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Formulation and Evaluation of Solid Lipid Nanoparticles containing Clotrimazole

Madhushri M*[1], R. S. Thakur[1], Kiran K Jadhav[2], Ronak N. Patel[1]

1. Department of Pharmaceutics, Krupanidhi College of Pharmacy, Chikkabellandur, Carmelram, Bangalore-570035, Karnataka, India

2. Sr. Executive, Plethico Pharmaceuticals Limited, Indore, MP, India

ABSTRACT

The purpose of this research was to develop a desired topical formulation containing clotrimazole for treatment of fungal infections like eczema, itching, pruritis etc. Topical formulation enriched with SLN of clotrimazole were prepared. The solid lipid nanoparticulate dispersion of clotrimazole was prepared by hot homogenization technique using polymers like Carbopol 934, mannitol and PEG 6000. The nanoparticulate dispersion was evaluated for various parameters such as physical evaluations, particle size, diffusion studies, DSC, SEM, stability studies. The solid lipid nanoparticulate dispersion showed mean particle size less than 1000 nm. Differential scanning Calorimetry studies revealed no drug excipient incompatibility. Diffusion studies release profile of clotrimazole from nanoparticulate dispersion showed prolonged drug release. And all other evaluations were found to be complied the limits. Thus it can be concluded that formulation of SLN containing clotrimazole can be successfully formulated to localize the drug in the skin for to treat topical fungal infections.

KEYWORDS: Solid lipid nanoparticles (SLN), Clotrimazole, Hot homogenization, fungal infections.

*Corresponding Author Email: madhushrimunireddy@gmail.com

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INTRODUCTION:

The main basic idea behind the research is to evaluate the potential use of solid lipid nanoparticles (SLN) in dermatology. Clotrimazole is a broad-spectrum antimycotic agent belongs to imidazole antifungal class commonly used in the treatment of fungal infections[1-3]. Effective topical treatment of a drug should be that which is sufficiently permeable into the skin to reach the desired location of infection. Solid lipid nanoparticles (SLN) can improve permeability of drug and further decrease irritation potential due to entrapment.

Most drugs are either absorbed into the body through the digestive system or injected into subcutaneous tissue or muscle. An alternative route, Topical drug administration, enables drug to pass across the epidemis and into blood vessels of the dermis. The drug is released continuously at a controlled rate over a period of one to several days. This method of administration is especially useful for drugs that are quickly eliminated from the body[4]. Absorption through the skin can be enhanced by suspending in an oily vehicle and rubbing the resulting preparation into the skin[5].

SLN'S are one among the nano carries, where the particle size is within the range of 10-1000nm. As the particle size is reduced to nanometer range, it can successfully penetrate through several anatomical barriers and had got good entrapment efficacy, penetrability and no skin irritation. Solid lipid nanoparticles appear promising as a drug carrier system, and therefore were investigated for topical application of clotrimazole. Stratum corneum is the main barrier in the percutaneous absorption of topically applied drugs. Small size and relatively narrow size distribution of SLN permit site-specific delivery to the skin. SLN have high affinity to the stratum corneum, and therefore an enhanced bioavailability of the encapsulated material to the skin is achieved. SLN enhance the penetration and transport active substances particularly lipophilic agents and thus intensify the concentration of these agents in the skin. Topically clotrimazole has anti-infective and anti-mycotic properties therefore incorporation of clotrimazole in SLN can successfully used in the treatment of topical fungal infections.

MATERIALS AND METHOD

Clotrimazole was gifted by Micro Labs Ltd, Hosur, PEG 6000 and Mannitol was obtained from S D Fine-Chem Ltd, Mumbai. Carbopol 934, Span 20, Tween 20, Stearic acid and Beeswax were obtained from Loba Chemie Pvt. Ltd, Mumbai.

Instruments: UV/VIS Spectrophotometer UV 2301 (Shimadzu Corporation, Japan), Polytron PT 1600E (Kinematica AG, Switzerland), Particle size analyzer (Malvern, U.K), Electronic

balance (Shimadzu Corporation, Japan), FTIR Spectrophtometer (Shimadzu 8400 series), Particle size analyzer (Malvern, U.K), Zeta potential instrument (Malvern, U.K), Scanning Electron Microscopy (Model JSM 840 A, Jeol, Japan).

