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Research Article

# PREPARATION AND EVALUATION OF NYSTATIN LOADED-SOLID-LIPID NANOPARTICLES FOR TOPICAL DELIVERY

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**ABSTRACT**

The purpose of present study was to prepare SLN incorporating an antifungal drug such as Nystatin and study its effects on skin localization of drug when administered through a suitable semisolid vehicle such as gel. Soby preparing solid lipid nanoparticles of Nystatin and theoptimize formulation was selected by using Box Behnken Design;and the characterizations of the gel for particle size, percent entrapment efficiency, XRD, FTIR and DSC to confirm the formation of SLN and entrapment of Nystatin in SLN which was formulated as a gel containing solid lipid nanoparticles. While for the selection of SLN delivery systemwas the maximum solubility of Nystatin in different lipids and also on melting point of lipid as the type of drug-lipid matrix and drug release pattern would depend on it. Various co-solvents were also used to aid the solubility of Nystatin in lipid. Out of different lipids used, Nystatin showed maximum solubility in mixture of glyceryl monostearate (GMS) and propylene glycol (PG). GMS, Span 60 and tween 80 were used as formulation ingredients determined by pre-optimization study. The optimized formulation by using different concentration of Carbopol 940 with 0.4 % gel which was finalized,and pseudo plastic behavior was observed by rheology study. After various process conditions, Nystatin in the Ny-SLN was found to be effective against *Candida albicans*.The result is the success of a new developed pharmaceutical formulation is able to deliver the active substance to the target organ at therapeutically relevant levels, with negligible discomfort and side effects; in addition the stability study indicates no significant difference between the parameters tested before and after the stability studies.

**Key words**: Nystatin, SLN, release box-Behnken design

## INTRODUCTION

Conventional formulations intended for topical and dermatological administration of drugs, such as creams, foams, pessaries and gels, are considered to reside for a relatively short period of time at the targeted site. To overcome many of these above-mentioned drawbacks, attempts have been made to introduce lipid nanoparticles into the cosmetic and pharmaceutical fields.

During the recent decades several studies have suggested that novel drug delivery systems based on lipid nanoparticles that have the potential of increasing cutaneous drug delivery of both hydrophilic and lipophilic drugs compared to the above mentioned conventional formulations[1, 2]. These lipid-based systems, well known as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC)[2], are composed of a solid matrix of physiological nature which might thereby fulfills the many promising aspects of the topical route and should in addition provide a controlled and prolonged release of drugs.

Their lipid based composition and small particle size contribute to the enhancement of penetration of compounds incorporated into these particles. With aging the stratum corneum barrier becomes fragile and the recovery is delayed [3], hence lipids play a crucial role for the water impermeability barrier function of the skin. In order to maintain locally a certain drug level and enable lower dosing frequency and lower amount of administered drug, Nystatin-loaded SLN and NLC formulations have been proposed in the present work as newly controlled and prolonged delivery systems

These properties bring many other advantages such as occlusion and skin hydration, absorption-increasing effects, active penetration enhancement, and controlled-release properties[4, 5]. SLN and NLC systems differ because SLNs were developed by exchanging the liquid lipid (oil) of oil-in-water (o/w) emulsions by a solid lipid[6], which can bring many advantages in comparison to a liquid core[7]. SLN is a less ordered lipid matrix with many imperfections, which can accommodate a higher amount of drug[8, 9].

The mixture obtained with solid and liquid lipids needs to be solid at least at 40$^0$C to make sure that it does not melt at room and body temperature if used for drug delivery. Advantages of using lipids as carrier systems for skin administration are related to their physiological and well-tolerated nature, which reduces the risk of toxicological problems and local irritancy[10]. A range of very different lipids with generally recognized as safe status has been used to produce SLNs and NLCs.

## OBJECTIVE

So the aim is,

1. To prepare solid lipid nanoparticles of Nystatin.
2. To reach at optimum formulation using Box Behnken Design.
3. To characterize the prepared optimum formulation.
4. To prepare and characterize gel containing solid lipid nanoparticles.

## MATERIAL AND METHODS

### Materials

Nystatin, Propylene Glycol, Polyethylene Glycol 400, Glyceryl Monostearate, Emulcire 61 Compritol 888 ATO, Precirol ATO 5, Gelucire, Stearic acid, Span 60, Tween 80, Soy lecithin, Poloxamer 188, Carbopol 940.

