INTRODUCTION

Solid lipid nanoparticles (SLNs) are one of the most promising particulate deliveries of pharmaceutical and other bioactive compounds [1–6]. SLNs have been successfully employed for efficient delivery of numerous therapeutic agents by various delivery routes [7–9]. These can also be modified for size and surface charges in order to achieve site-specific drug delivery designed for immediate or prolonged release. In addition, SLNs can be engineered to release their payload in response to a specific external trigger, such as temperature or pH [10–11]. Many different techniques for the production of lipid nanoparticles have been described in the literature, for example, high pressure homogenization [12], microemulsion technique [13], solvent emulsification-evaporation [14], solvent diffusion method [15], ultrasonication [16], double emulsion technique [17], solvent displacement method [18], and phase inversion [19]. Out of all these methods solvent emulsification-evaporation technique has several advantages for the preparation of SLNs particles [14] such as to reduce the exposure to high temperature of thermolabile compounds, like proteins and nucleic acids [20]. The solvent evaporation method is based on the evaporation of the organic solvent in which lipids are dissolved, allowing the formation of either solid microparticles or nanoparticles. To obtain nanoparticles, this process includes an additional step, such as high pressure homogenization [14] or sonication [21, 22].

Efavirenz (Fig. 1), (4S)-6-chloro-4-(cyclopropylethynyl)-4-(trifluoromethyl)-1,4-dihydro-2H-3,1-benzoxazin-2-one, is a drug that belongs to the category of non-nucleoside reverse transcriptase inhibitors (NNRTI) [23]. EFV is a first-choice non-nucleoside reverse transcriptase inhibitor used in the High Activity Antiretroviral Therapy (HAART) of the infection by the Human Immunodeficiency Virus (HIV) in both adults and children [24]. Due to its high lipophilicity (log P = 5.4) and consequently poor aqueous solubility [25] (4 g/mL), the drug shows relatively low oral absorption and bioavailability (40–45%) and high inter-subject variability [26]. Furthermore, EFV is not commercially available worldwide in aqueous solution for the oral management of the pediatric pharmacotherapy. The high efficacy and low dosage requirement of EFV has made it an attractive for a highly active antiretroviral therapy for the treatment of HIV infection in recent years [27]. Unfortunately, it exhibits low solubility in aqueous gastric fluid and imparts a strong and prolonged burning sensation to the mouth when incorporat-
-ed in water in the liquid formulations [28] and needs a potential drug carrier which can also mask its unpalatable taste. Literature survey revealed that EFV loaded SLNs (EFV-SLNs) have not been reported so far. Low solubility, extensive first pass metabolism, low bioavailability, high dosing frequency, and half-life makes EFV promising drug for formulation of SLNs to enhance its oral bioavailability. Delivering the existing drug molecules, by advanced technology will be the more preferred strategy so as to enhance its therapeutic efficiency. SLNs are prepared with glycerylmonostearate (Triglyceride), (GMS), by solvent emulsification/evaporation technique. Particle size, zeta potential measurements, FTIR Spectra, DSC, SEM, crystallinity studies, and in vitro release studies were performed.

2. MATERIALS AND METHODS

2.1. Materials

The drug, Efavirenz was a procured as gift sample from Hetero Drugs Ltd.,(Hyderabad, India). Glycerylmono-stearate, and Tween 80 were of analytical grade and purchased from LobaChemie Pvt. Ltd., (Mumbai, India). Chloroform, Methanol, and Acetonitrile were of HPLC grade purchased from Merck Specialties Ltd., (India).

2.2 Preparation of Efavirenz loaded Solid Lipid Nanoparticles

Efavirenz loaded solid lipid nanoparticles were prepared by using solvent emulsification and evaporation technique. In this method, accurately weighed amounts of glycerylmonostearate (100-250) and efavirenz(5 mg) drug were dissolved in 1mL of organic solvent like Chloroform. To the organic solvent 20 mL of (1%) Surfactant, Tween 80 was added and then homogenized (Ultra-Turex T25 homogenizer) at 7000 rpm for 3 min and in order to get a coarse O/W emulsion. The coarse emulsion obtained was sonicated using probe sonicator at 50 W (Vibra Cell, Sonics & Materials, Inc., USA) for 15 min to evaporate the solvent followed by magnetic stirring for 3 hr. The nanoemulsion was cooled to roomtemperature to obtain efavirenz-loaded solid lipid nanoparticles.

