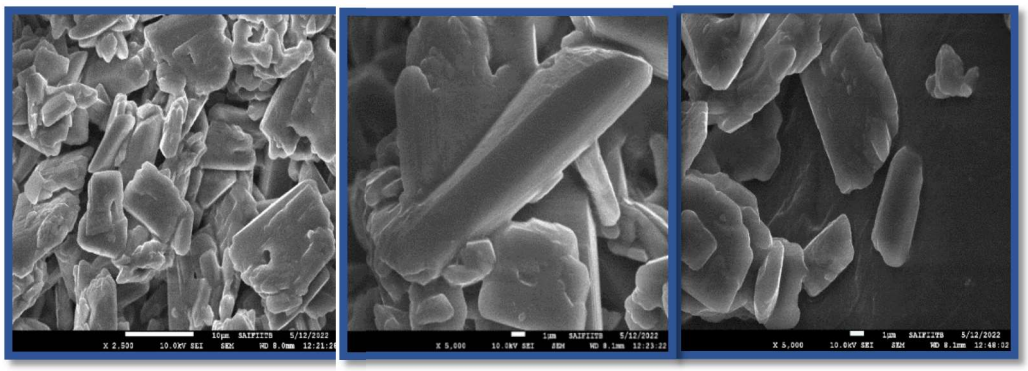

Fig. 6: SEM images of batch NS 18 Nanocrystals

5.12 Differential Scanning Calorimetry

The Standard DSC curve of Luliconazole shows sharp endothermic peak at 149^{0}C and degradation curve at 589^{0}C. Luliconazole nanocrystal (Batch SN 20) showed an endothermic peak at 590^{0}C with endothermic peak 151^{0}C DSC curve for nanocrystal reveals the crystalline state to be preserved.

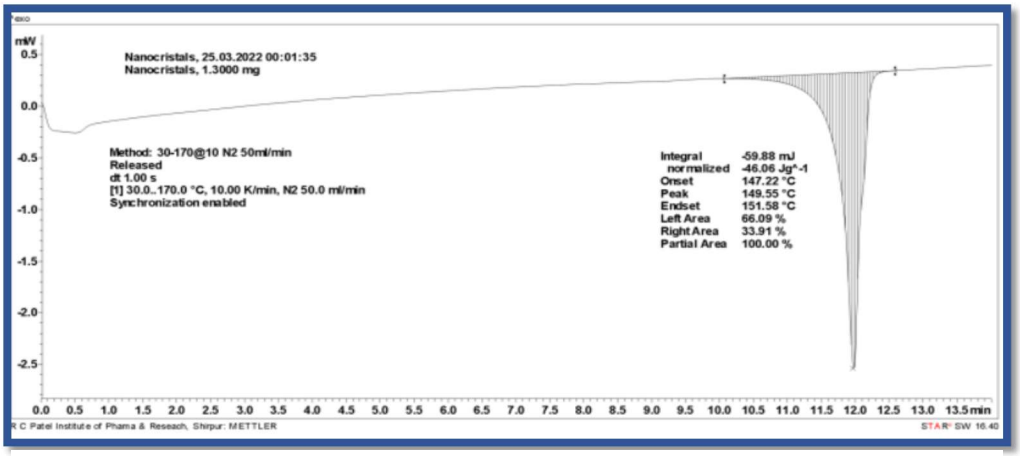

Fig. 7: DSC curve of SN20 Nanocrystal batch

5.13 powder X-Ray diffraction (PXRD)

X ray powder Diffraction studies were performed of batch SN18 to determine the stabilizers influence on the existing LZL state and analyse potential changes in crystalline state after drug formulations nanocrystals.

