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Investigations of the Effect of the Lipid Matrix on Drug Entrapment, In Vitro Release, and Physical Stability of Olanzapine-Loaded Solid Lipid Nanoparticles

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K. Vivek,[1] Harivardhan Reddy,[1] and Ramachandra S.R. Murthy[1]

[1]Drug Delivery Research Laboratory, Center of Relevance and Excellence in NDDS, Pharmacy Department, GH Patel Building, Donor's Plaza, Fatehgunj, M.S. University, Baroda – 390002, Gujarat, India

ABSTRACT

The purpose of this research was to study the effect of the lipid matrix on the entrapment of olanzapine (OL). OL-loaded solid lipid nanoparticles (SLNs) were prepared using lipids like glyceryl monostearate (GMS), Precirol ATO 5 (PRE), glyceryl tristearate (GTS), and Witepsol E85 (WE 85)—and poloxamer 407 and hydrogenated soya phosphatidylcholine as stabilizers—using a hot melt emulsification high-pressure homogenization technique, and then characterized by particle size analysis, zeta potential, differential scanning calorimetry (DSC), and powder X-ray diffraction (pXRD). Homogenization at 10 000 psi for 3 cycles resulted in the formation of SLNs with a mean particle size of ~190 nm for the 4 lipids investigated. The highest partition coefficient for OL between the melted lipid and pH 7.4 phosphate buffer (pH 7.4 PB) was obtained with GTS. The entrapment efficiency was in the following order: GTS SLNs > PRE SLNs > WE 85 SLNs > GMS SLNs. DSC and pXRD showed that much of the incorporated fraction of OL existed in the amorphous state after incorporation into SLNs. A sharp increase in the flocculation of the SLN dispersions was observed upon addition of 0.6 M aqueous sodium sulfate solution. Nanoparticle surface hydrophobicity was in the following order: GTS SLNs > PRE SLNs > WE 85 SLNs > GMS SLNs. A significant increase in size and zeta potential was observed for GTS SLN and WE 85 SLN dispersions stored at 40°C. Release of OL from the SLNs was sustained up to 48 hours in pH 7.4 PB and obeyed Higuchi's release kinetics.

KEYWORDS: Olanzapine, solid lipid nanoparticles, surface hydrophobicity, electrolyte-induced flocculation, in vitro release.

Corresponding Author: Ramachandra S.R. Murthy, Drug Delivery Research Laboratory, Center of Relevance and Excellence in NDDS, Pharmacy Department, GH Patel Building, Donor's Plaza, Fatehgunj, M.S. University, Baroda – 390002, Gujarat, India. Tel: +91-2434187; Fax: +91-265-2423898; E-mail: m_rsr@rediffmail.com

INTRODUCTION

Solid lipid nanoparticles (SLNs) have recently gained significant attention as potential alternate colloidal drug delivery systems for liposomes and lipid emulsions. The use of solid lipids is an attractive innovation that is advantageous because the solid matrix of the lipid provides more flexibility in controlling the drug release and protects the encapsulated ingredients from chemical degradation. Furthermore, in comparison to liposomes, SLNs have a slower degradation velocity in vivo because of their solid matrix.

SLNs are composed of physiological and biocompatible lipids and are free from the risk of acute or chronic toxicity. Lipids, including triglycerides[1,2] and hard fat waxes,[3,4] have been used for the formulation of SLNs. The choice of emulsifier and coemulsifier depends on the route of administration and is more critical in parenteral delivery.[5]

Various research teams[1-6] have studied the effect of lipid type on the final particle size of the SLN dispersions formed. Factors such as velocity of lipid crystallization, lipid hydrophilicity, and influence of self-emulsifying properties of the lipid on the shape of the lipid crystals (and hence the surface area) were found to affect the final size of the SLN dispersions.[6] To date, only a few attempts have been made to study the effect of lipid matrix on drug entrapment efficiency within and physical stability of the SLN dispersions.

Olanzapine (OL) is a psychotropic agent that belongs to the thienobenzodiazepine class and is indicated for acute and maintenance treatment of schizophrenia.[7]

In this study 4 chemically different lipids were selected: glyceryl monostearate (GMS), Precirol ATO 5 (PRE), glyceryl tristearate (GTS), and Witepsol E85 (WE 85). The rationale was to select a monoglyceride (GMS), a diglyceride (PRE), a triglyceride (GTS), and a lipid of low melting point (WE 85) and study the effect of lipid matrix type on the final SLN particle size, entrapment efficiency, in vitro release pattern, and physical stability on short-term storage. The nanoparticles were characterized by differential scanning calorimetry (DSC), powder X-ray diffraction (pXRD), and in vitro release profiling. Electrolyte-induced flocculation and rose bengal adsorption were performed to study the in vitro steric stability and surface hydrophobicity, respectively, of the nanoparticles.