2.3 Measurement of Size of SLNs

Size and zeta potential of efavirenz loaded SLN samples were measured by photon correlation spectroscopy using Zetasizer 3000 HAS (Malvern Instruments, UK). All the samples were diluted with aqueous phase of the formulation to get optimum kilo counts per second (Kcps) of 50–200 for measurements. Average particle size in nanometers, polydispersity index and zeta potential were measured.

2.4 Chromatographic conditions

Estimation of Efavirenz in phosphate buffer of pH 7.4 was conducted by using reverse-phase HPLC (JASCO, Inc., USA) in a binary mode with UV detector and a 2070 module. The analysis was performed at 274 nm with a Spherisorb ODS2, reverse-phase C18, 250 mm x 4.5 mm, 5 μm column (Waters Corporation, USA) maintained at 25°C (Column oven) employing a mobile phase of acetonitrile (70%) and potassium dihydrogen phosphate buffer (30%) and the pH was adjusted to 3.0 using orthophosphoric acid. The mobile phase was delivered at a flow rate of 1 mL/min. The retention time of the drug was found to be 4.0±0.1 min. The calibration curve was linear in the concentration range of 2-10 μg /mL (r² =0.99) in phosphate buffer of pH 7.4. Data analysis and processing were done by BORWIN software (Version No 1.50.04)

2.5 Determination of drug content

EFV loaded SLNs (0.5 mL) were diluted to 10 mL with chloroform/methanol mixture (1:1) and finally dilution was made with mobile phase (acetonitrile /buffer 70:30) mixture and total content was determined by HPLC (JASCO, Inc., USA) method as described above.

2.6 Determination of entrapment efficiency

The Percentage of drug entrapped in the lipid was determined by measuring the concentration in the aqueous phase by Ultra centrifuge (Remi Instruments Ltd., Mumbai, India) of the total formulation. About 1ml of undiluted SLNs sample was placed in the Eppendorf tube and ultra-centrifuged at 50,000 rpm for 30 min. The solid lipid nanoparticles along with encapsulated drug remained at the bottom of centrifuge tube and the unentrapped drug is remained in the upper supernatant layer or aqueous phase. The amount of drug in the aqueous phase was analyzed by using HPLC method as described above and subtracted from the total drug content to get the amount of entrapped drug. The entrapment efficiency was calculated by using following equations respectively.

\[
EE(\%) = \frac{Total\ mass\ of\ EFV - Mass\ of\ EFV\ in\ supernatant}{Total\ mass\ of\ EFV} \times 100
\]

2.7 Stability studies

Efavirenz loaded SLNs optimized formulation were stored at 25 °C for 3 months and average size and entrapment efficiency were determined.

2.8 Fourier transforms infrared spectroscopy (FTIR)

Fourier transforms infrared (FTIR) spectra of pure EFV, pure GMS and lyophilized SLNs formulation were recorded using samples embedded in KBr pellets and FTIR spectrophotometer (Bruker Optics, Germany). The scans were performed in the range of 400–4000 cm⁻¹.

2.9 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis was performed (using Mettler Toledo AG, Analitical, Switzerland) to investigate the melting point and crystalline behavior of crystalline materials. A heating rate of
10°C/min was employed in the range of 35–300°C. Analysis was performed under nitrogen purge (50ml/min). DSC studies were conducted for EFV, bulk GMS, Lyophilized SLNs optimized formulation batch.

2.10 Powder X-Ray Diffractometry (PXRD)
Powder X-ray diffraction analysis was carried out by using Siemens’ D-5000, Germany. PXRD studies were performed on the samples by exposing them to Cu Kα radiation (40 kV, 30 mA) and scanned from 2θ = 10° to 80° at a step size of 0.045° and step time of 0.5 s. Samples used for PXRD analysis were pure EFV, pure GMS and lyophilized SLNs optimized formulation.

