Formulation and Evaluation of Solid Lipid Nanoparticles of a Water Soluble Drug: Zidovudine

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The present investigation was undertaken to develop solid lipid nanoparticles (SLN) of a hydrophilic drug Zidovudine (an anti-human immunodeficiency viral agent) and improve the entrapment efficiency of the drug in SLN. The SLN were prepared with stearic acid by process of w/o/w double-emulsion solvent-evaporation method using 3^2 factorial design. Different triglycerides alone and in different combinations, with/without stearic acid were used to prepare SLN using similar procedure. Two operating variables, polyvinyl alcohol concentration and amount of lipid were found to have significant effect on the particle size and entrapment efficiency (EE) of the SLN. The maximum EE was found to be *ca.* 27% with particle size of 621 nm which was significantly higher than that reported earlier. The optimized batch was also analyzed for its morphological, physiochemical and drug release properties. EE of batches with triglycerides were significantly less than that achieved with stearic acid alone. This work indicates the possible advantage of fatty acids over triglycerides in the entrapment of hydrophilic drugs in SLN.

Key words solid lipid nanoparticle; zidovudine; entrapment efficiency; particle size; polyvinyl alcohol; lipid

The drug delivery system using solid lipid nanoparticles (SLN) came into being about two decades ago and since then lot of work has been done in this field.[1—5] SLN for oral drug administration are specifically used to target the uptake of the drug by lymphatic system which prevents its first pass metabolism. Lymphatic uptake of drugs follow two routes which include transcellular transport through the enterocyte and phagocytosis of the drugs by Mast cells of payer's patches lining the intestinal mucosa.[6—8]

The production of this nano particulate system is based on the principle of solidification of lipid nano emulsion.[9] The different technologies available for the fabrication of SLN are high shear homogenization, ultrasound, high pressure homogenization (cold homogenization and hot homogenization), solvent emulsification/evaporation and microemulsion method.[10] The major excipients used in the development of the SLN are fatty acids, mono, di and triglycerides, phospholipids *etc.*, which are part of the physiological composition and thus are biocompatible to the body.[11—13]

SLN have been shown to have superior advantages over polymeric nanoparticles, fat emulsion and liposomes.[14] SLN can be produced on large scale and are also biocompatible to the body as compared to polymers, the monomeric unit of which are cytotoxic to the body.[15,16] SLN exhibit sustained release effect due to the immobility of drug within lipid as compared to the emulsion formulations[17] and also exhibit better physical and chemical stability of drug compared to liposome.[18] This delivery system has been extensively used as carriers for proteins, protein drugs, vaccines and lipophilic water insoluble drugs.[19]

SLN are potential delivery system for lipophilic drugs where aqueous solubility of the drug is the limiting factor for its absorption.[20—22] Moreover, the incorporation of such drugs within SLN is easier due to their affinity for the lipid. On the other hand, entrapment of hydrophilic drug inside the hydrophobic matrix of SLN is a real challenge as the drug has maximum tendency to partition in the water during the fabrication process. Although a few hydrophilic molecules have been incorporated into SLN like thymocratin,[23] insulin,[24] diminazene[25] and thymopentin,[26] lot more scope still remains for the entrapment of hydrophilic drugs into lipid nanoparticles.

Zidovudine (AZT), a hydrophilic drug has been used in the present investigation. It belongs to the class of nucleoside reverse transcriptase inhibitor and is used in the treatment of AIDS. This drug has various disadvantages like short biological half life due to extensive first pass metabolism and dose related bone marrow toxicity. AZT is a potential candidate for delivery *via* lipid based nanoparticulate system as this would help to improve lymphatic uptake, avoid hepatic first pass metabolism and thus reduce the dose requirement and decrease the side effects.[27] Furthermore, sustained drug release would reduce dosing frequency and at the same time maintain plasma drug level within the therapeutic window to avoid sub therapeutic or toxic concentration. AZT is a Biopharmaceutical Classification System (BCS) class III drug (high solubility and low permeability) having water solubility of 20.1 mg/ml (World Health Organization Document QAS/03.077/final) and log P of 0.05. In an earlier study, a tedious approach of synthesizing a prodrug of AZT (AZT palmitate) was carried to improve the lipophilicity of the molecule. The prodrug was then incorporated into triglyceride (trilaurin) based SLN and an entrapment efficiency of $37 \pm 6\%$ was achieved.[28]