The nanoparticles were subjected to short-term stability studies to determine the optimum stability conditions.

Materials and Methods

Materials

OL was a gift from Sun Pharmaceuticals (Baroda, India), and poloxamer 407 was a gift from BASF (Ludwigshafen, Germany). Hydrogenated soya phosphatidylcholine (HSPC) was purchased from Lipoid Gmbh (Ludwigshafen, Germany). GMS (monoester of 16-carbon fatty acid [stearic acid] and glycerol) was purchased from Loba Chemie (Mumbai, India). PRE (mono-, di-, and triglycerides of palmitostearic acid with the diester fraction predominating) was a gift from Colorcon India Limited (Mumbai, India). GTS (triglyceride derivative of 16-carbon fatty acid [stearic acid] and glycerol) and WE 85 (hard fat in pastille form, consisting of mono-, di-, and triglycerides of saturated fatty acids [10 to 18 carbon] of plant origin along with some amount of emulsifiers) were kindly provided by Sasol Chemicals (Hamburg, Germany). All other chemicals used were of analytical grade. Water was distilled and filtered before use through a 0.22-μm nylon filter.

Methods

Partitioning Nature of Olanzapine Between the Lipids and pH 7.4 Phosphate Buffer

Ten milligrams of OL was dispersed in a mixture of melted lipid (1 g) and 1 mL of hot pH 7.4 phosphate buffer (PB) and shaken for 30 minutes, incorporating a mechanical shaker using a hot water bath maintained 10°C above the melting point of the lipid under investigation (Table 1) as previously reported by Venkateswarlu and Manjunath.[8] The aqueous phase of the above mixture was separated from the lipid by centrifugation at 25 000 rpm for 20 minutes in a high-speed centrifuge. The clear supernatant obtained after centrifugation was suitably diluted (to a concentration of 25 μg/mL of OL) with 0.1 N HCl, and the OL content was determined in a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) at 258 nm against a solvent blank. The partition coefficient (PC) of OL in lipid/pH 7.4 PB was calculated using Equation 1:

$$PC = (C_{OLI} - C_{OLA})/C_{OLA} \quad (1)$$

where C_{OLI} = the initial amount of OL added (10 mg), and C_{OLA} = the concentration of OL in pH 7.4 PB.

Table 1. Differential Scanning Calorimetry Values for Bulk Lipids and OL-Loaded SLN Formulations*

Formulation	Enthalpy Jg^{-1}	Melting Endotherm of Lipid (°C)	
		Peak Onset	Peak Maximum
Bulk GMS	–118.75	64.08	68.53
OL-loaded GMS SLNs	–95.46	—	63.75
Bulk PRE	–124.21	63.79	67.91
OL-loaded PRE SLNs	–97.50	—	63.42
Bulk GTS	–245.52	72.78	76.87
OL-loaded GTS SLNs	–204.74	—	71.42
Bulk WE 85	–130.15	46.39	49.56
OL-loaded WE 85 SLNs	–107.21	—	47.02

*OL indicates olanzapine; SLN, solid lipid nanoparticle; GMS, glyceryl monostearate; PRE, Precirol ATO 5; GTS, glyceryl tristearate; WE 85, Witepsol E85.

Preparation and Optimization of SLNs

OL-loaded SLNs were prepared by a slight modification of the previously reported melt emulsification and high-pressure homogenization method.[9] Briefly, the lipid was melted (10°C above the melting point of the lipid used), and OL was dissolved therein to obtain a drug-lipid mixture. HSPC was dissolved in chloroform and added to this lipid-drug mixture. The mixture was then warmed at ~60°C to 65°C to completely evaporate the chloroform. The clear lipid melt containing HSPC was added to the hot aqueous surfactant solution preheated to 10°C above the lipid's melting point under high-shear homogenization at 9500 rpm for 1 minute to yield a crude emulsion. The crude emulsion was subsequently homogenized in a high-pressure homogenizer (Emulsiflex C5, Avestin, Ottawa, Canada) in a water bath maintained at 10°C above the melting point of the lipid. The hot nanoemulsion obtained was then cooled to room temperature to recrystallize the lipid back to the solid state in the form of an aqueous SLN dispersion. The SLN dispersions had a lipid content of 5%, stabilized by 1% to 3% surfactant (poloxamer 407). To study the entrapment efficiency, the OL content with respect to the lipid matrix was varied from 2% to 5%.