2.11 Scanning Electron Microscopy (SEM)
The surface morphology of EFV, GMS, freeze-dried sample of EFV-SLNs was analyzed by scanning electron microscope (JSM-5800 SEM, JEOL Ltd., Tokyo, Japan). The powdered samples were uniformly spread on double-sided carbon tape, fixed on a stainless steel stub, and coated with gold/palladium to prevent charge buildup by the electrons absorbed by the specimen. The micrographs were obtained at an excitation voltage of 12 kV and magnification factors of x1,000.

2.12 Invitro drug release from SLNs
Invitro drug release studies of the selected optimized formulation were performed by using dialysis bag method. Dialysis membrane having molecular weight cut off 12,000-14,000 was used. Membrane was soaked in phosphate buffer solution for 12 hours prior using for drug release study. A volume of 2 mL of SLNs formulation was placed in the dialysis bag and the release media used is 50mL of phosphate buffer having pH of 7.4. At fixed intervals of 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48 h, 1mL of sample was withdrawn from release media and analyzed by using HPLC method as described above.

3.0 RESULTS AND DISCUSSIONS
For the preparation of stable solid lipid nanoparticles of efavirenz with glycerylmonostearate, process variables and formulation variables were optimized. Process variables include homogenization time, sonication time, stirring time and formulation variables including amount of lipid, concentration and volume of surfactant (Tween-80).

3.1 Preparation of efavirenz loaded SLNs
Solvent emulsification followed by evaporation technique is a reliable, simple and reproducible method for preparing SLNs. The drug and lipid mixture was dissolved in 1 mL of chloroform was found to be effective in homogenously dispersing the drug in the lipid phase. A hot aqueous phase was prepared by dissolving required amount of Tween 80 in 100 mL of double distilled water to get the concentrations of 0.5, 0.75, 1.0 and 1.5% v/v. Homogenization of the lipid phase with hot aqueous solution for 3 min was sufficient to produce a coarse emulsion with average particle size between 3.12 and 3.23µm. Further increase in homogenization time did not show any significant decrease in the particle size (3.11–3.20 µm). Thus a homogenization time of 3 min was selected for all the formulations and further reduction of size was observed with sonication. Sonicating the coarse emulsion for 15 min resulted in particles between 89 and 124 nm with narrow size distribution. In order to optimize the lipid to drug ratio, different amounts of lipid (100,150,200 and 250 mg) were tried with fixed amount of drug (5mg). At lipid content of 150 mg there was no deposition occurred and resulted particles had a substantial good nano size. The various experimental conditions used in the optimization of SLNs of EFV were shown in Table 1. Based on these results 150 mg of the lipid with 5 mg of the drug containing formulation was used for further studies.

3.2 Measurement of size
To obtain stable and smaller SLNs, Tween 80 concentration was varied from 0.5% to 1.5% and their effect on particle size was measured and shown in Table 2. It is evident from Table 2 that Tween 80 concentration of 1% was effective in producing smaller size SLNs (89.09 nm for F1, 92.58 nm for F2, 85.55 nm for F3 and 98.02 nm for F4). Further increase in Tween 80 concentration to 1.5% did not reduce the particle size. These results clearly suggest that an optimum concentration of 1% Tween 80 was sufficient to cover the surface of nanoparticles effectively and prevent agglomeration during the homogenization process. High concentration of surfactant (1.5%) was avoided to prevent decrease in the entrapment efficiency and also toxic effects associated with surfactants.

3.3 Total content and entrapment efficiency
The total content of the drug present in the formulation was determined as described in section 2.5 using the standard graph of the drug in the phosphate buffer saline 7.4. The total drug content in the formulation was found to be 13.91mg /ml. The entrapment efficiency of F1, F2, F3, F4 were shown in Table 2. Entrapment efficiency of the optimized formulation F3 was found to be 92±9.72%.