Therefore, the objective of the present study was to adopt a simple approach for the fabrication of stearic acid (SA) (fatty acid) based SLN of the basic molecule AZT, using 3^2 factorial design. Two formulation variables polyvinyl alcohol (PVA) concentration (as stabilizer) and amount of lipid were studied to optimize the formulation for maximum entrapment efficiency (EE). In addition to EE, the particle size and zeta potential were also considered as response using MINITAB software. Further, the optimized operating parameters were employed for the fabrication of AZT SLN using SA alone and in combination with various triglycerides (TGs) (tripalmitin (TP), tristearin (TS) trilaurin (TL) and trimyristin

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(TM)) alone and their combination with SA.

Experimental

Materials AZT was a kind gift from Ranbaxy (Gurgaon, India). SA was purchased from CDH (Delhi, India). TP and TS were purchased from SRL (Mumbai, India). TL and TM were purchased from Sigma (Germany). Tween 80 (T80) and polyvinyl alcohol (PVA) were purchased from Himedia (Mumbai, India). All other chemicals were of analytical grade and used as received.

Method. Preparation of Aqueous SLN Suspension The experiments were based on a 3^2 factorial design consisting of two factors (PVA concentration and lipid amount) at three levels (low (−), medium (0) and high (+)) as shown in Tables 1 and 2.

The SLN were fabricated using w/o/w double emulsion solvent evaporation method. SA was dissolved in 5 ml solvent mixture consisting of DCM : MeOH (2 : 3). Twenty milligrams of AZT was dissolved in 1 ml aqueous solution containing 0.125% v/v T80. Both the solutions were mixed and sonicated using ultasonicator (Hielscher, Ultrasound Technology, Germany) for 2 min at 60 °C amplitude and 0.5 frequency. The primary emulsion obtained was then poured into 100 ml PVA solution at room temperature (25 °C) and stirred for 2 h at 1500 rpm. Finally, the suspension was filtered and used as such for further analysis.[29,30]

The optimum parameters which resulted in maximum EE and minimum particle size of the nanoparticles were employed for the fabrication of TG based SLN using similar method. The ratios of SA to TG used were as follows; 1 : 3, 1 : 1 and 3 : 1.

Solubility of AZT in Different Medium Initially standard curves of AZT were prepared in distilled water, methanol, 1% PVA solution and phosphate buffer pH 6.8 in the range of 5—35 μg/ml. Linearity of standard curves were found to be 0.999, 0.998, 0.999 and 0.9999, respectively. Saturation solubility of AZT was determined in the above media except methanol, by adding excess amount of AZT to 10 ml of each medium in a tightly corked conical flask. The solutions were agitated separately at 25 °C and 37 °C for 48 h on a rotary shaker. The samples were filtered through 0.45μ filter and diluted appropriately to analyze the drug content in the linear UV absorption range using a UV spectrophotometer (U-1800 spectrophotometer Hitachi, Japan) at 265 nm (λ_{max}). This study was conducted in triplicate and the average of the three values was recorded.

Particle Size Analysis and Polydispersity Index (PDI) Particle size distribution (mean diameter and polydispersity index) was determined by photon correlation spectroscopy (PCS) using a Malvern instrument Serial Number: MAL500962 (Malvern Instruments, U.K.). Mean particle size and polydispersity index were calculated for each sample. Distilled water was used as dispersant and the system was maintained at 25 °C and at a measurement position of 4.65 mm.

Zeta Potential Measurement The zeta potential of nanoparticles was determined using a Malvern Zetasizer instrument Serial Number: MAL500962 (Malvern Instruments, U.K.). The system was maintained at 25 °C.