Preparation of SLN:

Clotrimazole containing SLN was prepared by hot homogenization technique[6-8] at temperatures above the melting point of the lipid. Weighed quantity of stearic acid, beeswax and span 20 were taken and melted at 65 – 70 ^{o}C and then drug was dispersed in hot lipid phase. In other side aqueous mixture of polymers and surfactants were heated to 65 – 70 ^{o}C. When both the phases attained same temperature i.e 65 – 70 ^{o}C lipid phase was added to aqueous phase under magnetic stirrer. Once pre emulsion is formed, it was kept under high-shear mixing device (like Polytron PT 1600E homogenizer) above the lipid melting point at 30000 rpm for 10 mins. Up on cooling down to room temperature leads to solidification of nanoemulsion.

Initially F1-F6 formulations were formulated by keeping different polymer ratios. Among all these 6 formulations F4 and F6 were found not stable. High drug content and release studies of F1 was selected for further standardization by formulating it into 4 batches and coded as A, B, C and D. All these observations are listed in Table 1.

Table 1: Formulation Design

Ingredients	F1 (grams)	F2 (grams)	F3 (grams)	F4 (grams)	F5 (grams)	F6 (grams)
Clotrimazole	1	1	1	1	1	1
PEG-6000	2	2	4	2	4	4
Mannitol	2	2	2	4	4	4
Carbopol 934	2	4	4	4	2	4
Stearic acid	2.26	2.26	2.26	2.26	2.26	2.26
Bess wax	5	5	5	5	5	5
Span 20	3.5	3.5	3.5	3.5	3.5	3.5
Tween 20	6.5	6.5	6.5	6.5	6.5	6.5
Distilled water q.s to	100	100	100	100	100	100

Physicochemical study on the drug:

Melting point determination

The Thiel's tube method of melting point determination liquid paraffin was used in present study.

UV Spectrum

UV scanning was done for pure drug from 200-400 nm in the dilution medium of methanol and in the dilution medium of phosphate buffer pH 7.4.

Medium- Methanol[9]

100mg accurately weighed CT was dissolved in the methanol and volume was made up to 100ml with methanol [Stock 1]. From stock 1, different dilutions were prepared in the concentration range of 5, 10, 15, 20 and 25µg/ml using methanol as dilution medium. The absorbance of these solutions was measured against blank as methanol in UV spectrophotometer at 261 nm.

Medium- Phosphate buffer pH 7.4 solution[10,11]

100mg accurately weighed CT was dissolved in the Glacial acetic acid and volume was made up to 100ml with glacial acetic acid [Stock 1]. From stock 1, different dilutions were prepared in the concentration range of 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500µg/ml using Phosphate buffer pH 7.4 solutions. The absorbance of these solutions was measured against Phosphate buffer pH 7.4 solutions as blank in UV spectrophotometer at 264nm.

Compatibility studies of drug and polymers:

FTIR spectra of pure clotrimazole, physical mixtures, and SLN formulations are carried out to determine if there was any interaction between the drug and the other formulation components[12].

EVALUTION OF SLN:

Physical Evaluations:

Visual appearance

pH:

The pH of SLN formulations were measured using pH paper.

Rheological studies

Rheological properties (study of deformation and flow of matter) are required in various pharmaceutical areas. It helps to monitor the effect of vehicles consistency on release of drug from the preparations and subsequent percutaneous absorption. Also it is important from the manufacturing point of view. Viscosity measurements were carried out using a Brookfield viscometer (T – bar spindle). The formulation of SLN was kept in 100ml beaker and dial readings was noted at 3, 5, 6, 10, 12, 20, 30, 50 and 60 rpm. The speed was then successively lowered and the corresponding dial readings were noted.

Particle Size Analysis[13]:

The particle size should be less than 1000 nm in nanoparticles. It was analyzed by using Malvern particle size analyzer. Particles in the size range of colloids display constant random thermal motion which is known as Brownian motion. This motion causes the intensity of light scattered by the particles to vary with time. The larger the particle slower their motion and hence the smaller the variation in intensity of light scattered.

Zeta potential measurement

Zeta potential of the SLNs was measured by Malvern zeta sizer. The zeta sizer mainly consists of laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the centre of the sample cell, and the scattered light at an angle of about 13^0 is detected. when an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed and this information is passed to the digital signal possessor and then to a computer. Zeta sizer software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potential is calculated.