3.4 Stability studies
Based on the results of optimization studies of all process and formulation variables, stable SLNs were prepared using optimized formulation and kept for stability studies for one month at 25°C temperature. The particle size evaluated can be used to predict the stability of the preparation; small particle size provides a better stability. Furthermore, difference in particle sizes with time would be strong evidence to the stability of solid lipid nanoparticle. The mean values of these parameters were compared with that obtained at time zero. Particle size and zeta potential were measured at different intervals of 1, 5, 10, 15 and 30th day and results are shown in Table.3. There was no significant change in the mean particle diameter at the storage temperature after one month of SLNs. It was observed that the total increase in size was less i.e., 3.5%. Though there is a small increase in particle size after one month storage, Zeta potential values were almost constant indicating the stability of the formulation.

3.5 Fourier Transform Infrared Spectroscopic Analysis
Infrared spectroscopy has been widely used to investigate drug lipid interactions in solid lipid nanoparticle systems. In order to evaluate any possible chemical interactions between the drug and carriers, FTIR spectra of EFV, GMS, and SLNs formulations were examined as shown in Fig.2. The FTIR spectrum of EFVs showed the characteristic peaks of alkyne at 2250 cm⁻¹, C-F stretch at 1400 cm⁻¹, N-H stretch at 3320 cm⁻¹, C-H stretch in the range of 2850–3000 cm⁻¹ and carbonyl functional group at 1750 cm⁻¹. The
The solubility of the EFV was not changed, which indicate that there were no chemical interactions after formation of SLNs.

### 3.6 Differential Scanning Calorimetry

DSC was a tool to investigate the melting and crystalline behavior. DSC curves of efavirenz, glycerylmonostearate and lyophilized SLNs formulation are shown in the Fig 3a, 3b, and 3c. The thermogram of efavirenz shows melting peak at 138.7°C and that of glycerol monostearate 65.9°C. The thermogram of lyophilized SLNs formulation did not show any peak for the efavirenz. This shows that efavirenz is not in crystalline state but it is in amorphous state. There was homogenous dispersion of drug in the lipid. It is observed that the drug is dispersed in amorphous state in the lipid as seen from DSC curves.

### 3.7 Powder X-ray Diffraction (PXRD)

P-XRD patterns of efavirenz have given sharp multiple peaks at 2θ - scattered angles, indicating crystalline nature of drug as shown in Fig 4a. The crystallinity of pure GMS is not clearly distinguishable, indicating the presence of a new solid phase.

### 3.8 Scanning Electron Microscopy (SEM)

The scanning electron microphotographs of EFV, GMS, and lyophilized SLNs are presented in Fig.5. In the microphotograph of EFV, the drug was appeared to be in the form of distinct regularly sized crystals. Generally, all freeze-dried products appear to have less crystalline structure with a uniform appearance. It was evident from the microphotographs of lyophilized SLNs that the typical EFV crystals, which were mixed with the GMS particles or coated on to their surface, lost their crystallinity and were not clearly distinguishable, indicating the presence of a new solid phase.

### 3.9 In vitro drug release

In vitro drug release study of the EFV loaded SLNs showed the sustained release behavior in 7.4 pH phosphate buffer. Optimized formulation batches have shown the burst release with the 35.76% of the drug release within first two hours followed by the sustained release from the EFV loaded SLNs. The presence of the free EFV in the external phase and on the surface of the nanoparticles may be the reason for the burst release. The solubility of the EFV in aqueous phase could be the reason for the burst release. The sustained release behavior in 7.4 pH phosphate buffer is shown in the P-XRD patterns (Fig.4b). The crystalline peaks of efavirenz were absent in the lyophilized SLNs samples Fig.4c, indicating that the drug was not in crystalline form. Intensity of pure lipid peaks was also decreased in the lyophilized SLNs samples. This reduced intensity indicates the decreased crystallinity of lipid in the lyophilized SLNs formulations.