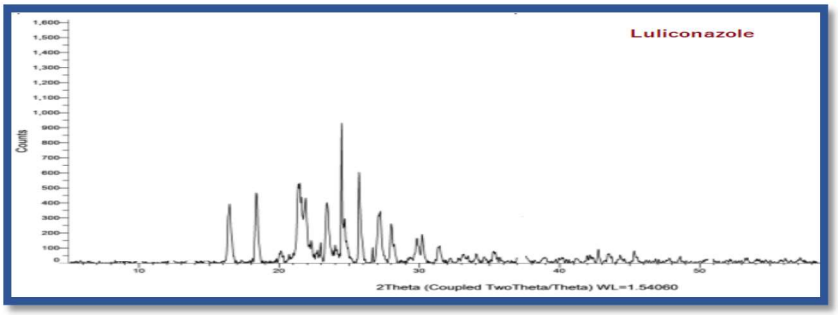

Fig. 8: PXRD of LZL

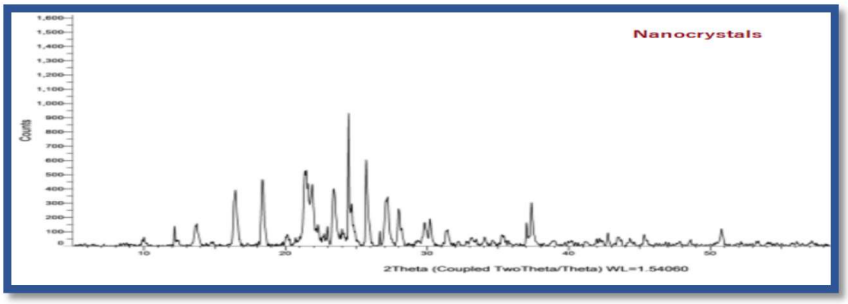

Fig. 9: PXRD of Nanocrystal

Table 7: peaks of Luliconazole and Nanocrystal at 2θ

Sr no	Luliconazole peaks at 2θ	Nanocrystals peaks at 2θ
1	15.6850	9.997
2	17.5486	12.172
3	20.667	13.736
4	21.1064	16.471
5	22.6711	18.374
6	23.8431	21.401
7	25.0047	23.420
8	26.2416	24.451
9	27.2611	25.700
10	29.1011	27.235
11	42.6418	27996
12	-	29.383
13	-	30.197
14	-	37.389
15	-	42.784
16	-	50.751

The patterns observed for Luliconazole and Nanocrystals were found to be quite similar. The peak values for luliconazole also show the crystalline nature of the drug. Patterns observed for Luliconazole and Nanocrystals were found to be quite similar due to non-homogenous distributions of the drug to the stabilizers' interior and surface.

5.14 FTIR STUDY

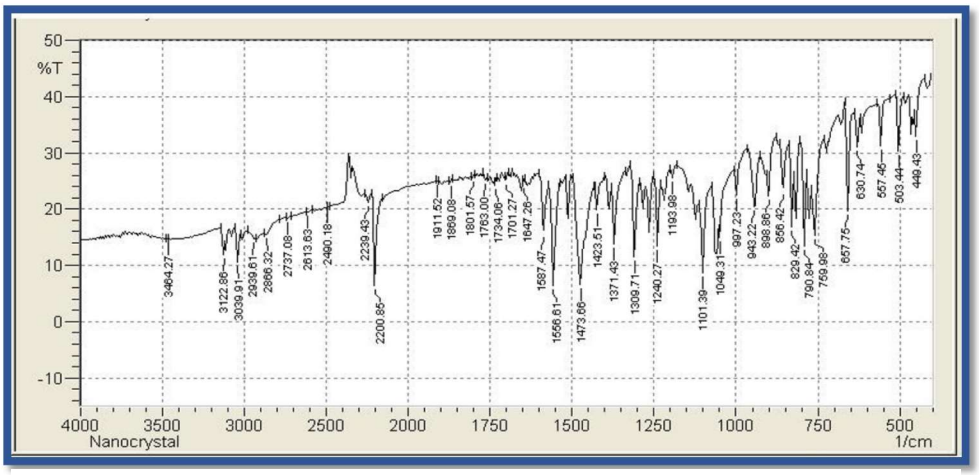

Figure 10: FTIR Spectra of batch SN18 Nanocrystal

Table 8: FTIR Spectra of batch SN18 Nanocrystal

Sr no	Functional group	Wave Number(cm^{-1})	Observed
1	C-H(Aromatic)	**2850-3300**	**3039,3124**
2	C-H(Aliphatic)	**2500-3300**	**2925**
3	C≡N	**2190-2260**	**2205,2240**
4	C-Cl	**850-550**	**759**
5	O-H	**3230-3550**	**3440**

5.16 Short term stability study of Luliconazole Nanosuspension

For stability studies, prepared nanosuspensions were stored at room temperature, 20C, and 400C. Total drug content and entrapment efficiency were analysed at 0, 1, and 7 days of storage. The accelerated stability study revealed that there were no significant changes in the attributes of the nanosuspension; hence, we conclude that the formulation was stable during all days.