Estimation of Olanzapine in SLNs

The SLNs in dispersion were aggregated by adding 0.1 mL of 10 mg/mL protamine sulfate solution and centrifuged at 8000 rpm for 10 minutes to obtain a pellet. The supernatant was suitably diluted with 0.1 N HCl, and the free drug content was determined spectrophotometrically at 258 nm against a solvent blank. The pellet obtained was dried by lyophilization, and the dry powder was dissolved in a mixture of methanol-chloroform (1:1) and analyzed spectrophotometrically at 276 nm for the entrapped drug against a solvent blank.

Characterization

Particle size and zeta potential of the SLN dispersions were measured by photon correlation spectroscopy using a Malvern

Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK), which works on the Mie theory. All size and zeta potential measurements were carried out at 25°C using disposable polystyrene cells and disposable plain folded capillary zeta cells, respectively, after appropriate dilution with distilled water.

Differential scanning calorimetry (DSC) analysis was performed using a differential scanning calorimeter (DSC-60, Shimadzu) at a heating rate of 10°C per minute in the range of 30°C to 250°C under an inert nitrogen atmosphere at a flow rate of 80 mL/min. DSC thermograms were recorded for OL, bulk GMS, OL-loaded GMS SLNs, bulk PRE, OL-loaded PRE SLNs, bulk GTS, OL-loaded GTS SLNs, bulk WE 85, and OL-loaded WE 85 SLNs.

Powder X-ray diffraction (pXRD) patterns were obtained using an X-ray diffractometer (Philips PW 1710, Tokyo, Japan) with Cu Kα radiation generated at 30 mA and 40 kV. pXRD diffraction patterns were recorded for plain OL, bulk GMS, bulk PRE, bulk GTS, bulk WE 85, OL-loaded GMS SLNs, OL-loaded PRE SLNs, OL-loaded GTS SLNs, and OL-loaded WE 85 SLNs.

To determine in vitro steric stability by electrolyte-induced flocculation test, sodium sulfate solutions ranging from 0 M to 1.5 M were prepared in a 16.7% wt/vol sucrose solution.[10] An appropriate volume of SLN dispersion was made up to 5 mL using sodium sulfate solutions of varying concentrations (0 M, 0.3 M, 0.6 M, 0.9 M, 1.2 M, and 1.5 M) to obtain a final concentration of 1 mg/mL lipid. The absorbance of the resulting dispersions was measured within 5 minutes at 400 nm using a UV-visible spectrophotometer against a respective blank.

To determine surface hydrophobicity by the rose bengal adsorption method, a fixed known amount of dye (rose bengal) was added to the SLN dispersions of various concentrations. The SLNs in dispersion were then aggregated by adding 0.1 mL of 10 mg/mL protamine sulfate solution and centrifuged at 8000 rpm for 10 minutes. The fluorescence of free dye in the supernatant was measured in a Shimadzu RF-540 spectrofluorophotometer (Shimadzu) using an excitation wavelength of 556 nm and monitoring the emission at 577 nm. The PC was calculated from the ratio of the amount of dye bound on the surface to the amount of dye in the dispersion medium. PC values obtained for each nanoparticle concentration were then plotted against the total surface area (in μm^2/mL) of the nanoparticles. The slopes of the straight lines obtained were taken as a measure of the surface hydrophobicity.[11]

Stability Studies

The initial particle size and zeta potential of the OL-loaded SLN dispersions were measured immediately after high-pressure homogenization using the Malvern Zetasizer Nano ZS. This batch was divided into 2 sample sets, 1 stored at 4°C (in a refrigerator) and the other stored at 40°C (in a temperature-regulated oven). All samples were stored in plain glass vials (USP type I) that were sealed and wrapped with black paper. Samples were withdrawn after 15, 30, 60, 90, and 120 days and subjected to particle size and zeta potential measurements.

In vitro release of OL from the OL-loaded SLNs was determined in both 0.1 N HCl and pH 7.4 PB by the dialysis bag method.[9] The dissolution medium was continuously stirred at 100 rpm and maintained at 37°C ± 2°C. The release of OL from solution in pH 7.4 PB containing 1% methanol (as a control) through the dialysis bag was also studied using the same medium. Samples were withdrawn at predetermined time intervals, and the volume withdrawn was replaced with the same volume of fresh dissolution medium. Samples withdrawn from 0.1 N HCL and pH 7.4 PB were analyzed for OL content spectrophotometrically at 258 nm and at 276 nm, respectively, against a suitable solvent blank. All experiments were repeated 3 times, and the average values were taken.