### Methods

### Selection of lipid

Selection of lipid was done on the basis of maximum solubility of Nystatin in different lipids and also on melting point of lipid as the type of drug-lipid matrix and drug release pattern will depends on it.

Various co solvents were also used to aid the solubility of Nystatin in lipid. Out of different lipids used, Nystatin showed maximum solubility in mixture of glyceryl monostearate (GMS) and propylene glycol (PG)[10]. Nystatin was only soluble in gelucire and GMS near about 65-70$^o$C, but after cooling the gelucire did not solidify up to desired extent hence the GMS was used.

The solubility was increased by addition of PG as Nystatin is soluble in PG at hot condition. Due to low melting point of GMS (60$^o$C) and high melting point of Nystatin (160$^o$C)[11], possible structure of SLN will be homogeneous matrix with prolong release of drug[12].

### Characterization of drug

Instrumental analytical techniques were performed to characterize the physicochemical properties of drug and to determine the changes that might occur in drug after preparation of solid lipid nanoparticle [13, 14].

**Differential Scanning Calorimetry study**

Differential Scanning Calorimetry (DSC) was performed on the bulk material i.e. pure drug nystatin to determine the melting point and exothermic and endothermic peaks. DSC was performed by Mettler-Toledo DSC 821$^{e}$ (Columbus, OH) instrument, and an empty standard aluminum pan was used as reference. DSC scans were recorded at heating rate of 10°C/min in temperature range 30°-300°C.

**X-ray diffraction**

X-ray scattering measurement was carried out on the pure drug Nystatin. XRD study was performed by Philips PAN analytical expert PRO X-ray diffractometer 1780[15].

**Fourier Transform Infra-Red (FTIR) Study**

A Jasco FTIR spectrophotometer (Jasco FTIR- 401, Japan) was used for infrared analysis of sample [16]. About 1-2mg of sample was mixed with 50 mg dry potassium bromide and the samples were examined at transmission mode over wave number range of 4000 to 400cm$^{-1.}$

**Melting Point Determination**

Melting Point was determined by the capillary method for the pure drug nystatin.

**Analytical method development (UV spectrophotometric method) Preparation of calibration curve of Nystatin**

UV spectrophotometric method for analysis of nystatin was developed. Calibration curve for Nystatin was done in Methanol and Saline pH 7.4 Phosphate buffer. Stock solution of nystatin (100 µg/ml) in methanol was prepared and suitably diluted to obtain concentrations ranging from 2 to 12 µg/ml with methanol and 2 to 16 µg/ml in saline pH 7.4 phosphate buffer. The absorbance of the prepared solutions was then measured at the wavelength, 304 nm, against reagent blank. The readings were recorded in triplicate and the experiment was repeated on 3 consecutive days using freshly prepared solutions each time. The mean values were recorded. The graph of absorbance against concentration was plotted.

**Selection of Solid Lipid Nanoparticle preparation method**

Solid Lipid Nanoparticles (SLN) can be prepared by various methods. The method of choice is pre-emulsion probe sonication method.

**Optimization of Pre-emulsion**

Pre-emulsion optimization studies were done with different concentration of lipids along with various surfactants at different concentration. These studies were used for initial optimization of pre-emulsion with optimum lipid concentration and surfactant concentration.

**Experimental Design**

Box Behnken Design was used for the optimization studies.Box-Behnken designs are response surface designs, specially made to require only 3 levels, coded as -1, 0, and +1. Box-Behnken designs are available for 3 to 10 factors. Here we have taken three factors such as drug to lipid ratio, span 60 concentrations and Sonication time, each of these factors at three different levels.