### Table 1 - Experimental conditions for the preparation of SLNs loaded with Efavirenz.

<table>
<thead>
<tr>
<th>SLNs formulation</th>
<th>Efavirenz (mg)</th>
<th>GMS (mg)</th>
<th>Concentration of Tween 80 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5</td>
<td>100</td>
<td>0.5</td>
</tr>
<tr>
<td>F2</td>
<td>5</td>
<td>200</td>
<td>0.75</td>
</tr>
<tr>
<td>F3</td>
<td>5</td>
<td>150</td>
<td>1.0</td>
</tr>
<tr>
<td>F4</td>
<td>5</td>
<td>250</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Table 2 - Particle size, zeta potential and Entrapment efficiency of SLNs formulation loaded with Efavirenz (Mean ± S.D, n=3).

<table>
<thead>
<tr>
<th>SLNs formulation</th>
<th>Particle size (nm)</th>
<th>Zeta potential (mv)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>89.00 ± 1.8</td>
<td>-23.1 ± 1.2</td>
<td>86 ± 0.11</td>
</tr>
<tr>
<td>F2</td>
<td>92.55 ± 0.1</td>
<td>-25.4 ± 0.2</td>
<td>80 ± 2.3</td>
</tr>
<tr>
<td>F3</td>
<td>85.55 ± 0.8</td>
<td>-24.4 ± 0.4</td>
<td>92 ± 9.72</td>
</tr>
<tr>
<td>F4</td>
<td>98.02 ± 0.2</td>
<td>-24.2 ± 1.0</td>
<td>66 ± 0.22</td>
</tr>
</tbody>
</table>

### Table 3 - Effect of storage time at (25°C) on size of the optimized SLNs formulations (Mean ± SD, n=3).

<table>
<thead>
<tr>
<th>SLNs formulation</th>
<th>Particle size (nm)</th>
<th>Zeta potential (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th day</td>
<td>10th day</td>
</tr>
<tr>
<td></td>
<td>0th day</td>
<td>10th day</td>
</tr>
<tr>
<td>Blank SLNs</td>
<td>89.80 ± 1.7</td>
<td>91.27 ± 0.5</td>
</tr>
<tr>
<td>Efavirenz SLNs</td>
<td>85.77 ± 1.15</td>
<td>86.00 ± 2.4</td>
</tr>
</tbody>
</table>

Fig.2 FT-IR Spectra of EFV, GMS, Efavirenz lyophilized SLNs.

Spectra of GMS showed the characteristic peaks of -OH group at 3384 cm⁻¹, C-H stretch at 2950-2910 cm⁻¹ and ester carbonyl functional group at 1733 cm⁻¹. After formation of SLNs, the major peaks of EFV and GMS were not changed, which indicate that there were no chemical interactions after formation of SLNs.

Fig.3 DSC thermograms of (a) EFV (b) GMS (c) physical mixture of EFV loaded SLNs.
Fig. 4. Powder XRD of (a) EFV (b) EFV lyophilized SLNs (C) GMS

The increase in lipid concentration had significant effect on the EFV release which prolonged the release of the EFV from SLNs. It may be due to the equal distribution of drug within the lipid matrix and good entrapment efficiency. The surfactant (Tween 80) concentration was optimized at 1% based on the particle size and entrapment efficiency. The drug from the lipid matrices after initial burst release. SLNs were thus prepared for systematic drug delivery with the intention of obtaining better therapeutic efficiency by sustained drug release, thus by improving bioavailability, decreased dosing and fewer side effects. It may be concluded that the EFV-SLNs showed good controlled release and optimum residence time, thus seems to be a potential candidate for the development of SLNs for effective therapeutic use.

ACKNOWLEDGEMENTS
The authors wish to thank Madhusudhanchari, National Institute of Nutrition, Hyderabad, India and Dr. JayatheerthaRao, IICT, Hyderabad, India for providing SEM and HPLC facilities respectively.

