Chapter - 6
DISCUSSION
# Chapter – 6

## DISCUSSION

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Sub-Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Analytical investigation of butenafine hydrochloride</td>
<td>163</td>
</tr>
<tr>
<td>6.2</td>
<td>Pre formulation study for butenafine hydrochloride</td>
<td>163</td>
</tr>
<tr>
<td>6.3</td>
<td>Experimental design of Butenafine Loaded Solid lipid nanoparticles (BUTE-SLN)</td>
<td>164</td>
</tr>
<tr>
<td>6.4</td>
<td>Evaluation of optimized BS9 BUTE-SLN dispersion</td>
<td>168</td>
</tr>
<tr>
<td>6.5</td>
<td>Characterization of lyophilized BS9 BUTE-SLN</td>
<td>168</td>
</tr>
<tr>
<td>6.6</td>
<td>Formulation and evaluation of BS9 BUTE-SLN gel</td>
<td>169</td>
</tr>
<tr>
<td>6.7</td>
<td>Analytical investigation of Sertaconazole nitrate</td>
<td>173</td>
</tr>
<tr>
<td>6.8</td>
<td>Pre formulation study of Sertaconazole nitrate</td>
<td>174</td>
</tr>
<tr>
<td>6.9</td>
<td>Optimization of sertaconazole loaded solid lipid nanoparticles (SERT-SLN)</td>
<td>174</td>
</tr>
<tr>
<td>6.10</td>
<td>Evaluation of optimized SS10 SERT-SLN dispersion</td>
<td>178</td>
</tr>
<tr>
<td>6.11</td>
<td>Characterization of lyophilized SS10 SERT-SLN</td>
<td>179</td>
</tr>
<tr>
<td>6.12</td>
<td>Formulation and evaluation of SS10 SERT-SLN gel</td>
<td>179</td>
</tr>
</tbody>
</table>
6. Discussion of results

The aim of present research was to formulate and evaluate the antifungal drug loaded solid lipid nanoparticles made up of natural origin material (OLML and OLMS) for effective drug delivery via topical route. Different formulations were prepared and evaluated for the characteristics of solid lipid nanoparticles for topical delivery as presented in chapter 5. In this chapter detailed discussion on results of each investigation is presented.

Butenafine

6.1 Analytical investigation of butenafine hydrochloride

In analytical method development, an absorbance maximum for butenafine hydrochloride (BUTE) was found to be at 252 nm by UV Visible spectrophotometer and showed the linear curves with regression value of 0.999 for butenafine hydrochloride in methanol and phosphate buffer pH 6.8: methanol (60:40). A sharp endothermic peak was observed at 217.3°C by DSC studies for butenafine hydrochloride confirming the crystalline nature of butenafine. The 2θ value of 16.0768 suggests the crystalline nature of butenafine hydrochloride by XRD pattern. Different confirmation, identification and compatibilities studies were carried out by FTIR and identified at 3047.63 cm⁻¹ (C-H stretching), 1072.46 cm⁻¹ (C-N bending) and 1365.65 cm⁻¹ (C-C bending).

6.2 Pre formulation study

The preformulation study commences with the screening of lipid and surfactant to determine maximum solubilizing potential for butenafine molecule. Solubility of butenafine in melted lipid influences the loading capacity, drug release pattern, loading pattern of drug inside lipid matrix and stability of formulation. Among the different solid lipid screened stearic acid, GMS, compritol 888 ATO and OLML showed maximum
solubilizing potential for butenafine. TPGS (co-surfactant), poloxamer 188, poloxamer 407 and stearyl amine (charge inducer) were selected to induce the charge on the SLN formulation.

Different surfactants and cosurfactants were screened for maximum solubilization of drug butenafine. The natural origin surfactant OLMS was selected for the further study. The OLMS was reported to have low human toxicity. It is more compatible with other ingredient and with skin. It is safe and has more potential to heal the damaged skin conditions due to infection. Dimethyl sulphoxide and acetone were shown complete solubilization of butenafine, hence these are added in the SLN formulation to improve the entrapment of drug.

6.3 Experimental Design

6.3 Optimization of Butenafine Loaded Solid lipid nanoparticles (BUTE-SLN)

Different formulations of BUTE were prepared by solvent emulsification technique and evaluated to determine the influence of varying concentration of excipients and process parameters on the properties of SLN dispersion. The formulations were evaluated for particle size, PDI, zeta potential and in vitro drug release study. In the formulations B1 and B3 use of 5% GMS and 5% GMS:OLML (1:1) showed the larger particle size and higher PDI as compare to B2 formulation with 5% OLML. Optimum concentration of surfactant was found to be 3% OLML which sufficiently reduces the interfacial tension between two phases, although higher concentration of surfactant up to 6% in formulation the B6 did not showed any further reduction in particle size and PDI. DMSO and acetone were used in formulation as they showed good solubilizing potential for butenafine. Formulation B8 with 5% DMSO was found to have minimum particle size with narrow particle size distribution. Formulations B11 and B12 with 0.5% poloxamer 188 and 0.5% poloxamer 407 respectively showed a strong negative zeta potential which is required for stability of nanoparticles, but the use of poloxamer series was restricted by considering the less penetration potential of SLN for topical application. Among the B13 to B17 formulations containing 0.05% to 5% TPGS, B15 showed optimum particle size and narrow PDI. Stearyl amine was used to induce positive charge on SLN. The formulations
B18-B20 containing 0.1% to 0.3% stearyl amine were evaluated for particle size, PDI and ZP and formulation B20 (0.3% stearyl amine) was found to have maximum zeta potential 17 mV and used for further study.