**Preparation of Ny-SLN dispersions**

Ny-SLN was prepared by using pre-emulsion probe sonication method. For preparing pre-emulsion the amount of drug, propylene glycol and Tween 80 was kept constant while the quantities of GMS, span 60 and sonication time were varied according to Box Behnken design. The lipid was heated to at least 10°C above its melting point. Nystatin was dissolved in the melt of lipid i.e. GMS and propylene glycol by stirring until the melt appeared clear. Tween 80 was dissolved in aqueous phase which was previously heated to the same temperature as lipid and pre-emulsion of the lipid in this aqueous phase was prepared by stirring with mechanical stirrer (REMI Instruments) at 10,000 rpm for 30 min. This pre-emulsion was sonicated with probe sonicator (Sonics VCX 750) for specified time according Box Behnken design. The hot dispersions were then quickly cooled down to 2-4°C to form Ny-SLNs suspensions

**RESULTS AND DISCUSSION**

The main purpose of this thesis is the investigation of the latest developments of innovative solid lipid carriers, particularly solid lipid nanoparticles (SLN) for topical delivery of antifungal drug. Fungal infections to the skin due to various fungi can be treated by antifungal drugs. Although Nystatin is one of the active molecules in case of various fungal infections it is given topically. To get symptomatic relief, fast action and also patient compliance, topical drug delivery of Nystatin is desirable. Hence, in the present work an attempt has been made to prepare topical formulation of Nystatin loaded SLN to improve the drug targeting to skin and accumulating the drug in skin for prolonged release.

**Characterization of drug**

Analytical techniques were performed to characterize the physicochemical properties of drug and excipient to determine the changes during the preparation of solid lipid nanoparticle.

**1. Differential Scanning Calorimetry study**

DSC is a highly useful means of characterizing material with respect to crystalline behavior and physical changes in the formulation. DSC gives the specific thermogram for Nystatin (Fig. 1) which is used to identify the Nystatin in Ny-SLN.

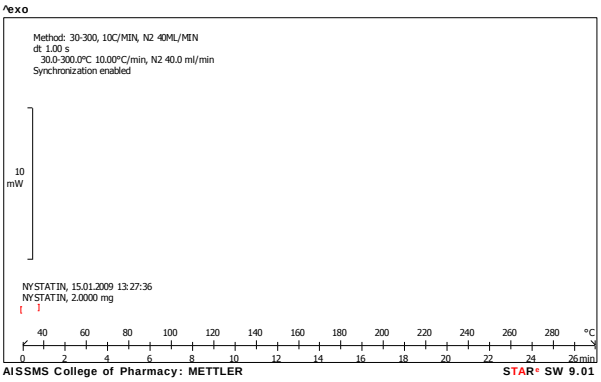


**Fig. 1: It shows Differential Scanning Thermograms of pure drug Nystatin.**

**2. Fourier Transforms Infrared (FTIR) Study**

FTIR shows the characteristic peak for NH, OH stretch – 3350-3331cm$^{-1}$, Lactone carbonyl stretch- 1701cm$^{-1}$, Carboxylate ion – 1566-1546cm$^{-1}$, C-OH stretch-1068cm$^{-1}$ (Fig.2).

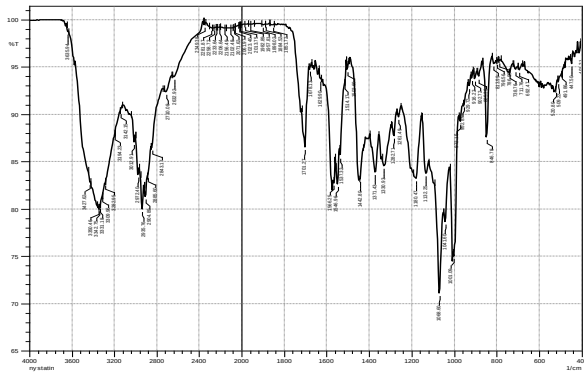


**Fig. 2: It shows Fourier Transform Infrared spectrums of Nystatin.**

**3. Melting Point Determination**

Melting point of pure drug Nystatin was found to be between 158-160$^{0}$C

**4. Analytical method development (UV spectrophotometric method).**

**Preparation of calibration curve of Nystatin**

Calibration curve was prepared in methanol and saline phosphate buffer pH 7.4, at $\lambda_{max}$ 304 nm where Nystatin shows the maximum linearity. For the Nystatin in methanol, the concentration range 2 - 12 µg/ml has regression coefficient ($R^2$) 0.999 and regression equation y = 0.0899x – 0.01 ; while the Nystatin in saline phosphate buffer pH 7.4 in the concentration range 2- 16 µg/ml has regression coefficient ($R^2$) 0.9991 and regression equation y = 0.0239x + 0.0823.