REFERENCES
3. VV. Kumar, D. Chandrasekar, S. Ramakrishna, V.Kishan, YM.Rao, PV.Diwan, Development and evaluation of nitrendipine loaded solid lipid nanoparticles: influence of wax and glyceride lipids on...
4. V. Venkateswarlu, K. Manjunath, Preparation, charac-
terization and in vitro release kinetics of clozapine 
5. K. Manjunath, K.Venkateswarlu, Pharmacokinetics, 
tissue distribution and bioavailability of nitrendipine 
solid lipid nanoparticles after intravenous and intraduodenal administration. J. Drug Target.14 (2006) 
632–645.
evaluations of norfloxacin-loaded solid lipid nanoparticles for oral delivery. Drug Deliv. 18(2011) 
441–450.
7. H. Yuan, J. Chen, YZ. Du, Hu FQ, S.Zeng, HL. Zhao, Studies on oral absorption of stearic acid SLN by 
9. A. Sosnik, DA. Chiappetta, AM.Carcaboso, Drug 
delivery systems in HIV pharmacotherapy: what has 
been done and the challenges standing ahead.J.Control. 
10. B. Gazzard, British HIV association (BHIVA) 
496.
11. U. Wintergerst, F. Hoffmann, A.Jansson, G.Notheis, K. 
Huss, M. Kurowski,et al : Antiviral efficacy, tolerability and pharmacokinetics of efavirenz in an 
12. V. Teeranachaideekul, P. Boonme, E.B.Souto, RH. 
muller, V.B. Junyaprasert, Influence of oil content on 
physicochemical properties and skin distribution of 
134–141.
13. S.D. Mandawgade, V.B.Patrawale, Development of 
SLNs from natural lipids: Application to topical 
14. B.Sjostrom, B. Bergenstein, Preparation of submicron 
drug particles in lecithinstabilizedO/W emulsions. 1. 
Model studies of the precipitation cholesteryl acetate. 
15. F.Q Hu, Y. Zhang, Y.Z. Du, H. Yuan, Nimodipine 
loaded lipid nanospheres prepared by solvent diffusion 
method in a drug saturated aqueous system. Int. J. 
16. C. Puglia, P.Blasi, L.Rizza, A.Schoubben, F.Bonina, 
C. Rossi, M. Ricci,Lipidnanoparticles for prolonged topical delivery: An in vitro and in vivo investigation. 
17. M. Garcia-Fuentes, D. Torres, M.J Alonso, Design of 
18. M.A. Schubert, C.C. Muller-Goymann,Solvent 
injection as a new approach for manufacturing lipid nanoparticles—Evaluation of the method and process 
131.
19. B. Heurtault, P.Saulnier, B.Pech, J.E. Proust, J.P. 
Benoit, A novel phase inversionbased process for the 
preparation of lipid nanocarriers. Pharm. Res.19 ( 
20. R. Cortesi, E.Esposito,G. Luca, C.Nastruzzi, 
Production of liposomes as carriers for bioactive 
21. M. Garcia-Fuentes, D. Torres, M. Martin-Pastor, M.J. 
Alonso,Application of NMRspectroscopy to the 
characterization of PEG-stabilized lipid nanoparticles. 
22. A.E. Mendoza, M. Rayo, F.Mollinedo, M.J. Blanco-
Prieto, Lipid nanoparticles for alkyl 
23. Ede. Clercq, The history of antiretrovirals: key 
Huss, M. Kurowski,et al : Antiviral efficacy, tolerability and pharmacokinetics of efavirenz in an 
25. N.A. Kasim, M. Whitehouse, C.Ramachandran, M. 
Bermejo, H.Lennernaes, AS.Hussain, ‘et al.’ Molecular 
properties of WHO essential drugs and provisional 
biopharmaceutical classification. Mol. Pharm.1 
26. C. Csajka, C.Marzolini, K. Fattinger, LA.Décosterd, 
A.Telenti, J. Biollaz, ‘et al ‘ Population 
pharmacokinetics and effects of efavirenz in patients 
with human immunodeficiency virus infection. Clin. 
27. M. Markowitz, BY. Nguyen, E.Gotuzzo, F.Mendo, 
W.Ratanasuwun, C. Kovacs, et al : Antiretroviral 
activity, pharmacokinetics, and tolerability of MK-
0518, a novel inhibitor of HIV-1 integrase, dosed as 
monotherapy for 10 days in treatment-naive HIV-1 
infected individuals. J Acquir Immune DeficSyndr.52 
Formulation and food effects on the oral absorption of 
a poorly water soluble, highly permeable antiretroviral 

Source of support: Nil; Conflict of interest: None declared