Total Drug Content (TDC) Total amount of drug in formulation was determined by dissolving 1 ml of suspension in 10 ml of methanol. The amount of AZT in each sample was determined spectrophotometrically by measuring the absorbance of the clear supernatant at λ_{max} of 265 nm. Each experiment was performed in triplicate. Placebo formulation treated similar to that of the sample was used as blank for UV absorbance. The total drug content was calculated by using the equation given below.

$$\text{TDC} = \text{concentration} \times \text{dilution factor} \times \text{volume of formulation}$$

Entrapment Efficiency The EE was determined by analyzing the free drug content in the supernatant obtained after centrifuging the SLN suspension in high speed centrifuge at 16000 rpm for 30 min at 0 °C using Remi cooling centrifuge (Mumbai, India). The EE was calculated as follows:

$$\text{EE} = \left\{ \frac{\text{total drug (assay)} - \text{free drug}}{\text{total drug}} \right\} \times 100$$

Differential Scanning Calorimetry (DSC) The DSC thermograms of AZT, SA, PVA and the freeze-dried SA based AZT nanoparticles were made in nitrogen environment by a Differential Scanning Calorimeter (TA instrument, model Q1000 V9.4 Build 287, U.S.A.). The heating rate employed was 10 °C per minute.

Scanning Electron Microscopy (SEM) SEM analysis of the optimized formulation was carried out for morphological studies. The formulation was poured into circular aluminum plate and dried in vacuum oven to form a dry film which was then observed under the scanning electron microscope (FEI, Quantum 200E Instrument).

***In-Vitro* Release Studies** *In vitro* release studies were performed in pH 6.8 phosphate buffer by dialysis bag method using dialysis membrane having molecular weight of 12000—14000 Da. Five milliliters of suspension was placed inside the dialysis bag, tied at both ends and dipped in the dissolution medium. Stirring of the medium was maintained at 100 rpm using magnetic bead and the temperature at 37±0.2 °C. Two milliliters aliquot were withdrawn at pre-set time intervals (0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24 h) and replaced by an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed spectrophotometrically at 265 nm. The concentration of AZT in test samples was corrected and calculated by using the regression equation of the calibration curve.

Stability Study Batch C4 was lyophilized at −30 °C under vacuum (2.5 mbar) for 24 h in a freeze dryer (Decible Technologies, India). The dried powder was then packed in glass vials sealed with rubber caps and were kept under ambient temperature and moisture condition (25 °C and 60% RH) for a period of 1, 2 and 3 months. Every month, the dried powder of the stability samples were re-dispersed in distilled water and the stability of the SLN was evaluated on the basis of measurement of particle size and the EE of the suspension.

Table 1. Factorial Design

Factors	Levels		
	Low (−)	Medium (0)	High (+)
PVA conc.	0.25%	0.5%	1%
Amount of lipid	200	300	400

Table 2. Factorial Design Operating Variable and Their Response

Batches	Formulation parameters		Evaluating parameters		
	SA	PVA concentration (w/v %)	Entrapment efficiency (%)	Particle size (nm)	Zeta potential (mV)
B2	−	−	15.65±1.49	765±41	−2.9
B3	−	0	17.31±4.38	654±24	−2.4
B4	−	+	20.54±3.11	620±24	−1.7
C2	0	−	21.50±1.69	882±10	−3.1
C3	0	0	24.03±0.98	664±03	−2.6
C4	0	+	27.34±0.42	621±13	−1.8
D2	+	−	19.63±1.71	905±13	−3.5
D3	+	0	21.94±1.41	703±19	−3.8
D4	+	+	25.87±0.85	689±10	−2.8

Entrapment efficiency and particle size values are expressed as mean±S.D. (n=3).