Various process parameters have great influence on the formation of SLN. Among the studied parameters, the stirring rate, time and use of sonication has negative influence on particle size and PDI. This could be due to strong sonic waves leads to collision SLN particles which lead to growth of smaller particle on the surface of larger particle. Formulation F1 to F10 were studied for particle size and PDI on the basis to determine influence of process parameter, hence these formulations were not studied for release study. F12 SLN dispersion was found to have narrow particle size distribution. Sudden cooling could lead to quick solidification of SLN hence uniform and small nanometric particles were obtained. In vitro drug release were performed for the all formulations to understand the influence of type of ingredients, their concentration and effect of process parameters on loading efficiency of BUTE in SLN and the possible structure of SLN. The drug release was studied up to 48 hr and the release was found to be between 61.84±2.84 to 67.94±2.91 %. Higher concentration of surfactant, TPGS and cosolvents showed the erratic diffusion pattern with initial burst release and then sustained release.

A 2³ factorial design was used to obtain the optimized BUTE-SLN dispersion. 3 factors at 2 levels were analyzed and evaluated for particle size, PDI, ZP, EE and DL. Eight formulations were drawn from the design expert software. The responses of all 8 formulations were fed to software and results of ANOVA were discussed below.

In the optimization study of BUTE-SLN, the regression equation \( Y_1 = +255.625 - 3.125*X_1 + 14.375*X_2 + 3.875*X_3 + 3.125*X_1*X_2 + 2.125*X_1*X_3 \) generated for Particle size was significant with F value of 43.08 (\( p < 0.0001 \)) and correlation coefficient value of 0.9908. The model generated for particle size revealed that the concentration of lipid, surfactant and organic solvent and their interaction has significant impact on the formation of nanometric particles. Figure 5.11 illustrates the response surface plot for influence of factors \( X_1 \) and \( X_2 \) on particle size \( (Y_1) \) of BUTE-SLN. It was observed that the increase in the concentration of lipid showed an increase in particle size while the
increase in the concentration of surfactant showed negligible effect on the particle size. The possible reason behind increase in particle size could be due to the increase in lipid concentration which might lead to increase in globule size of primary emulsion. The smaller droplet goes into emulsion and redeposit onto surface of nanoparticles in order to reach a more thermodynamically stable state. The insufficient surfactant concentration was unable to cover the formed new interfaces hence the particle size increases. Reasonable explanation for increase in surfactant concentration leads to slight increase in particle size could due to presence of organic solvent in the BUTE-SLN formulation. The surfactant present in SLN formulation is in equilibrium with the organic solvent hence the surfactant covers the SLN at interface and reduces the interfacial tension so that the nanolipid particles are formed but the additional rise in surfactant concentration goes into organic solvent hence rise in surfactant slightly increases the particle size.

The regression equation $Y_2=-0.302125+0.002875X_1+0.014875X_2+0.045625X_3-0.001125X_1X_3+0.005875X_2X_3$ generated for PDI was significant with F value of 240.33 ($p < 0.0001$) and $r^2$ value of 0.9983. The model generated for PDI revealed that the % concentration of lipid and organic solvent and their interaction has significant impact on PDI. The response surface plots (figure 5.12) illustrate that the PDI increases with the increase in the concentration organic solvent and lipid concentration. Increase in organic solvent concentration causes non uniform precipitation of SLNs leading to Polydisperse particles. This could be due to ostwald ripening that causes the formation of heterogeneous SLN dispersion. This result into variable particle with bi or tri modal size distribution, hence PDI increases.

The regression equation $Y_3=+18.5038+2.07875X_1+3.74625X_2-0.47125X_3-0.67875X_1X_2+0.47875X_2X_3$ generated for ZP was significant with F value of 87.80 ($p < 0.0001$) and regression value of 0.9955. The model generated for ZP revealed that the only concentration of lipid has significant impact on zeta potential. The response surface plot given in figure 5.13 for optimized formulation illustrates that increase in concentration of lipid increases the zeta potential while increase in concentration of surfactant has less effect on the charge of particle. Charge on nanolipid dispersion is the prime important factor for stability of formulation of any colloidal system. Generally
particles with zeta potential of ±30 mV could be considered as stable due to electric repulsion between particles. The individual zeta potential for BUTE-SLN ingredients were -16.9 mV for butenafine, -4.81 mV for surfactant and 20.5 for lipid OLML. From the given data it can be predicted that the lipid have more influence on zeta potential. The surfactant induces comparatively less negative charge on zeta potential of particles. The surface covered butenafine, surfactant, TPGS and organic solvent collectively induce charge on particle.

The regression equation generated for entrapment efficiency and drug loading ($Y_1 = +89.7075 - 0.17X_1 + 1.1825X_2 + 0.3575X_3 - 0.565X_1X_3 - 0.2975X_2X_3$) and ($Y_2 = +18.8625 - 0.21X_1 - 1.7075X_2 - 1.585X_3 + 0.175X_1X_2$) were significant with F value of 848.75 and 12.22 with $r^2$ of 0.9995 and 0.9422 respectively. Figure 5.14 and 5.15 illustrates the response surface plot of influence of concentration of organic solvent and lipid on entrapment efficiency and influence of lipid and surfactant concentration on drug loading respectively. Increase in concentration of lipid and DMSO showed marked increase in entrapment efficiency as the solubility profile of drug showed higher solubility of drug in selected lipid. While rise in concentration of surfactant give slight increase in entrapment efficiency due to partitioning of drug into aqueous and lipid phase. The increase in concentration of surfactant causes the formation of micelles which have a property to dissolve the lipophilic drug hence reduces the solubility in lipid phase. Cosurfactant also helps to dissolve drug into SLN as it has tendency to support the emulsifying property of surfactant and additionally improves the drug solubilization.