Table 9: Short term stability study

Sr. No.	Temperature	0^0C			40^0C		
1	**Sr No**	**0Days**	**1Days**	**7Days**	**0Days**	**1Days**	**7Days**
2	**1)Drug Content**	**90.25**	**90.05**	**89.90**	**90.25**	**89.64**	**88.67**

3	**2) Entrapment Efficiency**	**85.64**	**85.44**	**84.90**	**85.64**	**84.31**	**83.71**

5.17Evaluation of Aloe vera Gel

Based on the viscocity and spreadability of the trial batches, it was determined that batches G2 and G3 with a concentration of 1 and 1.5 percent w/v had good viscocity and spreadability. The final formulation used nanocrystal-loaded aloegel with a concentration of between 1% and 1.5% w/v.

5.18 Determination of pH

Table 10: pH of final formulations

Sr no	Batch No	Carbopol 934p(gm) %(w/V)	pH
1	G5	1	5
2	G6	1.5	5.5

5.19 Determination of viscosity

Table 11: Viscosity of final formulations

Sr no	batch No	Carbopol 934p(gm) %(w/V)	Viscocity(cP)	
1			50R.P.M	100R.P.M
2	G5	1	2096	1347
3	G6	1.5	4169	2694

Batch G6(1.5%W/V) shows good viscosity than Batch G5

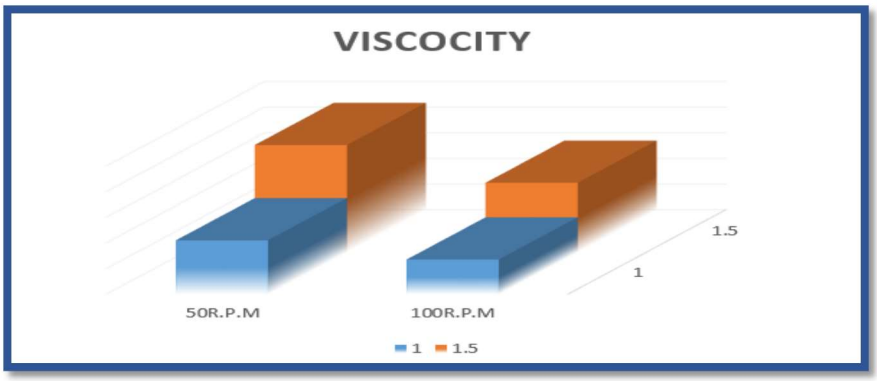

Fig. 11: viscosity of G5 and G6

5.19 Drug Content Uniformity

Drug content uniformity for gel was done by taken out 1g gel from each batch and dissolved in sufficient quantity of methanol, followed by centrifuged for 10 min, and supernatant can be assessed by using UV at absorbance maxima.

Table 12: % Drug content of G5 and G6

Sr no	Batch No	Drug Content
1	G5	89.37%
2	G6	92.71%

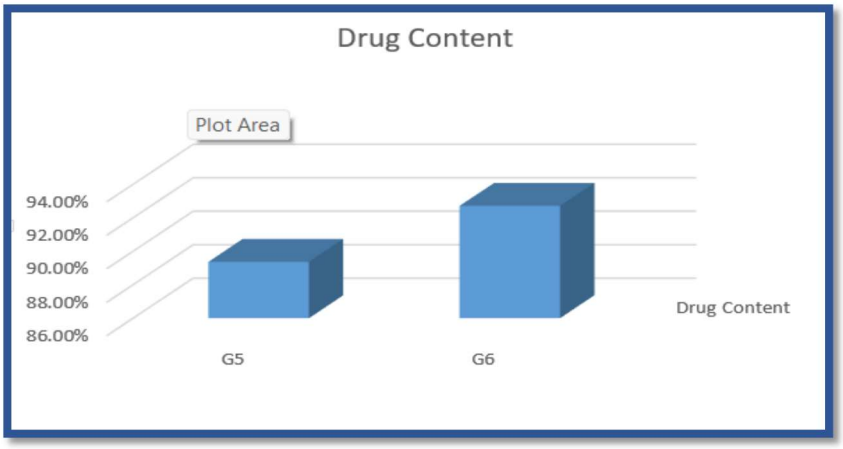

Fig. 12:%Drug content of G5 and G6

The batch G6 shows the maximum total drug content of 92.71% and batch G5 shows minimum drug content of 89.37%