Results and Discussion

Solubility of AZT in Different Medium The solubility of AZT in different medium at 25 °C and 37 °C is presented in Table 3. The data suggest that the solubility of the drug significantly changes with temperature. Moreover, the solubility of the drug is decreased in PVA solution as compared to pure aqueous system at 25 °C and 37 °C, respectively. The

Table 3. Solubility of AZT in Different Medium (n=3)

Medium	Solubility (mg/ml) at 25 °C	Solubility (mg/ml) at 37 °C
Distilled water	19.5±0.2	30.6±0.3
PVA solution (1%)	19.0±0.1	28.5±0.4
6.8 pH phosphate buffer	20.3±0.3	24.4±0.4

Solubility values are expressed as mean±S.D.

decrease in solubility may be due to competitive hydration between AZT and PVA (water soluble polymer) molecules for the aqueous phase. Therefore, it is evident that PVA while imparting emulsion stability will not increase the solubility of drug in external phase during formulation which is one of the major reasons for reduced loading efficiency in the fabrication of SLN. Based on the solubility data, the entire fabrication process of SLN using PVA as stabilizer was carried out at room temperature of 25 °C.

Particle Size, Entrapment Efficiency and Zeta Potential The aim of the factorial design was to determine the levels of the two factors which yield SLN with minimum particle size and maximum EE. The particle size of the fabricated batches was in the range of 620 to 882 nm, EE in the range of 13.87 to 27.34% and ZP in the range of −1.7 to −3.8 mV, as presented in Table 2. The composition of batch C4 with 300 mg SA and 1% PVA solution was found to be optimum as this batch showed lowest particle size of 621 nm (Fig. 1) and highest EE of *ca.* 27% with zeta potential (ZP) of −1.8 mV and polydispersity index of 0.241. The EE achieved in batch C4 was nearly 27 times higher than reported in an earlier study.[30] In an earlier study, it has been observed that the number of particles of mean diameter around 0.3 and 1.0 μm are preferably absorbed by payer's patches (which drains its content into the lymphatic system) in comparison to particles of 3.0 μm.[31] Since, the maximum particle size obtained in the present study is within the size range (600—700 μm) required for efficient lymphatic uptake, therefore, it may be expected that the size is acceptable and would not be a limiting factor in the lymphatic uptake of the prepared solid lipid nanoparticles.

SLN of TL, TP, TS and TM and their combinations with or without SA were fabricated and evaluated for their EE. The EE of all batches except TP and SA combination were found to range between 0.1 to 1% (data not shown). The particle size analysis of these batches was not carried out as there was no improvement in the EE of these batches. The maximum EE of 6.9% with particle size of 634 nm was obtained in batch J1 prepared using TP and SA combination in 1 : 3 ratio as shown in Table 4. The probable reasons for such low entrapment efficiency of the formulations can be attributed to the highly ordered crystal packing of pure triglycerides and the water soluble nature of the drug which gets easily expelled to the external phase during the process of re-crystallization.[32]

Effect of Method of Preparation on Particle Size The emulsion process used in the present study resulted in particle size of more than 500 nm (621—882 nm). This may probably be because the whole process was operated at room temperature which is quite lower than the melting point of SA (*ca.* 60 °C). Unlike hot microemulsion[33,34] wherein the emulsion droplets remains in the liquid state for a long period of time, the emulsion droplets by this method solidifies quicker as it comes in contact with the insoluble aqueous phase. As the solidified particles are difficult to break than the liquid droplets, the present study resulted in the generation of relatively larger sized nanoparticles.

Effect of Operating Variables on Particle Size The effect of the operating variables on the particles size of the SLN is presented in Fig. 3. The different concentrations of PVA used in the external medium as an emulsion stabilizer showed significant effect on the particle size of SLN. The particle size was found to decrease with increase in the PVA concentration at a constant amount of lipid. The effect is mainly attributed to the increasing viscosity of the PVA solution. During the process of emulsification, the droplet size reduces under the influence of high shear. At the same time they even have a tendency to aggregate in order to reduce their surface energy. However, the presence of surfactant molecules stabilizes the emulsion by forming a thick protective layer around the droplets which prevent the coalescence of the droplets.