Organic solvent and lipid concentration showed the positive influence on the drug loading. Organic solvent used here dissolves more amount of drug and at the time of cooling of SLN dispersion lipid core encapsulate DMSO in between imperfect crystal lattice structure of lipid. Solidified SLN showed Nanostructured Lipid Carrier like structure which is composed of solid lipid in amorphous form with various lattice defects in which drug gets dissolved in organic solvent is presented as pockets. Along with independent variables some practical experimentation work has been done for fabrication of modified SLN such as sudden cooling of hot SLN dispersion can lead to sudden
precipitation of lipid and drug which forms lipid in α form containing maximum amount of drug and organic solvent.

It was revealed that the best fitted model for all responses was 2FI. According to the results of ANOVA for dependent variables, the models were significant for all measured responses, as concluded from the insignificant lack of fit (P<0.05). Observed and predicted responses for optimized formulation parameters were found to be are in close agreement with predicted values having relative error less than 5%.

6.4 Evaluation of optimized BS9 BUTE-SLN dispersion

The optimized BS9 BUTE-SLN was composed of 5% OLML, 4% OLMS, 0.5% DMSO and 1.5% TPGS. The optimized BS9 BUTE-SLN dispersions were translucent, odorless and stable after centrifugation at 2000rpm for 30 min. The particle size and PDI of the BUTE-SLN were observed to be in range of 231±2.79 nm to 278±3.19 nm and 0.241±0.005 to 0.369±0.01 respectively. Particle size and PDI of optimized batch was found to be 261.25±2.38 nm and 0.268±0.01 respectively. The TEM results showed the presence of nanometric size spherical particles. The zeta potential of all the prepared eight batches BS1-BS8 was found to be in between 11.7±0.16 mV to 24.9±0.41 mV and for optimized batch it was observed to be 23.98±0.27 mV. The EE formulations BS1 to BS8 were found to be in between 87.45±2.26 % to 91.65±1.69 % and drug loading in between 15.05±0.58 % to 22.86±0.46 %. The EE and DL of optimized formulation BS9 was found to be 91.35±2.35 % and 19.69±0.95 % respectively. The BS9 BUTE-SLN dispersions were subjected to different storage conditions and found to be stable and compatible. The BS9 BUTE-SLN dispersions were subjected to freeze drying by using 5% w/w mannitol. Mannitol and lactose in the concentration of 5 and 8% w/w each were used as cryoprotectant. BS9 BUTE-SLN dispersion made from 5 % w/w mannitol had shown short reconstitution time 2.5 min, no signs of aggregation and less difference in particle size 292±2.89 nm and 0.289±0.009 PDI.

6.5 Characterization of lyophilized BS9 BUTE-SLN

The freeze dried BS9 BUTE-SLNs were characterized for DSC, XRD and FTIR analysis. DSC determines the physical properties of compound like crystalline or amorphous
nature. Thermal analysis of pure drug butenafine, Lipid OLML and BS9 BUTE-SLN are given in figure 5.20. DSC thermogram of pure drug exhibited a sharp endothermic peak of melting point at 217.35°C. Thermogram for pure lipid OLML showed a sharp endothermic peak at 63.20°C indicating lipid is present in pure crystalline form. Further small peaks are attributed to the decomposition of formed small liquid droplets. The curve for BS9 BUTE-SLN showed a left side shifted small and broad peak at 46.91°C. The results shows crystalline lipid is converted into amorphous state as the heat flow through larger and perfectly ordered crystal require greater energy hence it give large and sharp peak while heat flow through small and less ordered particle require smaller amount of energy to melt the particle. The BS9 BUTE-SLN formulation showed the small and broad peak with the difference of 16.29°C as compare to pure lipid suggesting presence of nanostructure in amorphous form along with surfactant and pockets of organic solvent inside it.

From the XRD data, it is observed that the sharp diffraction peaks of butenafine at 2θ value of 16.0768 with d value of 5.4996 and pure lipid 2θ at 21.664 with d value 4.1124 which indicate the highly crystalline nature of compound as shown in figure 5.21. Whereas BS9 BUTE-SLN formulation showed broader and shorter peak with no specific diffraction peak for butenafine revealing encapsulation of drug inside the lipid matrix which was existed in amorphous form.

The comparative FT-IR spectra of butenafine, pure lipid OLML and BS9 BUTE-SLN are shown in Fig. 5.22. FTIR spectra for butenafine gives a typical specific absorption character at 3047.63, 1365.65 and 1072.46 cm\(^{-1}\) corresponding to \(-\text{C-H stretch, }-\text{C-C and -C-N vibrations respectively, but the BS9 BUTE-SLN spectra showed absence of these peaks. The FTIR results reveals that there is no strong interaction and no incompatibility observed and butenafine is successfully encapsulated into lipid structure.}