**Optimization of Pre-emulsion**

In the preparation of SLN different surfactants were used to determine their effect on particle size, Span 60 and Soy Lecithin were used in lipid phase and Tween 80 and Poloxamer 188 were used in aqueous phase.

Lesser particle size was found in lecithin-Tween 80 combinations and Span 60-Tween 80 combinations, but while preparing pre-emulsion due to high temperature the sticky mass was found at the bottom of container and also the dispersion was not stable for longer period of time.

The Span 60-Tween 80 combination formulation exhibited lesser particle size as well as rigid particles and is stable over longer period of time.

The poly dispersity index was slightly greater than 0.3. Effect of various formulation variables on average particle size and entrapment efficiency was determined by varying the concentration of GMS, Span 60 and Sonication Time.

As the concentration of GMS increases, particle size and entrapment efficiency of SLN increases. While increase in concentration of surfactant i.e. Span 60, particle size decreases and entrapment efficiency increases due to more solubilization of drug in lipid. **Experimental design**

Solid lipid nanoparticles of Nystatin (Ny-SLN) were prepared using probe sonication technique. The experiments were designed to study the effect of three independent variables at three levels on particle size and percent drug entrapment efficiency.

**Experimental design**

Solid lipid nanoparticles of Nystatin (Ny-SLN) were prepared using probe sonication technique. The experiments were designed to study the effect of three independent variables at three levels on particle size and percent drug entrapment efficiency.

**Table 1: It shows formulation of SLN Dispersion with different lipid and aqueous surfactant**

| Formulation | Drug (%) | Lipid | Lipid (%) | Lipid Surfactant (1%) | Aqueous Surfactant (2 %) |
|---|---|---|---|---|---|
| F1 | 1 | GMS | 3 | Span 60 | Tween 80 |
| F2 | 1 | GMS | 3 | Lecithin | Tween 80 |
| F3 | 1 | GMS | 3 | Span 60 | Poloxamer 188 |
| F4 | 1 | GMS | 3 | Lecithin | Poloxamer 188 |
| F5 | 1 | SA | 3 | Span 60 | Tween 80 |

**Table 2: It shows formulation of SLN Dispersion with different concentration of lipid with lipidic and aqueous surfactant concentration.**

| Serial number | Drug (%) | Lipid (%) | Span 60 (%) | Tween 80 (%) | Sonication time (min) |
|---|---|---|---|---|---|
| **F6** | **1** | **3** | **1** | **0.5** | **5** |
| **F7** | **1** | **3** | **3** | **1** | **5** |
| **F8** | **1** | **3** | **3** | **1.5** | **5** |
| **F9** | **1** | **3** | **5** | **2** | **5** |
| **F10** | **1** | **3** | **5** | **2** | **10** |
| **F11** | **1** | **3** | **5** | **2** | **15** |
| **F12** | **1** | **7** | **5** | **2** | **10** |
| **F13** | **1** | **7** | **5** | **2** | **15** |
| **F14** | **1** | **10** | **5** | **2** | **10** |
| **F15** | **1** | **10** | **5** | **2** | **15** |

From the above studies the levels selected for lipid was 3-10%, surfactant Tween 80 was chosen as aqueous phase surfactant in concentration range 2 % and Span 60 for oil phase in concentration of 1-5 %.

**Table 3: It shows values of particle size and entrapment efficiency for Ny-SLN dispersion**

| Formulation Code | Trial No | Coded Factor Level | | | Average particle size (nm) | % EE | Free Nystatin % |
|---|---|---|---|---|---|---|---|
| | | Factor 1 | Factor 2 | Factor 3 | | | |
| $R_1$ | 1 | -1 | 0 | +1 | 105 | 83.6 | 15.26 |
| $R_2$ | 2 | 0 | +1 | +1 | 220 | 87.5 | 11.78 |
| $R_3$ | 3 | -1 | +1 | 0 | 89 | 84.2 | 15.03 |
| $R_4$ | 4 | 0 | -1 | +1 | 255 | 86.7 | 12.82 |
| $R_5$ | 5 | +1 | +1 | 0 | 492 | 91.2 | 7.37 |
| $R_6$ | 6 | -1 | -1 | 0 | 201 | 82.6 | 16.65 |
| $R_7$ | 7 | 0 | 0 | 0 | 182 | 88.8 | 10.92 |
| $R_8$ | 8 | 0 | +1 | -1 | 297 | 90.9 | 8.24 |
| $R_9$ | 9 | +1 | -1 | 0 | 562 | 89.2 | 9.72 |
| $R_{10}$ | 10 | +1 | 0 | -1 | 617 | 92.8 | 6.03 |
| $R_{11}$ | 11 | 0 | -1 | -1 | 431 | 88.1 | 10.32 |
| $R_{12}$ | 12 | +1 | 0 | +1 | 386 | 90.4 | 8.68 |
| $R_{13}$ | 13 | -1 | 0 | -1 | 130 | 85.9 | 13.22 |