The particle size was found to be significantly affected with increase in lipid amount at lower PVA concentration. This may be explained on the basis of the inability of the PVA solution to stabilize the emulsion at very low concentrations (<0.5%). Further higher concentrations of PVA are suf-

Table 4. Solid Lipid Nanoparticles Prepared with TP and SA Combination

Batches	TP : SA	PVA concentration	Entrapment efficiency (%)	Particle size (nm)
J1	1 : 3	+	6.93±1.2	634±14
J2	1 : 1	+	4.1±0.12	679±12
J3	3 : 1	+	1.6±0.04	654±9

Entrapment efficiency and particle size values are expressed as mean±S.D. (n=3).

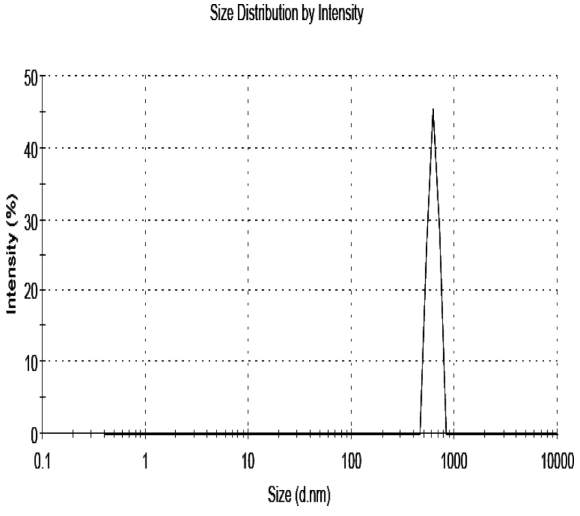

Fig. 1. Particle Size of Batch C4

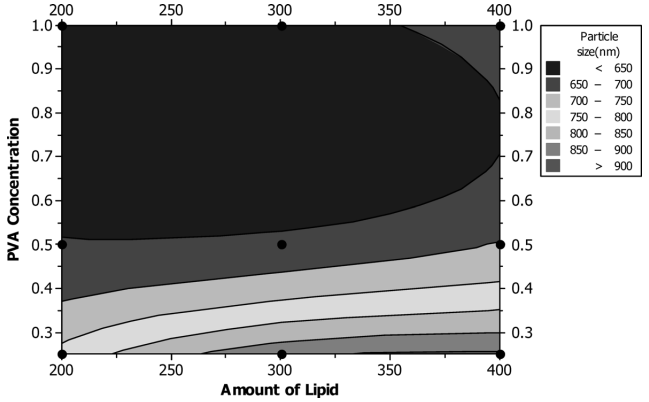

Fig. 2. Contour Plot of Particle Size *vs.* PVA Concentration and Amount of Lipid

ficient enough to stabilize the emulsion even with lipid load of 300 mg with consistent particle size. Figure 2 shows that a concentration of PVA solution in the range of 0.7—0.8% would have sufficient viscosity in preventing the coalescence between particles/droplets which occurs as a result of increase in number of particles per unit volume (lipid load) of the solution.

The above discussion clearly indicates that the viscosity imparted by the PVA solution plays a major role in dictating the particle size of the nanoparticles and, therefore, be applicable for a wide range of lipid amount.

Effect of Operating Variables on Entrapment Efficiency The effect of the operating variables on the entrapment efficiency of the SLN is presented in Fig. 3. Unlike particle size, the EE was found to be significantly affected by both, PVA concentration and the lipid amount at every level studied. The EE was increased with increase in PVA concentration. PVA is a polymer which imparts prominent effects on the SLN suspension formulation. It efficiently coats the particles and imparts viscosity to the external phase.[35,36] Interestingly, PVA was found to decrease the solubility of AZT in water (Table 3). The above mentioned factors together may be responsible for the stabilization of the nanosuspension and prevention of leaching of AZT in external phase which helps to improve the EE. As expected, the EE increased with increase in lipid amount which may be due to more availability of lipid to encapsulate the drug.

As indicated by the Figs. 2 and 3, a concentration of PVA solution above 0.8% and lipid amount within 300—370 mg are ideal conditions to achieve the highest entrapment efficiency with the minimum particle size as was exhibited by batch C4.