5.20 Entrapment Efficiency

1g of gel was diffused with ethanol and vortexed for 10 minutes to achieve effective drug extraction in ethanol, then centrifuged for 40 minutes, and the supernatant was decanted and spectrophotometrically assessed at 299nm.

$$\% EE = W_{(Initial\ drug)} - W_{(free\ drug)} / \times 100$$

Table 13: Entrapment Efficiency of G5 and G6

Sr no	Batch No	Entrapment Efficiency
1	G5	90.46%
2	G6	92.17%

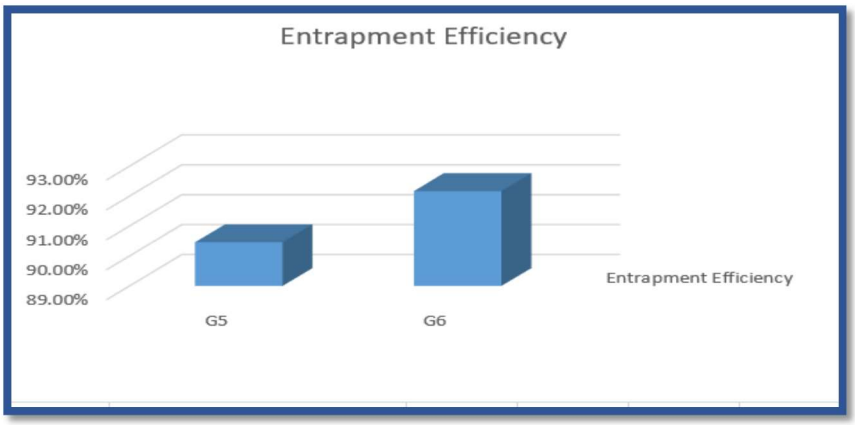

Fig. 13: Entrapment Efficiency of G5 and G6

Batch G6 shows better entrapment with 92.17% as compared to batch G5 which is 90.46%

5.21Spreadability

Table 14: Spreadability of G5 and G6

Sr no	Batch No	Spreadability
1	G5	4.12
2	G6	6.3

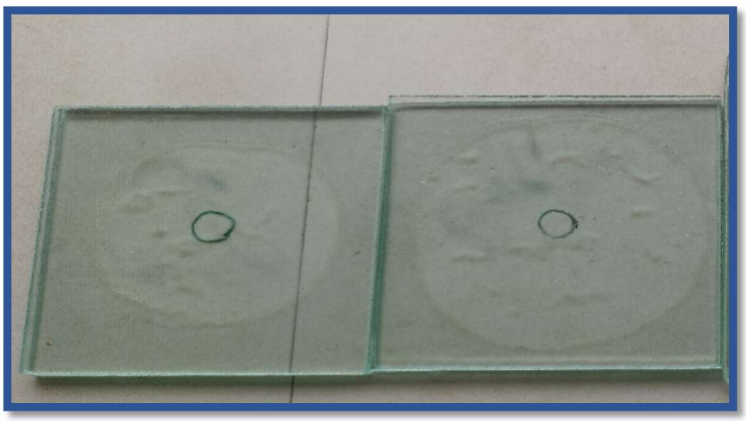

Fig. 14: Spreadability of G5 and G6

Batch G6 shows excellent spreadability as compared to G5.

5.22Antifungal Study

In vitro antifungal study was performed, LNC-loaded aloegel exhibited greater efficacy and diffusion against candida albicans, resulting in enhanced antifungal activity.