### 6.6 Formulation and evaluation of BS9 BUTE-SLN gel

For the effective topical delivery, some constrains should be considered which include physicochemical parameters of drug such as log P, pKa values, rheological study of formulation, occlusive and hydrating potential of formulation and disease state. The
natural origin *aloe vera* gel proved to be an excellent anti inflammatory, possesses natural healing ability and moisturizing effect which is beneficial for the treatment of diseased condition of skin. *Aloe vera* gel is useful and comfortable to treat exaggerated condition of skin. Hence the BS9 BUTE-SLN was incorporated into *aloe vera* gel. Percent assay for BS9 BUTE-SLN gel was found to be 99.18±3.27%. The prepared *aloe vera* gel was white translucent in color with pH 6.8. In the evaluation of semisolid formulation the Viscosity and spreadability parameter have given the more attention because the ultimate formulation was going to be used on the inflamed part of skin which should give the easy spread and soothing effect. BS9 BUTE-SLN *aloe vera* gel showed pseudoplastic rheological flow which is desirable for the topical application of gel. Small nanosize particle showed excellent slip property required for spreading of gel onto surface of wounded skin. The spreadability value of BS9 BUTE-SLN was found to be 4.6±0.37 gm.cm/sec and for plain gel base 4.06±0.41 gm.cm/sec. Entrapment of SLN into gel network slightly increases the rheology and spreadability of gel due to increase in amphiphilic surfaces upon which water can bind and immobilizes water molecules.

### 6.6.1 Occlusive study and Ex-vivo skin hydration study

The occlusion factor which represents the prevention of water loss from substance for BS9 BUTE-SLN were found to be 31.362 %, 41.1372%, 43.2659% and 45.7% as compared to plain gel 18.2795%, 23.0020%, 31.2094% and 31.5209% respectively after the 6 hr, 24 hr, 48 hr and 72 hr of study. The process of SLN permeation starts with occlusion and skin hydration. SLN composed of solid lipid and stratum corneum also contains intercellular lipids such as cholesterol, phospholipids and ceramides, hence SLN have strong affinity towards stratum corneum. Hence they form the impermeable occlusive film on the surface of skin which prevents the trans epidermal water loss. The impermeable layer prevents the release of vapor from skin surface which lead to condensation of vapors in the skin pores. The negative pressure build up that lead to attraction water molecules in the stratum corneum. This particular mechanism is very useful for the treatment of superficial fungal infections which are associated with scaling, dry skin, irritation etc. Stratum corneum is composed of compactly packed corneocytes and intercellular lipids. Due to occlusive film and reduction in trans epidermal water loss,
the hydration of stratum corneum takes place which reduces the compact packing between corneocytes and increases the pore size. This is the possible mechanism behind the transport of SLN through skin. The present formulation contains the SLN and *aloe vera* gel which will help to improve the skin barrier homeostatis and aids in healing process.

### 6.6.2 In vitro drug release study

*In vitro* drug release study of BS9 BUTE-SLN was compared with marketed cream FINTOP® as reference. BS9 BUTE-SLN gel showed higher drug release about 73.86±2.71% as compared to reference FINTOP 42.08±2.84%. The BS9 BUTE-SLN showed biphasic release pattern initially faster which becomes sustained after 2 hr.

### 6.6.3 Drug permeation and skin retention study

*In vitro* drug permeation was studied using franz diffusion cell. The BS9 BUTE-SLN showed 3.27 times higher drug release than the reference cream. The flux value calculated from the linear portion of graph of cumulative amount of drug release per unit area vs time was found to be 818.181 ± 0.392 ng/cm²hr for reference cream and 1666.7 ± 0.198 ng/cm²hr for BS9 BUTE-SLN gel. Results of butenafine retained after 48 hr were expressed in percentage of drug undiffused, deposited and permeated through skin and was found to be 5.96 times higher in BS9 BUTE-SLN applied pig ear skin than the reference cream. The skin retention study reveals the presence of higher quantity of butenafine for BS9 BUTE-SLN in the different skin strata as compared to reference cream. BUTE content in different skin layers were as stratum corneum 66.30%, epidermis 19.16% and dermis 14.53% for the BS9 BUTE-SLN. The above findings were useful for the topical drug delivery.

Drug release and permeation showed the biphasic release pattern of drug from SLN matrix. This can be due to use of some novel excipients such as surfactant OLMS, DMSO and TPGS which entraps the drug inside forming nanosize mini pockets in SLNs. The process of fabrication provoked the α state lipid transformation hence could include more amount of above said excipients. The formation of α state lipid structure slowly
convert into β’ form and then most stable β form then slowly expel the drug from lipid matrix which accounts for sustained release.

The possible mechanism for diffusion of drug from lipid matrix can be start with possible events like distribution of drug between lipid and DMSO, OLMS and TPGS, diffusion of drug through lipid, transformation of lipid from α state to most stable β form, distribution of drug in between lipid and dissolution medium, release of drug to dissolution medium. Incorporation of TPGS aids in the burst release from SLN matrix. This could occur at the time of sudden cooling of dispersion. The saturated emulsion when cooled down suddenly leads to solidification of drug and lipid and forms the drug lipid matrix while presence of TPGS and DMSO partially solubilizes the drug in aqueous phase from which drug get precipitated at the time of cooling. This form a uniform coat around SLN particle when such formulation was applied to skin show initial burst release. The efficacy of formulation was also depend upon the skin condition like diseased state, type of infection, inflammation, oedema and dry skin. All these pose the problem in permeation of drug in to the superficial layer of skin. In disease state the superficial layer of skin get disturbed exposing the epidermal cell along with existence of various metabolites of fungal cell, enzymes secreted by fungal cell and body immune system (cytokines) degenerate the drug into inactive fragments. Marketed cream showed the reduced diffusion of drug in to skin layer as compared to SLN which form a depot by concentrating in to SC and releases drug slowly. Skin retention studies revealed that the BS9 BUTE-SLN dominantly concentrates into stratum corneum which form reservoir for further release of drug and natural surfactant OLMS aid in partitioning of drug properly into epidermal cell.