Effect of Operating Variables on Zeta Potential ZP is the electric charge on particle surface which creates an electrical barrier, and acts as a 'repulsive factor' in the process of emulsion stabilization.[37] High surface energy plays a major role in the stability of the formulation as like charges at the interface resists coalescence of particles.[38] The ZP of SLNs was found to possess negative surface charges due to the negatively charged SA.[39,40] The change in the ZP with the operating variables was not found to vary significantly (−1.7 to −3.8 mV) with the change in either of the operating variables as shown in Fig. 4. Surprisingly, batch C4 was found to have considerable stability despite its very low ZP of −1.8 mV. This indicates some factor other than ZP is responsible for the stability of the formulation which is the presence of polymer dissolved in the external phase. The polymeric molecules coat and shield the charge on the particle surface due to which the nanoparticles exhibit very low ZP value and still do not coalesce during storage.[41—43]

Differential Scanning Calorimetry The DSC graphs of the individual excipients and batch C4 formulation are presented in Fig. 5. The DSC curve of the pure drug AZT is indicative of its crystalline anhydrous state, exhibiting a sharp endothermic peak at 125.09 °C (ΔH=126.33 J g^{-1}), corresponding to its melting point in the range of 120—124 °C.[44] The melting point of SA was obtained at 66.54 °C (ΔH=67.83 J g^{-1}) and that of PVA was obtained at 192.66 °C (ΔH=193 J g^{-1}). The melting point of SA in formulation was found to be at 59.70 °C (ΔH=61.16 J g^{-1}). The DSC curve of SA shifted downward in C4 batch and this might be due to decrease in crystalline nature of SA as a result of presence of other excipient (PVA) including drug which act as impurities and therefore, disturb the arranged crystalline lattice structure of the fatty acid.

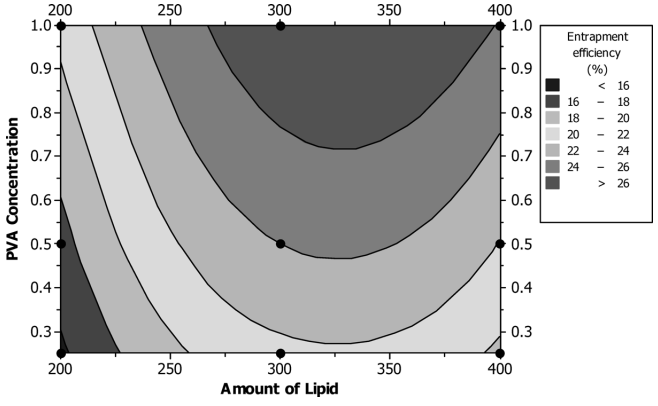

Fig. 3. Contour Plot of Entrapment Efficiency *vs.* PVA Concentration and Amount of Lipid

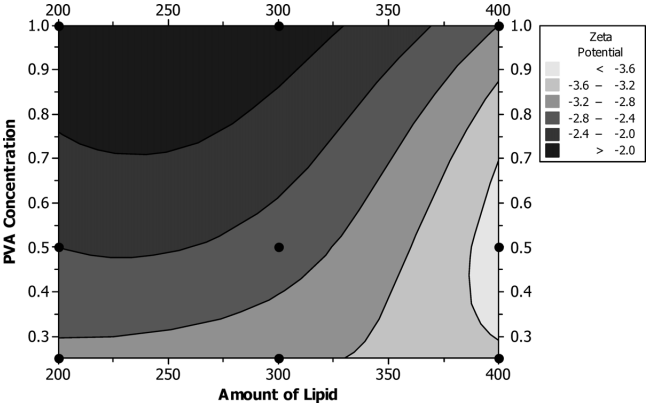

Fig. 4. Contour Plot of Zeta Potential *vs.* PVA Concentration and Amount of Lipid