Fig. 15: Zone Of Inhibition of Batch G6

5.23 In-Vitro drug release profile of Nano gel and marketed formulation cream

In vitro release tests were conducted to compare the improved formulation (nanocrystal loaded aloegel) to the commercial version of luliconazole cream (Luliford cream 1% w/w). The in-vitro drug release profile for the optimised batch and commercialized cream revealed that the cumulative skin permeated drug from LNC Aloe gel to cream was found to be

significantly different within 3 hours. Therefore, LNC Aloe gel showed more than % drug release compared to cream. Consequently, LNC Aloe gel retained a greater quantity of the drug in the skin.

Figure 16: In-Vitro drug release study

Figure 17: Snake shed

Table 15:13 % drug release of G6 vs Marketed cream

Sr No	Time in hours	% drug entrapment of G6	% drug entrapment of marketed cream
1	0	0	0
2	0.25	7.214	2.674
3	0.5	13.78	3.088
4	1	23.47	4.301
5	2	32.37	5.947
6	3	41.74	6.843

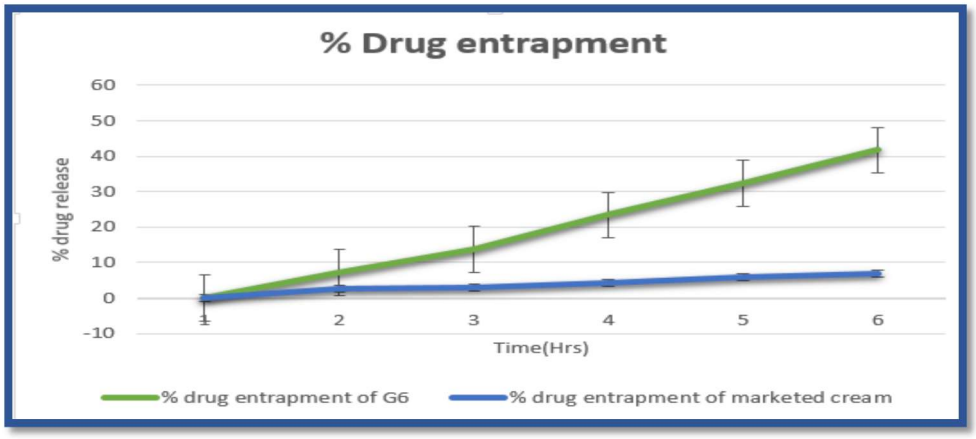

Figure 17:% drug entrapment of G6 and marketrd cream

6. CONCLUSION

By reducing the particle size of the medication, the modified nanoprecipitation approach can be an efficient strategy to generate Nanocrystals of Luliconazole to improve solubility and bioavailability. Drug Nanocrystals can be used to improve the solubility and bioavailability of poorly soluble medicines. To influence drug release profile using Vitamin E TPGS as stabilizing agent, polymers HPMC K100, carbopol 934P, and solvent methanol in this strategy, the first step is to reduce particle size using a modified

nanoprecipitation method, and the solubility and bioavailability profiles of the obtained nanocrystals are compared to pure drug.According to an in-vitro release study, the Nanocrystal formulation can boost bioavailability of the Luliconazole by improving its saturation solubility and increased penetration. to DSC studies confirmed that the crystalline state to be preserved. PXRD experiments revealed that Luliconazole and the physical combination of medication and stabiliser are both crystalline in form. SEM analysis validated the Nanocrystal formulation's morphology.

Because synthetic gels have so many detrimental effects when compared to natural gels, Aloe vera is a significant approach for natural gel. Aloe vera is a medicinal plant with the potential to do action against numerous fungus, and aloe vera gel extract has a higher absorbtion rate than other marketed gels. Furthermore, aloe gel promotes wound healing and cell development, and the plant's whole extract has antifungal and antibacterial effects. The aloe vera extract has been shown to be exceptionally resistant to a wide range of fungal and bacterial infections. The incorporation of nanocrystals into aloe gel could be a novel approach, as demonstrated by using trial batches to determine the concentration of gelling agent, and then evaluating the trial batches for viscocity, pH, and spreadability. Finally, a final batch of aloe gel was chosen for nanocrystal incorporation, and the final batches were evaluated for Ph, viscocity, spreadability, drug content, and drug entrapment. Further antifungal research demonstrated that LNC-loaded aloe gel had better dispersion and efficiency against *Candida albicans*, indicating that it had increased antifungal activity. also