6.6.4 Skin irritation study

The skin irritation study was performed according to OECD guidelines #404 and the study reveals the non irritant nature of BS9 BUTE-SLN gel explaining its usefulness in treatment of severe fungal infection. The formulation didn’t show any skin irritation, redness, inflammation and edema. In the skin irritation study, the formulation was found to be non irritant and safe for topical use.
6.6.5 *In vitro* antifungal activity

*In vitro* antifungal study was performed to investigate the comparative potency of butenafine SLN with marketed cream and to determine the effect of various process parameter on the formulation of BS9 BUTE-SLN. Zone of inhibition of marketed preparation was found to be 29.5 mm and for BS9 BUTE-SLN was found to be 32 mm which is greater than the marketed formulation proving the therapeutic efficacy of SLN formulation.

6.6.6 Stability studies of BS9 BUTE-SLN gel

Stability of the prepared BS9 BUTE-SLN gel was observed at different storage conditions. Increase in temperature showed slight leakage of drug. BS9 BUTE-SLN gel showed 98.73±2.39% butenafine content at all temperature during initial time period. The increase in temperature reduces the drug content in BS9 BUTE-SLN and decreases up to 96.16±3.42% at the end of 12 months. The pH, appearance, physical stability and organoleptics properties were also observed and showed no significant change. Thus the prepared formulations were found to be stable at studied temperatures.

Butenafine loaded solid lipid nanoparticles were prepared by using the novel excipients OLML and OLMS obtained from natural fruit olive. The modified solvent emulsification method was employed to prepare BS9 BUTE-SLN. Effect of various formulation ingredient and process parameters was evaluated by using factorial design. The formulation containing BS9 BUTE-SLN *aloe vera* gel was found to be safe and effective for the treatment of topical fungal infection.

**Sertaconazole**

6.7 Analytical investigation of sertaconazole nitrate

An absorbance maximum for sertaconazole nitrate was investigated using UV Visible spectrophotometer and found to be 260 nm. A graph of concentrations of sertaconazole in methanol and phosphate buffer pH 6.8: methanol (60:40) versus relative absorbance were plotted and showed linear curve with regression value 0.999. The DSC thermogram
showed a characteristic sharp endothermic peak at 156.98°C suggesting the melting of crystalline sertaconazole. In accordance with DSC study, XRD pattern showed two sharp peaks for sertaconazole were found at 2θ value of 16.826 and 24.458 respectively suggesting crystalline nature of sertaconazole. The characteristic functional group peaks were observed for sertaconazole FTIR at 3919.48 cm\(^{-1}\) (N-H stretch), 2877.89 cm\(^{-1}\) (C-S stretch), 1643.41 cm\(^{-1}\) (C=N stretch), 1188.19 cm\(^{-1}\) (C-O-C bend) and 640.39 cm\(^{-1}\) (C-Cl bend).

### 6.8 Pre formulation study

The preformulation study includes the screening of lipid and surfactant to determine the maximum solubilizing ability for the sertaconazole drug. Different lipids were screened for the maximum solubilization of sertaconazole, among these GMS and OLML were selected based on the minimum amount of solid lipid required to solubilize the 100 mg of drug. Both the lipids GMS and OLML reported to have self emulsifying property and saturated C\(_{16}\)-C\(_{22}\) chain length which aids in more solubilization of drug and hence entrap more amount of drug and will have uniform small nanometric particle. TPGS, poloxamer 188, poloxamer 407 and stearyl amine were added in the formulation to induce the charge and improve the the stability of SLN formulation. To impart the stability and to reduce interfacial tension at interface, different surfactants and cosurfactants were screened for maximum solubilization of sertaconazole. On the basis of safety, compatibility and maximum solubility of sertaconazole, the surfactant from natural origin OLML was selected and used for further study. Other cosolvents such as acetone and DMSO were selected to improve solubility of drug.

### 6.9 Optimization of sertaconazole loaded solid lipid nanoparticles (SERT-SLN)

With the aim to investigate the influence of different ingredients, their concentrations and process parameters on the fabrication of SLN, different formulations were prepared and evaluated for particle size, PDI, ZP and in vitro drug release study. Formulation S1 and S3 uses pure GMS 5% and combination of GMS:OLML (1:1) 5%as lipid phase and the results reveals the presence of polydisperse nanoparticle with larger particle size SLN. Due to larger particle size and PDI formulation S1 and S10 was not studied for
release. While S2 formulation with 5% OLML showed smaller particle size and narrow particle size distribution of SLN. Different concentration of surfactant OLML 1-6% was used to prepare SLN and it was observed that higher concentration of OLML did not reduced the particle size further. Formulation S5 showed optimum properties for particle size and PDI. DMSO and acetone in the concentration of 1.5 and 5% each were used to formulate SERT-SLN. 5% DMSO showed a promising reduction in particle size due to diffusion of drug and solvent from organic to aqueous phase. Poloxamer 188 and poloxamer 407 were used in the formulation to impart charge on the SLN and they showed the strong negative zeta potential on SLN. Although the stability is main concern of SLN, penetration is also one of the factor which affect formulation performance hence instead of negative charge inducer a positive charge imparter stearyl amine was used. The results S20 with 0.3% stearyl amine showed smaller particle size, reduced PDI and +26.4 mV. Formulation S13 to S17 were formulated using different concentration of cosurfactant TPGS, higher concentration of TPGS reduces the particle size but increases the drug partitioning behavior which could lead to low drug load. Hence S20 having 5% OLML, 3% OLMS, 5% DMSO, 1.5% TPGS and 0.3% stearyl amine was selected for further study.