Scanning Electron Microscopy (SEM):

Surface morphology of the specimen will be determined by using a scanning electron microscope (SEM).

Drug content[14]:

The drug equivalent to 25 mg of formulation was taken and dissolved in small quantity of methanol. Then the formulation is warmed on the water bath so that the drug present in the formulation was completely dissolved. Then the solution was filtered through Whattman filter paper in 25 ml. volumetric flask and volume was made up to the mark by methanol to give concentration of 1000 µg/ml. for Clotrimazole. Then 1 ml. was pipetted out in 100 ml. volumetric flask to give concentration of 10µg/ml. and then absorbance was measured at 261 nm.

***In-vitro* release studies[9, 10, 15]:**

In Franz diffusion cell, 2 gm of sample was kept in donor compartment. The entire surface of cellophane membrane was in contact with the receptor compartment containing 62 ml of phosphate buffer pH 7.4. The receptor compartment was continuously stirred using the magnetic stirrer. The temperature was maintained 35°C. The study was carried out for 24 hrs and the sample was withdrawn at 30 minute time interval and same volume was replaced with free phosphate buffer. The content of clotrimazole from withdrawn sample was measured after suitable dilution.

Stability studies:

Whenever a new formulation is developed, it is very essential to establish that the therapeutic activity of the drug has not undergone any change. To conform this, the selected formulations were subjected to stability studies. Generally, the observation of the rate at which the product degrades under normal room temperature requires long time. To avoid this undesirable delay, the

principles of the accelerated stability studies are adopted. The International Conference of Harmonization guidelines titled "stability testing for drug substance and product" describes the stability tests requirements for drug registration applications in the European Union, Japan and United States of America. Short term Stability studies were carried out at 40°C ± 2°C /75% RH ± 5% for the selected formulations for physical and chemical stability for 1 month.

RESULTS AND DISCUSSION:

Melting point:

Melting point of the pure drug was found to be 143-145 ^{o}C which complied the limits of IP and BP. It's shown in the table no 2.

Table 2: Melting point.

No	Reported	Observed		
1.	141^{o}C – 145^{o}C	Trial 1	Trial 2	Trial 3
		143^{o}C	145^{o}C	143^{o}C

UV spectrum

UV scanning of the drug revealed that the drug had λmax of 261 nm in methanol and 264nm with Phosphate Buffer pH 7.4. Also, the IR spectrum was concordant with the reference spectrum of Clotrimazole.

Standard Graph of Clotrimazole:

The standard graph of Clotrimazole was prepared by using both Methanol and Phosphate Buffer pH 7.4. The absorbance was measured using UV spectrophotometer. The linearity was found at the concentration range of 5-25 µg/ml in case of Methanol and 50-500 µg/ml in case of Phosphate Buffer pH 7.4. The data was subjected to regression analysis and regression coefficient (R^2) was calculated and was found to be 0.9999 with Methanol and 0.9989 with Phosphate Buffer pH 7.4. Their respective values are shown in table no 3and 4, Figure 1 and 2.