In-Vitro release study was done by doing comparison between prepared Lnc Aloe gel and marketed cream formulation, that cumulative skin permeated drug from Lnc Aloe gel to cream was found to be significantly different within 3hrs,thus LNC Aloe gel showed more % drug release compared to cream,thus LNC Aloe gel retained a larger amount of drug into the skin layer, therefore it was concluded that LNC loaded aloe gel formulation has a great potential for topical delivery with better drug penetration and retention as as it is safe, natural treatment for various skin diseases,and also LNC Aloe gel could be a new approach which can be applied in future to improve the dermal delivery of drugs with poor aqueous solubility[28,39,30,31]

5. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1.Roy I, Ohulchanskyy TY, Pudavar HE, et al. Ceramic–based nano particles entrapping water–insoluble photosensitizing anticancer drugs: a novel drug–carrier system for photodynamic therapy. J Am Chem Soc. 2003;125(26):7860–7865.

2. Luliconazole, Accession No. DB08933, Available at https://www.drugbank.ca/drugs/DB08933 [Accessed 27 September 2017].

3.Luliconazole, National Center for Biotechnology Information. PubChem Compound Database; CID=3003141, https://pubchem.ncbi.nlm.nih.gov/compound/3003141 (accessed July 3, 2018). Available at https://pubchem.ncbi.nlm.nih.gov/compound/3003141 [Accessed 27 September 2017].

4.. Mundstock A., Lee G. Saturation solubility of nicotine, scopolamine and paracetamol in model stratum corneum lipid matrices. Int. J. Pharm. 2014;473(1–2):232–238.

5.Samuel I, Mihail C Roco, William Sims Bainbridge. Societal Implications of Nano–science and Nano–technology. Stupp Northwestern University Materials and Life Sciences Building 2225 N, Campus Drive Evanston, IL 60208. 2001. p. 1–280.

6.Sarfaraz S, Bano T, Fatima W. Nanotechnology and it's therapeutic application-a review. MOJ Bioequiv Availab. 2018;5(1):24-27.

7.Patra, J. K., Das, G., Fraceto, L. F., Campos, E., Rodriguez-Torres, M., Acosta-Torres, L. S., Diaz-Torres, L. A., Grillo, R., Swamy, M.

K., Sharma, S., Habtemariam, S., and Shin, H. S. (2018). Nano based drug delivery systems: recent developments and future prospects. Journal of nanobiotechnology, 16(1), 71. https://doi.org/10.1186/s12951-018-0392-8.

8.ManishKumar,NithyaShanthia,Arun,KumarMahatoa,ShashankSonia,P.S.Rajnikanth. Formulation and evaluation of Luliconazole Nanocrystal loaded Heliyon.com ELSEVIER May :1-10

9.Garg AK, Maddiboyina B, Alqarni MH, Alam A, Aldawsari HM, Rawat P, Singh S, Kesharwani P. Solubility enhancement, formulation development and antifungal activity of luliconazole niosomal gel-based system. Journal of Biomaterials Science, Polymer Edition. 2021 May 28;32(8):1009-23.

10.T. Reynolds, A.C. Dweck Aloe vera gel leaf: a review update J Ethnopharmacol, 68 (1999), pp. 3-3

11.J.H. Hamman Composition and applications of Aloe vera leaf gel Molecules, 13 (2008), pp. 1599-1616

12.https://medlineplus.gov/druginfo/meds/a682858.html

13.https://www.drugbank.ca/drugs/DB00695

14.Saturation solubility of nicotine, scopolamine and paracetamol in model stratum corneum lipid matrices Drug Discov. Today, 23 (3) (2018), pp. 534-547

15.M. Malamatari, K.M. Taylor, S. Malamataris, D. Douroumis, K. KachrimanisPharmaceutical nanocrystals: production by wet milling and applications Int. J. Pharm., 473 (1–2) (2014), pp. 232-238

16.T. Hatahet, M. Morille, A. Hommoss, C. Dorandeu, R.H. Muller, S. Begu Dermal quercetin smartCrystals: formulation development, antioxidant activity and cellular safety Eur. J. Pharm. Biopharm., 102 (2016), pp. 51-63