Formulation of SLN was greatly influenced by various process parameters. The stirring rate, time and sonication time had negative impact on the particle size and PDI. The possible reason could be due to collision of molecule and then growth of larger particle on account of smaller particles. Formulation R1 to R10 were made to study effect of process variables on formation of SERT-SLN in terms of particle size and PDI. Hence these were not studied for drug release. The effect of cooling pattern was monitored in the formulation R11 and R12 and it was observed that sudden cooling leads to quick solidification of SLN hence uniform and small nanometric particles were obtained. The formulation R12 SLN dispersion was found to have narrow particle size distribution. All the ingredients and their concentration greatly affect the size of nanoparticles, entrapment efficiency and the drug release pattern from SERT-SLN. In vitro drug release was studied for the all formulations up to 48 hr and the release was found to be between 62.64±3.05 to 67.93±2.83 %. In vitro drug release study found to have biphasic release pattern with
initial burst release and then sustained release due to presence of higher concentration of surfactant, TPGS and cosolvents.

A taguchi experimental design was used to optimize the SERT-SLN formulation. The 4 factors at 3 different levels were studied and evaluated for particle size, PDI, ZP, EE and DL. The nine experimental runs were drawn from the design expert software and the dependant variables were measured. The results were analysed by ANOVA and discussed below.

The reduced two factor model \((Y_1=+211.81+14.23X_1+51.39X_2+27.40X_3-97.58X_1X_2+103.10X_1X_3+48.19 X_2X_3)\) generated for particle size was significant with F value of 279.91 (\(p < 0.0001\)) and correlation coefficient value of 0.998. The model generated revealed that the % concentration of lipid, surfactant and organic solvent and their interaction has significant impact on particle size. Figure 5.46 illustrates the response surface plot for influence of factors \(X_1\) and \(X_2\) on particle size \((Y_1)\) of SERT-SLN. Particle size was found to be increased with increase in surfactant concentration, increased lipid concentration and high level of charge modifier. Increase in particle size was due to increase in lipid concentration which in turn results into increased globule size of primary emulsion. Reasonable explanation for increase in surfactant concentration leads to increase in particle size was given by coating of surfactant molecules over the formed SLNs, the excessive surfactant concentration causes bilayer on SLN with the ultimate result of increase in particle size.

The regression equation \((Y_2=+0.30+0.056X_1-0.049X_2-0.057X_3-0.077X_1X_2+0.054X_2X_3)\) generated for PDI was significant with F value of 14.08 (\(p < 0.0001\)) and correlation coefficient value of 0.959. The reduced two factor model generated revealed that the % concentration of lipid, surfactant and organic solvent and their interaction has significant impact on PDI. The response surface plots (figure 5.47) illustrate that the PDI increases significantly as lipid concentration (%) increases while concentration of surfactant showed linear increase. The PDI is related to the particle size of SLN. Increase in lipid concentration causes non uniform precipitation of SLNs leading to deposits of smaller and larger particles.
The reduced two factor model \( Y_3 = +11.35 - 16.23X_4 + 11.93X_1X_3 \) generated for Zeta potential was significant with F value of 13.22 \((p < 0.0001)\) and correlation coefficient value of 0.8151. The model generated for zeta potential revealed that the % concentration of organic solvent and interaction of concentration of lipid and charge modifier has significant impact on zeta potential. The response surface plot given in figure 5.48 illustrates that increase in concentration of charge modifier and lipid increases zeta potential. Stearyl amine contains a lipophilic hydrocarbon chain which on adsorption onto particle projects its amine group towards aqueous phase which gives positive zeta potential.

The reduced two factor model \( Y_4 = +91.98 + 1.91X_4 - 0.58X_2X_3 \) generated for entrapment efficiency was significant with F value of 48.07 \((p < 0.0001)\) and \(r^2\) value of 0.9413. The model revealed that the % concentration of organic solvent and interaction of concentration of surfactant and charge modifier has significant impact on entrapment efficiency. The response surface plot (figure 5.49) illustrates that as concentration of surfactant and charge modifier increases entrapment efficiency increases. Here lipid showed high drug encapsulation efficiency as the maximum solubility of drug in lipid. High amount of Surfactant shows maximum drug loading capacity this is due to micelles of surfactant have solubilising potential.

The organic solvent dissolves drug in itself and at the time of emulsification encapsulated in lipid core due to its lipophilicity and imperfect lattices present in lipid. Solidified SLN showed NLC like structure incorporating drug in organic solvent which was encapsulated into lipid core as a pockets.

The reduced two factor model \( Y_5 = +16.53 + 0.77X_1 - 5.38X_4 + 1.37X_2X_3 \) generated for drug loading was significant with F value of 214.20 \((p < 0.0001)\) and \(R^2\) value of 0.992. The reduced two factor model generated revealed that the % concentration of lipid, organic solvent and interaction of concentration of surfactant and charge modifier has significant impact on drug loading. The response surface plots illustrate that as concentration of surfactant and charge modifier has linear relationship with drug loading capacity.
It was revealed that the best fitted model was 2FI for all responses. The results of ANOVA for dependent variables demonstrate that the models were significant for all measured responses, as concluded from the insignificant lack of fit (P<0.05). Observed and predicted responses for optimized formulation parameters was found to be in close agreement with predicted values having relative error less than 5% demonstrating the reliability and reproducibility of this method.