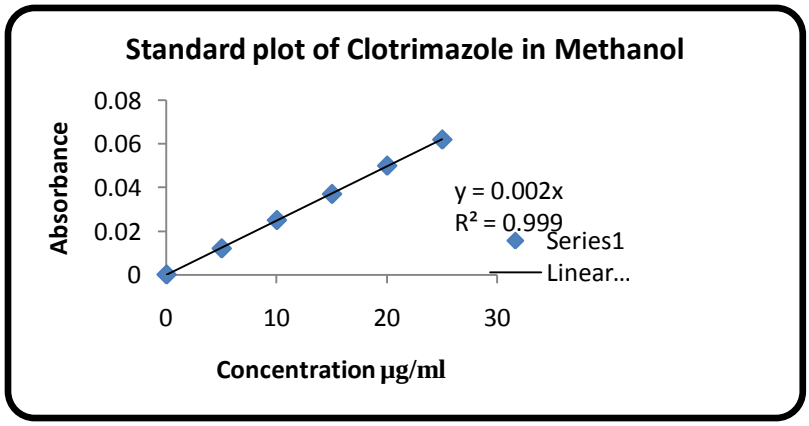

Figure 1: Standard plot of Clotrimazole in Methanol

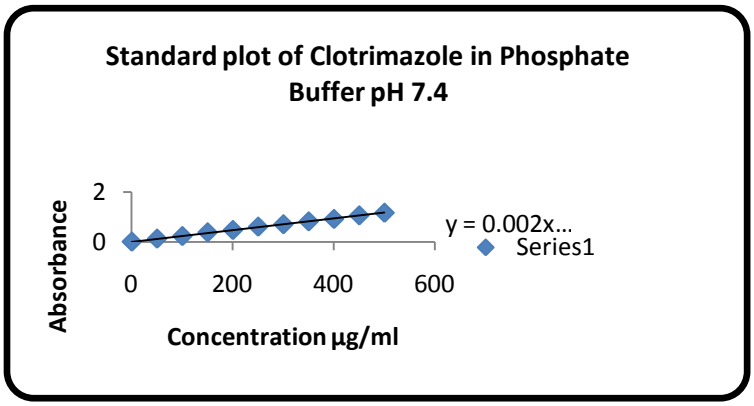

Figure 2: Standard plot of Clotrimazole in Phosphate Buffer pH 7.4

Table 3: Standard plot of Clotrimazole in Methanol

Sr No	Concentration (mcg/ml)	Absorbance at 261nm
1	5	0.012
2	10	0.025
3	15	0.037
4	20	0.05
5	25	0.062

Table 4: Standard plot of Clotrimazole in Phosphate Buffer pH 7.4

Sr.No	Concentration (mcg/ml)	Absorbance at 264nm
1	50	0.0123
2	100	0.227
3	150	0.378
4	200	0.472
5	250	0.608
6	300	0.704
7	350	0.82
8	400	0.919
9	450	1.063
10	500	1.17

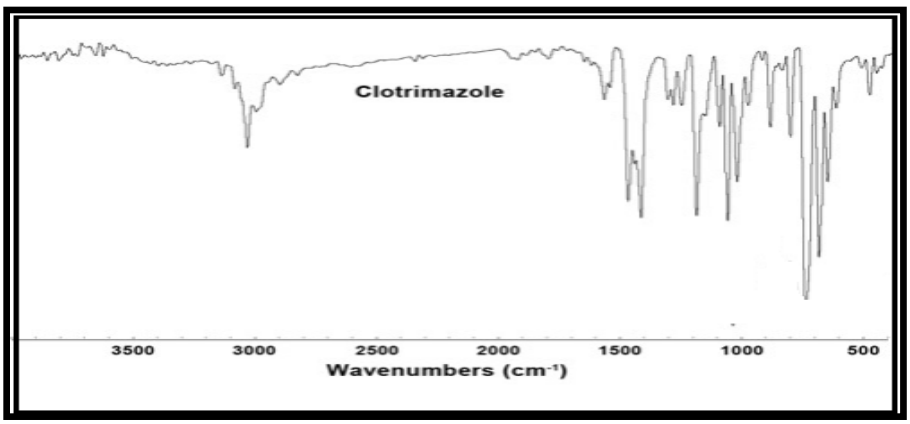

Figure 3: FTIR Spectrum of Clotrimazole

Compatibility studies

Physical mixture of drug and polymer and optimized formulations (A, B, C and D) were characterized by FTIR spectral analysis for any physical as well as chemical alteration of the drug characteristics. From the results it was concluded that there was no interference of the functional groups as the principal peaks of the Clotrimazole were found to be unaltered in the spectra of the drug-polymer physical mixtures as well as in optimized formulations. The FTIR spectrum of pure clotrimazole is shown in Figure 3.

EVALUTION OF SLN:

Physical Evaluations:

Visual appearance and pH

All the formulations were found to be off white colored and semi solid consistency. The pH of the formulations was in the range of 7.0 to 8.0. All these observations are listed in Table 5.

Table 5: Visual appearance and pH of the formulations

Formulation	Appearance	pH
F1	Off white	
F2	Off white	
F3	Off white	
F4	Off white	
F5	Off white	7 to 8
F6	Off white	
A	Off white	
B	Off white	
C	Off white	
D	Off white	

Rheological studies

Rheological behaviour for optimized formulations (A, B, C and D) indicated that the systems were non -Newtonian in nature showing decrease in viscosity at the increasing shear rates. The viscosity of the formulations is mentioned in Table 6 & Figure 4.