Results and Discussion

Partitioning Nature of Olanzapine Between Lipids and pH 7.4 PB

OL is a hydrophobic drug with a log P value of 2.199. The PC was in the order of GTS (2.77) > PRE (2.31) > WE 85 (2.23) > GMS (1.91). GTS is the most lipophilic of the 4 lipids and had a higher affinity for OL. PRE, being a mixture of mono-, di-, and triglycerides, is suspected of having more space, that is, more imperfections in its lipid matrix to accommodate the drug. WE 85, which is also a mixture of mono-, di-, and triglycerides, had a partition value comparable to PRE's. Furthermore, the presence of emulsifiers in WE 85 may have played a role in the solubilization of OL in the lipid matrix. The PC values obtained for the different lipids correlated with the entrapment efficiency of SLNs. GTS SLNs exhibited the highest entrapment of OL (94.31%), while GMS SLNs showed the lowest entrapment efficiency (82.17%). There was no significant difference in the entrapment efficiency of PRE SLNs and WE 85 SLNs. Hence, an initial study of the partitioning nature of the drug between the melted lipid and aqueous media can provide some clues about the entrapment in the SLN formulation.

The prerequisite to obtain adequate drug loading is high solubility of the drug in the lipid melt. Generally, solubility decreases after cooling down the lipid melt and might even be lower in the solid lipid. The presence of mono- and diglycerides in the lipid used as matrix material promotes drug solubilization.[12] The chemical nature of the lipid is also important because lipids that form highly crystalline particles with a perfect lattice (eg, monoacid triglycerides) lead to

drug expulsion.[3] Lipids that are mixtures of mono-, di-, and triglycerides and lipids containing fatty acids of different chain lengths form less perfect crystals with many imperfections, offering space to accommodate the drugs.

Influence of Homogenization Pressure and Homogenization Cycle Number

The optimum homogenization pressure was determined by passing the crude emulsion (stabilized using 1.5% wt/vol poloxamer 407) at different homogenization pressures ranging from 5000 to 10 000 psi. As the homogenization pressure was increased from 5000 psi to 10 000 psi, a decrease in mean particle size of SLN dispersions was observed. Homogenization pressures above 10 000 psi did not result in a significant decrease in the mean particle size in any of the 4 SLN dispersions. Reduction in particle size is mainly due to the development of cavitational forces in the homogenization gap, resulting in diminution of the lipid droplets to the nano size.[13]

The mean particle size of nanoparticles was measured for the lipid dispersions (stabilized using 1.5% wt/vol poloxamer 407) homogenized at 10 000 psi after different homogenization cycles. It was observed that, as the number of homogenization cycles increased, the size of SLN dispersions decreased. The size distribution curve moved toward a narrow particle size distribution up to 3 cycles, and a slight increase in size was observed after the fourth cycle, accompanied by a broad size distribution. The optimum number of homogenization cycles resulting in smaller-sized nanoparticles was 3 cycles.

Optimization of Surfactant Concentration

The mean particle size of OL-loaded SLN dispersions stabilized with 0.5% wt/vol, 0.75% wt/vol, 1.0% wt/vol, 1.5% wt/vol, and 2.0% wt/vol poloxamer 407 was measured. There was a gradual decrease in SLN particle size with an increase in the surfactant concentration from 0.5% wt/vol to 1.5% wt/vol poloxamer 407. Increasing the surfactant concentration to 2.0% did not result in a significant reduction in the nanoparticles' size. Thus, the optimum surfactant concentration for all 4 lipids investigated in this study was fixed as 1.5% wt/vol poloxamer 407.

The primary role of the surfactant is stabilization of the nanoparticles in the colloidal state and prevention of particle size growth during storage. The choice of stabilizers is an important parameter to be considered in optimizing any nanoparticle formulation, not only to control the particle size and stabilization of the dispersions but also to control the crystallization and polymorphic transitions.[2] Bunjes et al demonstrated that the crystallization temperature of nanoparticles made from triglycerides depends on the stabilizer, which can lead to homogenous or surface heterogeneous nucleation.[2] The crystallization tendency of the particles increases with the length of the (saturated) hydrophobic chain of the stabilizer. The crystallization-promoting effect of certain surfactants is believed to be caused by an ordering process of the surfactant molecules in the stabilizer layer.