6.10 Evaluation of optimized SS10 SERT-SLN dispersion

The optimized SS10 SERT-SLN dispersions having 4% OLML, 4% OLMS, 1.122% DMSO and 0.288% stearyl amine were found to be odorless, translucent and stable after centrifugation at 2000 rpm for 30 min. The particle size and PDI of the SERT-SLN obtained from taguchi design were observed to be in range of 148.2±3.12 nm to 327.4±3.28 nm and 0.228±0.014 to 0.428±0.020 respectively. Particle size and PDI of optimized SS10 SERT-SLN was found to be 153.8±2.08 nm and 0.23±0.016 respectively. In accordance of particle size analysis the TEM results reveals that the nanoparticles are spherical in shape and confirmed to be near to 200 nm. The zeta potential of all the nine batches was found to be in between -9.42±0.35 mV to 27.7±0.16 mV and for optimized batch it was observed to be 9.76±0.08 mV. Presence of DMSO and TPGS showed higher loading capacity of sertaconazole into SLN. The EE of all formulations were found to be in between 89.206±2.35 % to 94.25±1.86 % with the drug loading in between 11.6388±0.76 % to 22.6213±0.71 %. The EE and DL of optimized formulation was found to be 93±2.32 % and 15.4±0.53 % respectively. The SS10 SERT-SLN dispersions were subjected to different storage conditions and found to be stable and compatible in terms of particle size, PDI and %EE. 5%w/w mannitol was used as cryoprotectant for the preparation of freeze dried SS10 SERT-SLN. Different cryoprotectants like mannitol and lactose in the concentration of 5 and 8% w/w each were screened for freeze drying. Lyophilization of SS10 SERT-SLN made from 5 % w/w mannitol have been selected for further study as it had shown short reconstitution time 2.5 min, no signs of aggregation and less difference in particle size 175±1.49 nm and 0.286±0.019 PDI.
6.11 Characterization of lyophilized SS10 SERT-SLN

The freeze dried SS10 SERT-SLNs were characterized for DSC, XRD and FTIR analysis. Thermal analysis of SS10 SERT-SLN, Lipid OLML and pure drug SERT are given in figure 5.55. DSC thermogram of pure drug exhibited a sharp endothermic peak of melting point at 156.98°C. Thermogram for pure lipid OLML showed a sharp endothermic peak at 63.20°C indicating lipid is present in pure crystalline form. Further small peaks are attributed to the decomposition of formed small liquid droplets. The curve for SS10 SERT-SLN showed a left side shifted small and broad peak at 48.35°C suggesting that crystalline lipid is converted into amorphous state. A small peak near 160°C reveals the encapsulation of sertaconazole inside SLN and also physical adsorption of the same.

From the XRD data, it can be observed that the sharp peaks of XRD represents the highly crystalline form of sertaconazole. The peak for lipid OLML was appeared as sharp peak with d value of 4.11249 and 2 theta value of 21.591, revealing presence of highly pure crystalline nature of OLML. XRD data for SS10 SERT-SLN suggest the existence of sertaconazole and lipid OLML in amorphous state and encapsulation of sertaconazole into lipid OLML.

A typical characteristic functional peaks were observed for FTIR of sertaconazole at 3919.48 (N-H stretch), 3147.93 (C-H stretch Aromatic), 2877.89 (C-S stretch), 1643.41 (C=N stretch), 1581.68 (C=C stretch), 1188.19 (C-O bend), 1087.89 (C-N bend) and 749.76 (C-Cl bend) cm⁻¹. SS10 SERT-SLN showed absence of these peaks while infrared spectrum of SS10 SERT-SLN was found to be super imposable with lipid OLML. Therefore it can be concluded that the SLN matrix of lipid OLML has successfully encapsulated sertaconazole.

6.12 Formulation and evaluation of SS10 SERT-SLN gel

For successful topical delivery, the drug must reach to the site of action at effective concentration. This transport of drug from the semisolid topical formulation to the target tissue can be affected by various chemical, mechanical and biological processes. For the
effective drug delivery and for maximum penetration of drug, drug has to pass through various chemical and biological environments beneath the superficial layer of skin such as toxins of fungal cell, inflammatory enzymes, dead tissues, pus and altered skin defence mechanism. Hence for the topical route drug delivery is very complex. In the light of this, drug properties (molecular weight, pka, log p), biological conditions of infected skin and effective drug carrier system should be optimized to deliver the drug to site of infection. *Aloe Vera* works as natural penetration enhancer and healing agent. It has some chemical constituents element like lignin and saponin which helps it to penetrate right down to the cellular level and increases the penetration of drug or carrier to reach the cellular level of the skin. In addition to this, it also nourishes the skin and replenishes it with the much needed nutrients. Hence the *aloe vera* gel was used as a semisolid carrier for the SS10 SERT-SLN formulation. Percent assay for SS10 SERT-SLN gel was found to be 98.54±1.54%. The prepared *aloe vera* gel was translucent in color with pH 6.7. In the evaluation of semisolid formulation, Viscosity and spreadability give prime information about application and performance of semisolid formulation which ultimately leads to drug release from vehicle. SLN enriched gel showed decrease in viscosity with consistent increase in shear stress which can be clearly seen from rheology profile.

SS10 SERT-SLN *aloe vera* gel showed pseudoplastic type of flow which is expected for the exaggerated skin condition. Small nanosize particle showed excellent slip property required for spreading of gel onto surface of inflamed skin. The spreadability value of SS10 SERT-SLN was found to be 4.5 ±0.11 gm.cm/sec and for plain gel base 4.03 ± 0.04 gm.cm/sec. Entrapment of SLN into gel network slightly increases the rheology and spreadability of gel.