**Analysis of Experimental results**

Analysis of experimental results was done by using the Stat-Ease Design Expert. After filling the data in the design, linear and quadratic model were suggested to run the design.

$$Y = Bo + B1X1 + B2X2 + B3X3 + B4X1X2 + B5X1X3 + B6X2X3 + B7X21 + B8X22 + B9X23$$

**Search for optimum formulations**

The results for the feasibility search to find the suitable region. The criteria for selection of suitable feasible region were primarily based upon the highest possible values of %EE and lowest possible values of particle size. A region was selected on the basis of following criteria:

$$For\ region: Particle\ size\ <\ 250nm, \%EE\ >\ 89.00\%$$

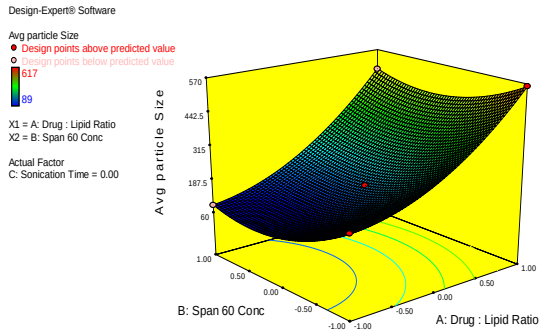


**Fig. 3: It shows 3-D Response surface plot showing the influence of span 60 and drug: lipid ratio on the value of average particle size Ny-SLN dispersion.**

Effect of drug: lipid ratio, Span 60 and sonication time on the % EE were determined by contour graph and 3-D response surface graph. As drug: lipid ratio and Span 60 concentration increases entrapment efficiency increases while as sonication time increases it show less significant effect on EE.

**Table 4: It shows values of particle size and %EE of optimized dispersion of Ny-SLN**

| Code | Dispersion composition | | Particle size (nm) | Entrapment efficiency (%) |
|---|---|---|---|---|
| | Drug: Lipid | Span 60 Soni. time | | |
| **S1** | 1: 7.2 | 3.45 11.125 | 210.33 | 89.4779 |
| **S2** | 1: 7.2875 | 3.45 11.125 | 210.206 | 89.4881 |
| **S3** | 1: 7.375 | 3.45 11.125 | 210.17 | 89.5361 |
| **S4** | 1: 7.2875 | 3.5 11.25 | 214.504 | 89.5369 |
| **S5** | 1: 7.375 | 3.5 11.25 | 214.49 | 89.5417 |

**Table 5: It shows comparison of experimental results with predicted responses**

| Formulation code | Response | Predicted value | Experimental value | Percenterror |
|---|---|---|---|---|
| S1 | Particle size (nm) | 210.33 | 219 | 0.0369 |
| | Entrapment (%) | 89.4779 | 91.87 | 0.011 |
| S2 | Particle size (nm) | 210.206 | 218 | 0.0385 |
| | Entrapment (%) | 89.4881 | 92.06 | 0.0107 |
| S3 | Particle size (nm) | 210.17 | 217.5 | 0.0297 |
| | Entrapment (%) | 89.5361 | 92.72 | 0.0098 |
| S4 | Particle size (nm) | 214.504 | 225 | 0.0404 |
| | Entrapment (%) | 89.5329 | 92.86 | 0.0117 |
| S5 | Particle size (nm) | 214.49 | 224.36 | 0.0422 |
| | Entrapment (%) | 89.5417 | 92.88 | 0.00119 |
| Mean (± S.E.M.) of Percentage Error | | | | 0.0338±0.002 0.00211 |