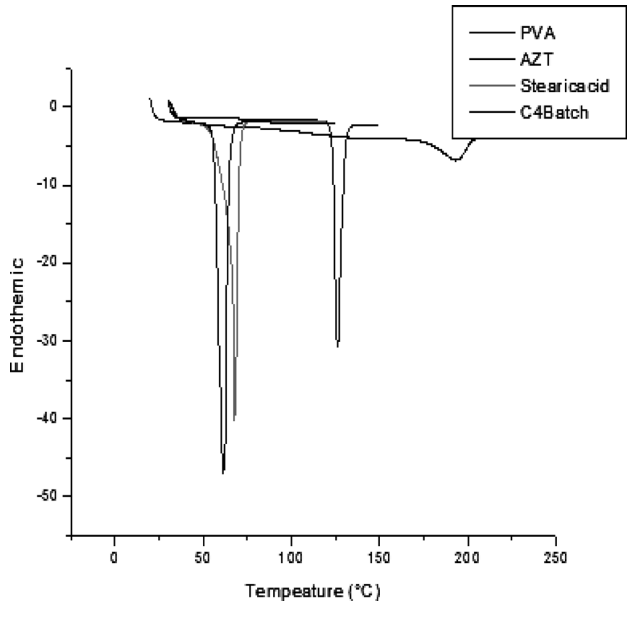

Fig. 5. DSC of AZT, PVA, SA and Batch C4

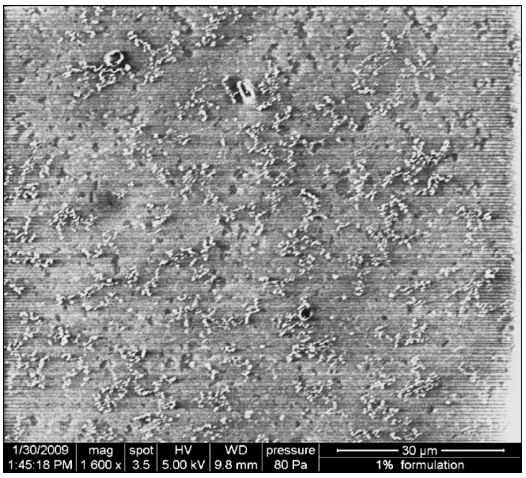

Fig. 6. SEM of Batch C4 (1600× Magnification)

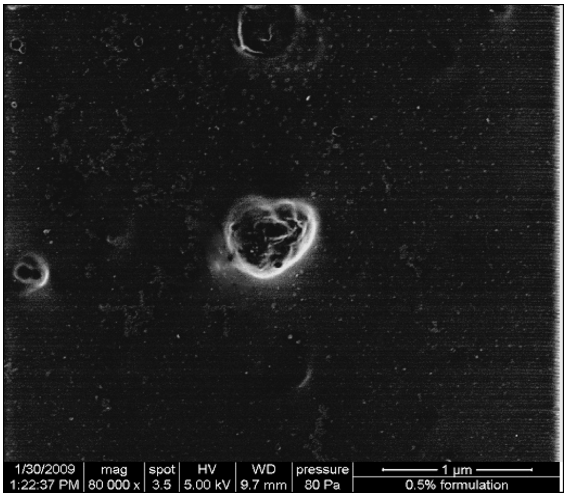

Fig. 7. SEM of Batch C4 (80000× Magnification)

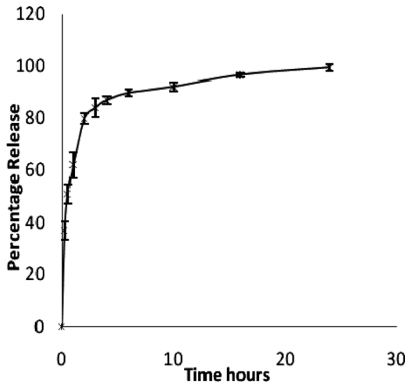

Fig. 8. Drug Release Profile of Batch C4

Scanning Electron Microscopy The shape and surface morphology of SA based nanoparticles (Batch C4) was studied by SEM. The microphotographs reveal that the particles are uniform in size and roughly spherical in shape (Figs. 6, 7).