### 6.12.1 Occlusive study and Ex-vivo skin hydration study

The occlusion factor which represents the prevention of water loss from substance for SS10 SERT-SLN were found to be 33.333±0.62%, 42.8791±0.35%, 46.1656±0.46% and 48.0099±0.42% as compare to plain gel 18.2795±0.37%, 23.0020±0.58%, 31.2094±0.33% and 31.5209±0.45% respectively after the 6 hr, 24 hr, 48 hr and 72 hr of study. The process of occlusion and skin hydration are important for the effective
permeation of drug molecules. The occlusion study result reveals the higher occlusivity of SS10 SERT-SLN gel, the possible consequences for the occlusive and hydration mechanism could be as given. The small nanometric SLN forms a layer over the surface of skin. As the particles are nanometric in size they form a compact pack layer which prevents the evaporation of water from skin. The compact layer of SS10 SERT-SLN and aloe vera gel additively form an occlusive film on skin surface and prevent the trans epidermal water loss. Due to water pore dynamic more water get concentrated towards stratum corneum hence showed more skin hydration. Stratum corneum consists of compact corneocytes and intercellular lipids like cholesterol, phospholipids and ceramides. Occlusion and hydration causes the reduction in compact packing between corneocyte and intercellular lipids and increase in pore size. Hence SS10 SERT-SLN can easily penetrate and concentrate in the stratum corneum and act as depot for further drug release. Aloe vera gel helps to regenerate skin natural barrier system and aids in healing.

6.12.2 In-Vitro drug release study

In-vitro drug release study of SS10 SERT-SLN was compared with marketed cream SERTAKON® as reference. SS10 SERT-SLN gel showed higher drug release about 75.81±2.76 % as compared to reference SERTAKON 39.41±2.81%. The SS10 SERT-SLN showed biphasic release pattern initially faster which becomes sustained after 2 hr. The SLN gel showed comparatively controlled release than the marketed cream.

6.12.3 Drug permeation and skin retention study

In vitro drug permeation was studied using franz diffusion cell. The SS10 SERT-SLN showed 3.74 times higher drug release than the reference cream. The flux value calculated from the linear portion of graph of cumulative amount of drug release per unit area vs time was found to be 7.5 ± 0.392 mcg/cm²hr for SS10 SERT-SLN gel and 4 ± 0.198 mcg/cm²hr for reference cream. The amount of sertaconazole retained in donor, receptor and skin was determined. It was found that the pig ear skin applied with SS10 SERT-SLN gel showed 3.6 times higher concentration of sertaconazole than the skin with marketed cream. The skin retention study reveals the presence of higher quantity of sertaconazole for SS10 SERT-SLN in the skin as compare to reference cream.
Sertaconazole content in different skin layers were as stratum corneum 57.35%, epidermis 27.89% and dermis 14.75% for the SS10 SERT-SLN. The above findings were useful in the topical drug delivery.

Drug release and permeation showed the dualphasic release pattern of drug from SLN matrix. The type of drug incorporation model and the penetration of drug through skin are the two prime factors that determine the permeation of SS10 SERT-SLN aloe vera gel. According to literature available in the text of drug incorporation model, cooling of nanoemulsion leads to a precipitation of the supersaturated drug molecule from the lipid phase and drug precipitate first prior to lipid recrystallization. Further cooling leads to precipitation of lipid around drug core as membrane. In SS10 SERT-SLN dispersion, other excipients (DMSO and TPGS) could affect this process which can lead to drug entrapment in DMSO nanodroplet inside lipid matrix forming nanostructured lipid carrier like model, while cosurfactant TPGS partly solubilizes the drug in itself which at the time of precipitation forms coat around SLN. This coat contains the precipitated drug and TPGS, hence give biphasic release pattern with initial burst and then sustained release.

The sudden cooling of nanoemulsion leads to solidification of lipid in to the α state, most unstable state which contains less ordered lipid arrangement hence encapsulate the more drug. The α state lipid slowly transform into more stable β’ form and then most stable β form during which the drug slowly get expelled from the lipid matrix. At the time of release of drug from lipid, DMSO and TPGS matrix, it could follow the diffusion and dissolution mechanism for sustained release of drug.

6.12.4 Skin irritation study

The skin irritation study scores were found to be 0 for erythema and 0 for edema as per OEDC guidelines. The formulations were free from skin irritation and redness. Hence the SS10 SERT-SLN gel was safe and effectively used for the treatment of fungal infection.

6.12.5 In vitro antifungal activity
In Vitro antifungal study was performed against *Candida spp.* and evaluated on the basis of zone of inhibition. The antifungal activity of SS10 SERT-SLN formulation showed higher zone of inhibition 25 mm as compared to marketed 24 mm. Hence the present formulation have enhanced antifungal activity.

6.12.6 Stability studies of SS10 SERT-SLN gel

Stability of the prepared SS10 SERT-SLN gel was observed at different storage conditions. SS10 SERT-SLN gel showed 99.23±2.42% sertaconazole content at all temperature during initial time period and 96.81±2.57% at the end of 12 months. Thus the prepared formulations were found to be stable and showed no significant change at studied temperatures.

In present experiment, modified solid lipid nanoparticles were prepared as novel carriers for topical antifungal drug delivery for sertaconazole as a treatment strategy for superficial fungal infection. SS10 SERT-SLN system was prepared by simple and novel modified solvent emulsification technique. Process and formulation variables were optimized using Taguchi experimental design approach. SS10 SERT-SLN was formulated into aloe vera gel for topical drug delivery and was found to have higher penetration of SERT, quick onset of action and effective in management of fungal infection.

The BUTE-SLN and SERT-SLN were successfully prepared for the treatment of topical fungal infection. The formulations were found to have enhanced penetration of drug and rejuvenating the infected skin.