Table 6: Viscosity of optimized formulation

RPM	Viscosity(cps) (n=4)
3	51975.25
5	31683.75
6	26347.5
10	15022.5
12	11137.25
20	8880.25
30	6906
50	4315.75
60	3446.75

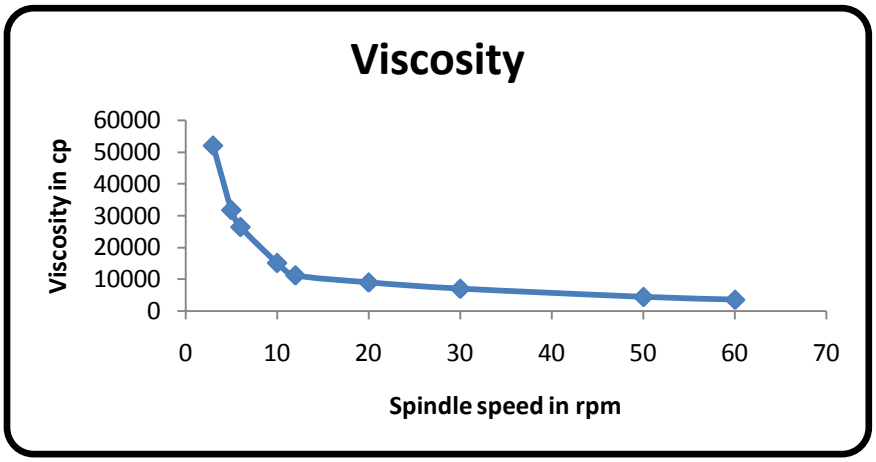

Figure 4: Viscosity of optimized formulation

Drug Content:

The drug content analysis for formulations were carried out and found to be 96.87%, 95.44%, 96.54%, 94.56%, 93.66% and 94.23% for F1, F2, F3, F4, F5 and F6 respectively. And for the optimized formulations it was found to be 96.86%, 96.87%, 96.87% and 96.86% for A, B, C and D respectively. The drug content of the formulations is shown in the Table 7.

Table 7: Drug content

Sr No.	Formulation	Drug content :(%)
1	F1	96.87
2	F2	95.44
3	F3	96.54
4	F4	94.56
5	F5	93.66
6	F6	94.23
7	A	96.88
8	B	96.86
9	C	96.85
10	D	96.87

Particle Size Analysis:

The particle size analysis was done for optimized formulations (A, B, C and D) in order to find the diameter of the particles. The mean particle size for optimized formulations (A, B, C and D) were found to be 962nm, 992nm, 872nm and 977nm respectively. Indicating, that the particles fell in an acceptable nanometer range (Table 8).

Table 8: Particle size and Zeta potential for optimized formulations

Formulation	Particle size in nm	Zeta potential
A	962	-36.3
B	992	-39.4
C	872	-43.2
D	977	-41.6

Zeta potential measurement

Zeta potential of the SLNs was measured by Malvern zeta sizer. The Zeta potential studies were carried out and the results were found to be -36.3, -39.4, -43.2 and -41.6 for A, B, C and D formulations with respectively, which indicated that formulations were stable (Table 8).

Differential Scanning Calorimetry:

The thermal behaviour of the Clotrimazole and its solid lipid nanoparticles were studied using DSC to observe the effect of lipids on the Clotrimazole. The DSC thermogram of the clorimazole exhibits an endothermic peak at 147.17 ^{o}C. In the case of optimized formulations (A, B, C and D) endothermic peak shifted towards lower temperature i.e 145.05 ^{o}C, 145.09 ^{o}C, 140.40 ^{o}C and 140.40 ^{o}C respectively. This indicates the lipids (Beeswax and Stearic acid) and polymers (Mannitol, Carbopol 934 and PEG-6000) may have decreased the melting point of the clotrimazole and there could be a chance for formation of the rigid solid lipid nanoparticles of clotrimazole. However the drug melting peak was retained almost nearer as that of pure drug. The DSC of the pure drug and one of the optimized formulations is shown in the Figure: 5 and 6.