17.Dermal quercetin smartCrystals: formulation development, antioxidant activity and cellular safety Eur. J. Pharm. Biopharm., 102 (2016), pp. 51-63

18.Vivek P. Chavda, Chapter 4 - Nanobased Nano Drug Delivery: A Comprehensive Review, Editor(s): Shyam S. Mohapatra, Shivendu Ranjan, Nandita Dasgupta, Raghvendra Kumar Mishra, Sabu Thomas, In Micro and Nano Technologies, Applications of Targeted Nano Drugs and Delivery Systems, Elsevier, 2019, Pages 69-92, ISBN 9780128140291, https://doi.org/10.1016/B978-0-12-814029-1.00004-1.

19.Kumar, S., and Bhatnagar, T. (2014). Studies to Enhance the Shelf Life of Fruits Using Aloe Vera Based Herbal Coatings: A Review, 5, 211–218.

20.Nilesh S. Zarekar, Vishal J. Lingayat, and Vishal V. Pande, "Nanogel as a Novel Platform for Smart Drug Delivery System." Nanoscience and Nanotechnology Research, vol. 4, no. 1 (2017): 25-31.

21.Armstrong-James D, Meintjes G, Brown GD. Aneglected epidemic: Fungal infections in HIV/AIDS. Trends Microbiol. 2014; 22(3):120- 127. doi:10.1016/j.tim.2014.01.001

22.Kulkarni, Samir and Myerson, Allan. (2017). Methods for Nano-Crystals Preparation. 10.1007/978-94-024-1117-1_16.

23.Salma A. Fereig, Ghada M. El-Zaafarany, Mona G. Arafa and Mona M. A. Abdel-Mottalab(2020), Tackling the various classes of nano-therapeutics employed in topical therapy of psoriasis, Drug Delivery, 27:1, 662-680.

24. Kanwar AJ, De D. Superficial fungal infections. In:Valia G, ed. IADVL Textbook of Dermatology.Mumbai: Bhalani Publishing House India, 2008;252-293.

25.Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry. 2019 Nov 1;12(7):908-31.

26.Prashantkumar K. Parmar, Jhanvi Wadhawan, Arvind K. Bansal, Pharmaceutical nanocrystals: A promising approach for improved topical drug delivery, Drug Discovery Today, Volume 26, Issue 10, 2021, Pages 2329-2349, ISSN 1359-6446, https://doi.org/10.1016/j.drudis.2021.07.010.

27.Thakur Nishi, GargGarima, Sharma P.K. and Kumar Nitin. Nanoemulsions: A Review on Various Pharmaceutical ApplicationsGlobal Journal of Pharmacology. 2012;6 (3): 222-225

28.Baghel, S., Nair, V. S., Pirani, A., Sravani, A. B.,Bhemisetty, B., Ananthamurthy, K., Lewis, S. A.(2020). Luliconazole-loaded NanostructuredLipid Carriers for Topical Treatment of Superficial Tinea infections. Dermatologic ahiiTherapy. doi:10.1111/dth.13959

29.Savardekar, P., and Bajaj, A. (2016). Nanoemulsions-a review. Inter J Res Pharm and Chem, 6, 312-322.

30.Bahram, M., Mohseni, N., and Moghtader, M. (2016). An Introduction to Hydrogels and Some Recent Applications. In (Ed.), Emerging Concepts in Analysis and Applications of Hydrogels. IntechOpen. https://doi.org/10.5772/64301.

31.Yu YQ, Yang X, Wu XF, Fan YB. Enhancing permeation of drug molecules across the skin via delivery in nanocarriers: novel strategies for effective transdermal applications. Frontiers in bioengineering and biotechnology. 2021 Mar 29;9:646554.

32. Patel, K. D., Singh, R. K., and Kim, H. W. Carbon-based nanomaterials as an emerging platform for theranostics. Materials Horizons, 2019, 6(3), 434-469.

33.Zielińska A, Carreiró F, Oliveira AM, Neves A, Pires B, Venkatesh DN, Durazzo A, Lucarini M, Eder P, Silva AM, Santini A. Polymeric nanoparticles: production, characterization, toxicology and ecotoxicology. Molecules. 2020 Aug 15;25(16):3731.