**Validation of optimum formulations**

(Table 4) indicates the formulations as per the predicted responses preparedusing GMS and span 60 as per the optimum region from intensive grid search shown in (Table3), the values of entrapment efficiency ranged between 89-90 % indicating high entrapment of drug in SLN. The values of particle size ranged from 205 to 245 nm indicating that nanoparticle size obtained with this technique. From the intensive grid search the values for drug to lipid ratio, span 60 concentration and sonication time were calculated and accordingly experiment for the predicted responses were reproduced. Comparative table of the observed responses with that of the predicted responses along with percentage error is listed in Table 4.All the plots were found to be highly linear as the values of $r^2$ ranged between 0.964 to 0.985.Hence, the prognostic ability of the experimental design to predict entrapment efficiency and particle size of prepared lipid nanoparticles dispersions of Nystatin is validated.

**Evaluation of solid lipid nanoparticles**

The prepared Ny-SLN dispersion was characterized with respect to the particle size, shape, entrapment efficiency, crystallinity and stability study.

**Particle size analysis** the particle size analysis of the optimized sample S3 was 217 nm. Particle size of other samples is shown in above Table 5. The effect of Span 60 concentration, sonication time and Drug: Lipid ratio on the particle size can be seen from values for sample S1, S2, S4 & S5 (219 nm, 218 nm, 225 nm and 224.36 nm respectively). Increased span 60 concentration decreases the particle size which can be explained by reduction in interfacial tension between aqueous and lipid phase which lead to the formation of emulsion droplet of smaller size thereby effectively stabilize the particles by forming a steric barrier on particle surface and by protecting the particle from coagulation. A particle size distribution curve of sample S3 is shown in Fig. 4having average particle size 217 nm. The poly dispersity index was slightly greater than 0.3 as the SLN was prepared by the probe Sonication method and homogeneous distribution of power density is necessary to obtain narrow size distribution. In the particle size distribution curve, the small peak observed near the 7000 nm which is due to poly dispersity of nanoparticles because the particles located in different volume of sample will experience different dispersing forces and therefore degree of particle disruption will vary within the sample volume.

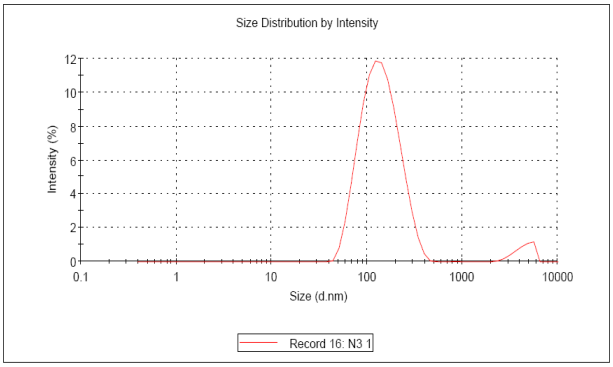


**Fig. 4: It shows particle size distribution curve of Sample S3.**

**Entrapment efficiency**

Entrapment efficiency of Ny-SLN sample S3 was found to be 92.72%. A high amount of drug could be incorporated in nanoparticle dispersion. Such high incorporation was possible because of lipid solubility of Nystatin and use of PG as cosolvent and also span 60 as a lipid surfactant helps to solubilize the Nystatin in to lipid which further increases entrapment of drug. It can be seen that, high lipid and span 60 concentrations show positive influence on entrapment efficiency while sonication time has less impact. Sample S3 was selected as optimized SLN dispersion since it showed less particle size & high entrapment efficiency (92.72 %) as compared to other dispersions.

**Differential scanning calorimetry (DSC)**

DSC is a highly useful means of characterizing material with respect to crystalline behavior and physical changes in the formulation.

Nystatin alone and in formulation was studied using DSC. The DSC thermogram of GMS show the melting process taking place at 62°C and Nystatin show peak at 160°C (Fig. 5). Thermogram of Ny-SLN showed an endotherm at 60.55°C, which can be attributed to melting of Nystatin in GMS.