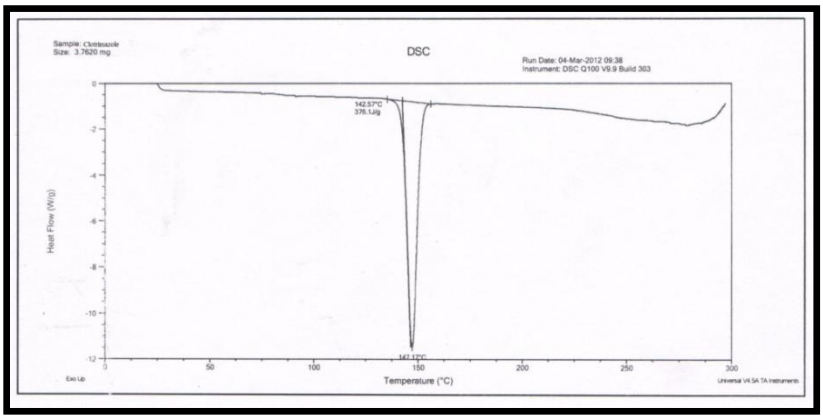

Figure 5: DSC of the pure drug (Clotrimazole)

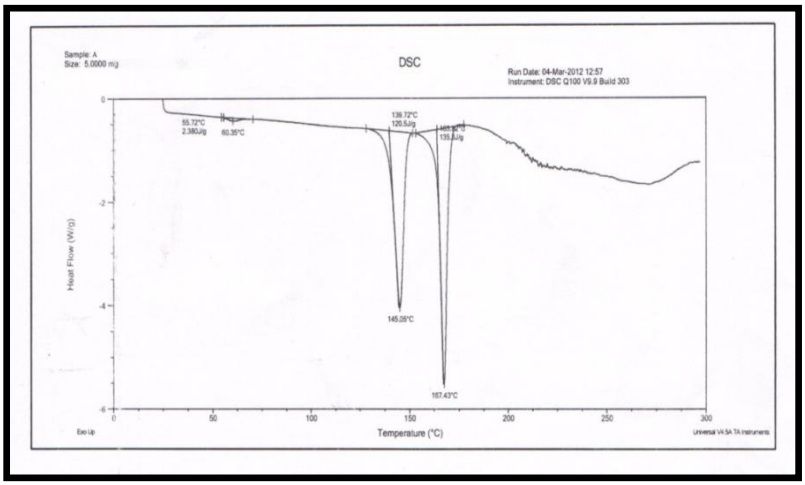

Figure 6: DSC of the optimized formulation

Scanning Electron Microscopy (SEM):

Scanning Electron Microscopy (SEM) studies were carried out for the selected formulations (A, B, C and D), the pictures revealed that the Clorimazole- SLNs were smooth and spherical. The particles were found to be in clusters as shown in the Figure: 7.

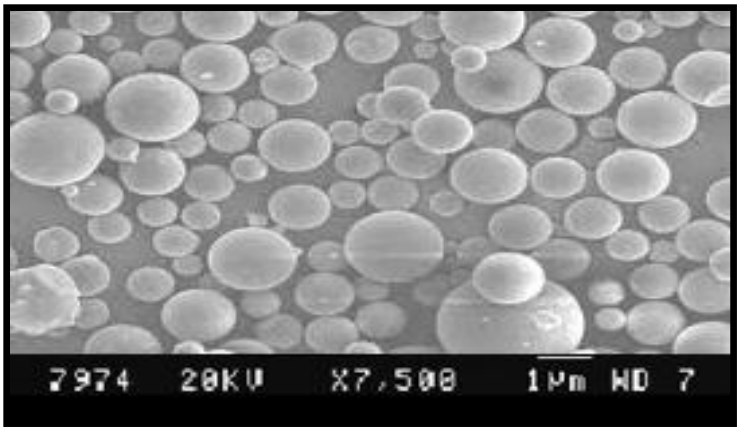

Figure 7: SEM for optimized formulation

***In-vitro* release studies:**

Formulations F1, F2, F3, F6, A, B, C and D were subjected to in-vitro release studies. The release studies were performed in a phosphate buffer of pH 7.4, suspending the formulation with 25mg equivalent of the drug. The results revealed that, about 86.18%, 72.7%, 41.7%, 56.6%, 86.18%, 80.86%, 84.91% and 86.18% of drug was released from F1, F2, F3, F6, A, B, C and D formulations respectively, in a span of 8hrs of study. But the In-vitro release of the Clotrimazole cream I.P showed about 95.6% at 5.5 hr. This indicated the In-vitro release pattern of the formulations prepared happened to be sustained in comparison to that of the Clotrimazole cream I.P. The release profiles are shown in Figure: 8, 9 and Table 9.