***In Vitro* Release Studies** The drug release study of batch C4 was carried out as it showed the maximum EE. The dissolution profile showed biphasic behavior consisting of initial burst release followed by a slow release phase (Fig. 8). The initial burst release can be attributed to the presence of free drug in the external phase and drug adsorbed on the surface of particles while the slow release was due to drug encapsulated within lipid matrix. The release pattern indicates that using this delivery system, a loading dose of around 80% drug could be made available within 2—3 h of its administration while the remaining would support the maintenance dose for a considerable duration and thus may prevent excessive fluctuations in the drug plasma level. The release kinetics followed higuchi model (r^2=0.997) and almost 98% of the drug was released in a period of 24 h. The release of drug from SLN may be majorly due to the diffusion of drug from the pores on the particle surface. Being slightly soluble in water, SA has less chance of drug release by the process of surface erosion in the buffer.[45)]

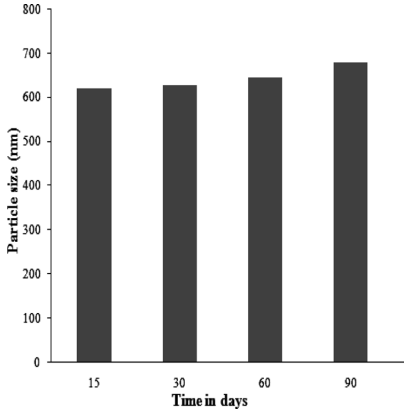

Fig. 9. Particle Size of Batch C4 during Stability Study

Stability Studies The effect on particle size during storage is shown in Fig. 9. It was found that the formulation was stable for more than 3 months. The particle size was increased slightly from 621 to 670 nm during stability studies. The difference in particle size was insignificant which indicates that the stability of the system for a period of 3 months under accelerated conditions. Unlike the fact that low ZP is indicative of unstable colloidal formulation, the SLN formulation of the present study with low ZP was found to be stable for 3 months which indicates that the ZP had no significant role in the stability of the fabricated batches. Here, the contributing factor for the stability of the system may be due to the protective nature and viscosity imparted by the polymer–PVA. The EE of the batch after 3 months (26.1%) indicated that the drug was retained within the nanoparticles throughout the stability period.

Conclusion

The major outcome of this work was the successful entrapment of a hydrophilic drug within a lipid core. The SA based SLN of AZT (batch C4) was successfully prepared by using w/o/w double emulsion solvent evaporation method with maximum EE of 27.37%, particle size of 621 nm and stability for 3 months. A comparative study of the fabricated SLN using fatty acid (SA) and TG and their combinations clearly shows the superior capacity of the fatty acids over TGs to encapsulate the model hydrophilic drug. This may be associated with the crystalline nature of the lipids and needs further

investigation. Despite of very low ZP the formulation was found to be stable which was due to the use of a polymer as a stabilizing agent. It was also found that PVA decreased the solubility of AZT which may be one of the reasons for attaining the highest entrapment efficiency reported so far for this drug.

Future Perspective The development of SLN for enhancement of therapeutic efficacy has been limited to water insoluble lipophilic drugs. However, similar benefits could also be extended to water soluble drugs which require targeted drug delivery to the lymphatic system for local action and avoidance of first pass metabolism. Zidovudine, used in the present study is an example of such a drug. The major limitation in the fabrication of SLN of water soluble drugs is its poor entrapment efficiency which has resisted research in the field. Interestingly, our attempt to fabricate SLN of a water soluble drug using the simple conventional manufacturing technique revealed that the use of fatty acids was significantly superior to the triglycerides to achieve considerable entrapment efficiency. Additionally, the use of polyvinyl alcohol as a stabilizer was effective in the SLN development by preventing the increase in the saturation solubility of the drug in the external phase. Therefore, a combination of fatty acids as the drug carrier and polyvinyl alcohol as an emulsion stabilizer may be employed to improve the drug entrapment efficiency in SLN of water soluble drugs. Although the data presented in this work is applicable to Zidovudine we hope that the present investigation will assist pharmaceutical researchers to select suitable lipid carriers and stabilizers for formulation development of SLN of other water soluble drugs as well.

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