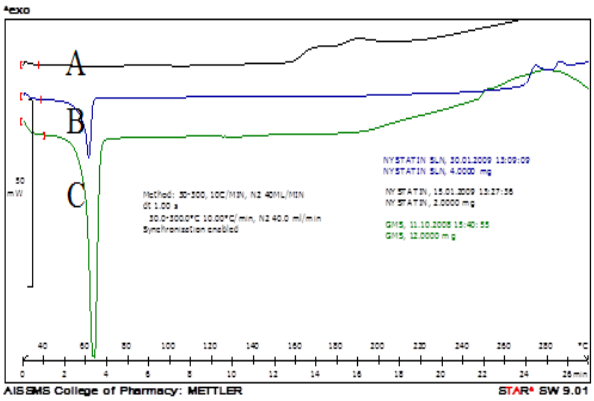


**Fig. 5: It shows differential scanning thermograms of bulk material of (A) Nystatin, (B) Ny-SLN and (C) GMS**

**X-ray diffraction**X-ray investigations have been most valuable in the elucidation of the manner of arrangement of lipid molecules, their multiple-melting phenomena, phase behavior and the characterization and identification of the structure of lipid and drug molecules. X-ray Diffraction data Fig. 6[A.B] were good in agreement with results established by DSC asurements.

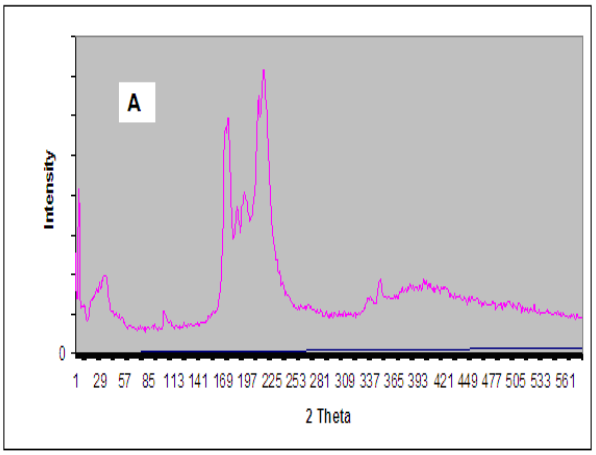


**Fig. 6 A: It shows X-ray Diffractograms of (A) GMS.**

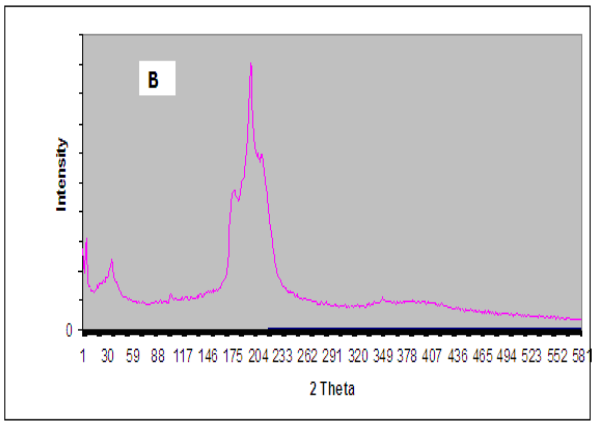


**Fig. 6 B: It shows X-ray Diffractograms of (B) Ny-SLN.**

**Fourier Transform Infrared study**

From FTIR study, the characteristic peak of drug such as of NH,OH stretch (3350-3331 $cm^{-1}$), lactone carbonyl stretch (1701 $cm^{-1}$), carboxylate ion stretch (1566-1546 $cm^{-1}$) disappeared and were replaced by the peak of GMS of H-OH (3300-3311 $cm^{-1}$), COOH OH stretch (2914-2848 $cm^{-1}$) and C=O stretch (1730 $cm^{-1}$) while remaining peaks also either shifted or replaced in the IR shown in Fig. 7[A.B] spectra of formulation. This established drug entrapment in lipid matrix.