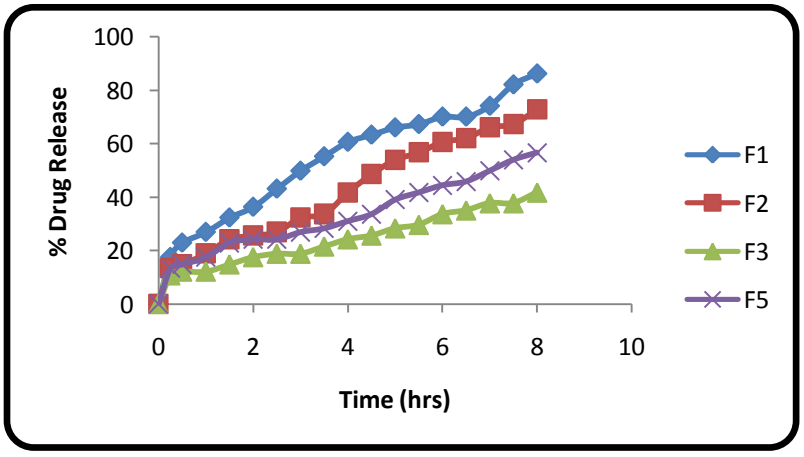

Figure 8: Release Profiles of Formulations F1, F2, F3 and F5

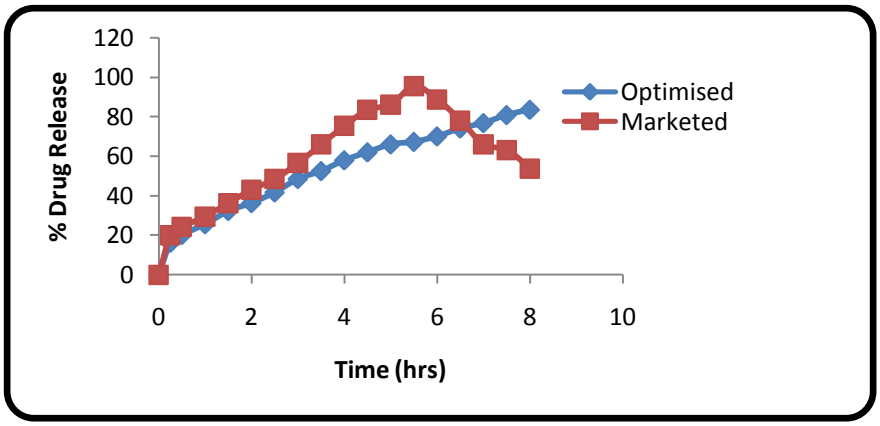

Figure 9: Comparative Drug Release Profiles of Marketed and Optimized formulations

Table 9: Dissolution Study of Formulations F1, F2, F3, F5, Optimized and Marketed in phosphate buffer pH 7.4

Time	% DR Of Formulation code					
in hr	F1	F2	F3	F5	Marketed	Optimized (n=4)
0.25	17.52	13.4	10.7	13.4	20.2	16.17
0.5	22.9	14.8	12.1	14.8	24.2	20.2
1	26.9	18.8	12.1	17.52	29.6	25.6
1.5	32.3	24.2	14.8	22.9	36.3	32.3
2	36.3	25.6	17.52	24.2	43.1	36.3
2.5	43.1	26.9	18.8	24.2	48.5	41.7
3	49.8	32.3	18.8	26.9	56.6	48.5
3.5	55.2	33.6	21.5	28.3	66.0	52.5
4	60.6	41.7	24.2	31.0	75.47	57.95
4.5	63.3	48.5	25.6	33.6	83.56	62.0
5	66.0	53.9	28.3	39.0	86.26	66.0
5.5	67.27	56.6	29.6	41.7	95.6	67.27
6	70.06	60.6	33.6	44.4	88.95	70.06
6.5	70.06	62.0	35.0	45.8	78.17	74.09
7	74.09	66.0	37.7	49.8	66.0	76.82
7.5	82.1	67.27	37.7	53.9	63.3	80.86
8	86.18	72.7	41.7	56.6	53.9	83.56
23.5	14.8	32.3	21.5	10.7	10.78	14.8
24	13.4	26.9	20.21	9.4	9.43	13.4

Data analysis

The release data obtained from optimized formulations was subjected for data analysis. The average of all 4 (A, B, C and D) optimized formulations were subjected to data analysis and it was found to be higuchi model of release profile. The values are shown in Table 10.