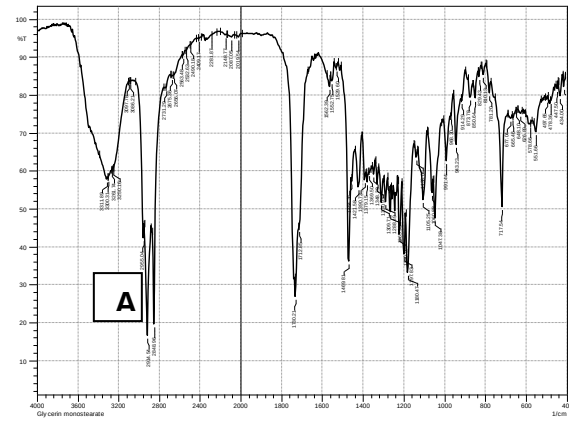


**Fig. 7A: It shows Fourier transforms infrared spectrums of (A) GMS Stability study.**

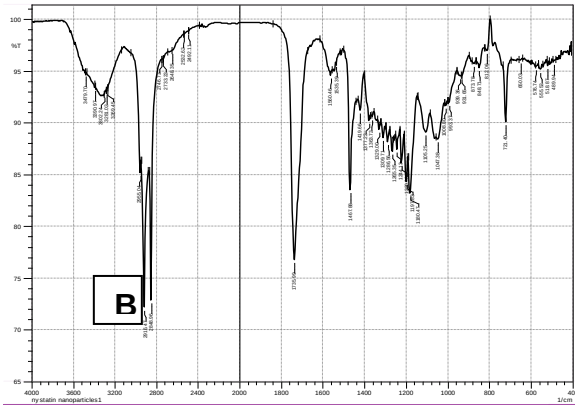


**Fig. 7B: It shows Fourier transforms infrared spectrums of (B) Ny-LN. Stability study**

After one month storage the SLN dispersion at various temperature parameters showed little difference in particle size and entrapment efficiency. There is no change in clarity and phase separation was observed. The average particle size and entrapment efficiency of optimized sample S3 stored for 1 month was 229 nm ± 2.88 and 89.37 % ± 0.5263 content. Centrifugation at 3000 rpm for 30 min showed there is no precipitation and the Ny-SLN had a good physical stability. Changes in particle size and entrapment efficiency were due to polymorphic transition of the lipid which leads to expulsion of drug from SLN (transformation of higher energy α and β' modification to the lower energy β modification).This may imply that the transition of dispersed GMS in SLN from ß´ form to stable ß form might occur extremely slowly.

**Preparation and evaluation of Ny-SLN gels**

Gel of Ny-SLN was prepared by using different concentrations of carbopol 940 (0.3%- 1%) out of that 0.4% concentration was selected. The criteria for the selection of 0.4 % Carbopol gel are the consistency of gel rheological pattern, drug release from the gel and hydrating and film forming properties.

Drug content in Ny-SLN gel was found to be 98.73 % ± 0.39 (n=3).

**Rheological study of gel**

The rheological behavior of 0.4 % Carbopol 940 gel containing Ny-SLN kept at different temperature. Rheological study was performed in Brookfield Viscometer CAP 2000+2, the results were recorded after one week of storage at 5ºC, 25ºC and at 40ºC, showpseudo plastic flow behavior at all temperature conditions.

**CONCLUSION**

Can be drawn from the study:

1. For the selected SLN delivery system GMS, Span 60 and tween 80 were used as formulation ingredients determined by pre-optimization study.
2. Box Behnken design was used for optimization study and searched optimum formulation with low particle size and high entrapment efficiency.
3. The optimized formulation was evaluated for particle size, percent entrapment efficiency, XRD, FTIR and DSC to confirm the formation of SLN and entrapment of Nystatin in it.
4. Nystatin SLN was formulated as gel by using different concentration Carbopol 940 from with 0.4 % gel was finalized and pseudo plastic behavior was observed by rheology study.
5. When Ny-SLN gel compared with the marketed gel, drug release was found to be sustained in case of SLN gel and more drug penetrated in to the skin layer which was desired.
6. To study the occlusive behavior of SLN, Ex vivo skin hydration and In vitro occlusion study were performed. From Ex vivo skin hydration study, it was found that due to formation of compact film trans epidermal water loss from skin reduces due to which moisture content of skin increases lead to more hydration with increase thickness of stratum corneum. Similar type of data obtained by occlusion study showing the change in weight of beakers which indicate loss of water.
7. After various process conditions, nystatin in the Ny-SLN was found to be effective against Candida albicans NCIM 3471.
8. Skin irritation test of Ny-SLN gel was performed on rabbit. The test article, Ny-SLN gel and plain gel was evaluated for primary skin irritation in accordance with the guidelines of the Consumer Product Safety commission and primary irritation index was calculated to be 0.00.
9. The result of stability study indicates no significant difference between the parameters tested before and after the stability studies.

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