Table 10: Regression co-efficient (r^2) value of different kinetic models for optimized formulation

Optimized formulation	Zero order	First order	Higuchi	Korsmeyer- Peppas
Regression co-efficient (r^2)	0.8217	0.9901	0.9927	0.9826

Stability studies:

The selected formulations were subjected to short term stability studies for a period of four weeks at 40°C ± 2°C /75% ± 5% RH. Both physical and chemical changes were observed during the study. Physical stability was analyzed by morphological appearance and chemical stability was analyzed by the change in the drug contents and release profile. The results revealed that no much changes in morphological appearance as well as in the drug content and release profile. As a result of which the formulations were found to be stable at 40°C ± 2°C /75% ± 5% RH. (Table 11, 12 and Figure: 10).

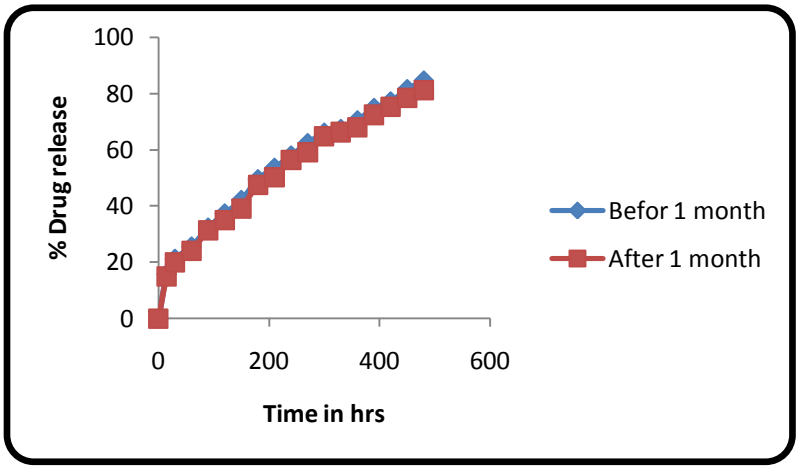

Figure 10: Release Profile of selected formulations after and before one month

Table 11: Release Profile of selected formulations after one month

Time in hr	% Drug release (n=4)
0.25	15.0
0.5	20.0
1	24.2
1.5	31.33
2	34.98
2.5	49.0
3	47.55
3.5	50.22
4	56.33
4.5	69.11
5	64.78
5.5	66.33
6	68.07
6.5	72.45
7	75.24
7.5	78.45
8	81.21
23.5	12.62
24	13.1

Table 12: Drug content and physical appearance for Stability studies samples

Formulation	Temperature and relative humidity	Appearance Weeks 0 1 2 3 4	Drug Content (%) After 4 weeks
A	40°C ± 2°C /75% ± 5% RH	No change	94.22
B	40°C ± 2°C /75% ± 5% RH	No change	94.85
C	40°C ± 2°C /75% ± 5% RH	No change	95.37
D	40°C ± 2°C /75% ± 5% RH	No change	94.93

CONCLUSION:

The formulation can be economically manufactured from relatively cheap raw materials like Carbopol 934, Mannitol, PEG 6000, Stearic acid, Beeswax, Tween 20 etc. Lipid Nanoparticles with suitable and desired characteristics may be prepared by hot homogenization technique. The release profile of Clotrimazole from the SLNs is amenable to slow delivery of the drug compared to marketed product to afford least time administration. The developed SLNs offers the advantage of high drug-lipid ratio, drug loading, minimal particle size and size-distribution and a good zeta potential of the particles. The nanoparticulate colloidal drug delivery system of Clotrimazole prepared from Beeswax, Stearic acid, Tween 20 and Span 20 is expected to provide the clinician with a new choice of an economical, safe and efficient regimen in the management of fungal infections. From the above experimental date it can be concluded that a successful SLNs containing Clotrimazole have been developed.

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