Entrapment Efficiency

SLN dispersions were prepared by keeping the lipid concentration constant at 5% wt/vol and varying the concentration of OL between 2% wt/wt and 5% wt/wt with respect

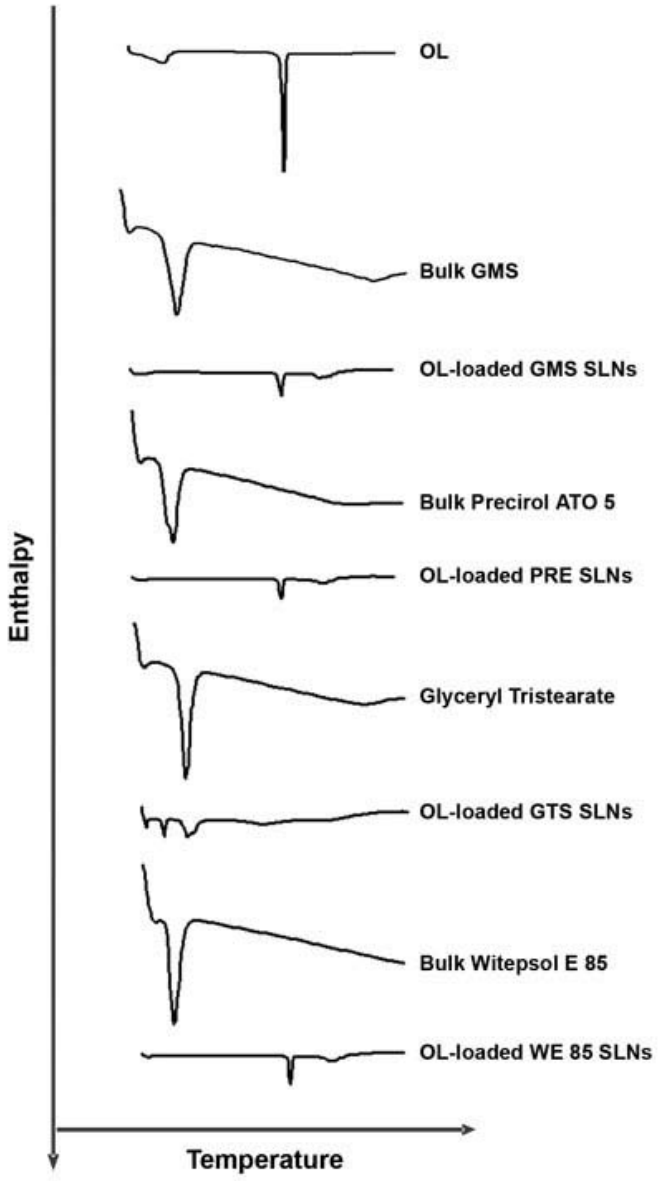

Figure 1. Differential scanning calorimetry curves of OL and the OL-loaded SLN formulations. OL indicates olanzapine; GMS, glyceryl monostearate; SLN, solid lipid nanoparticle; PRE, precirol ATO5; GTS, glyceryl tristearate; WE8, witepsol E85.

to lipid. The results showed that an increase in concentration of the drug led to an increase in drug entrapment efficiency of the SLN dispersions. The maximum drug loading possible was 4% wrt lipid. Efforts to load more drug, for instance 5%, led to a decrease in entrapment efficiency. This can be attributed to the fact that 4% drug loading led to a saturation of the lipid matrix and higher loading levels resulted in more free drug rather than drug encapsulated inside the lipid matrix. The order of entrapment was as follows: GTS SLNs (94.31%) > WE 85 SLNs (90.29%) > PRE SLNs (87.07%) > GMS SLNs (82.17%).

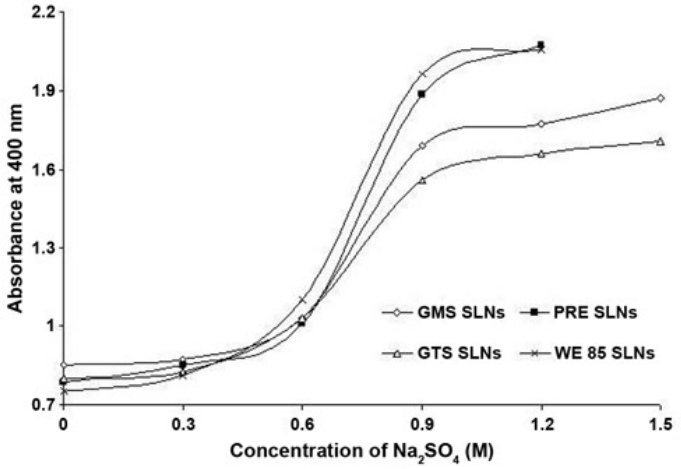

Figure 3. Steric stabilization effect of the OL-loaded SLN formulations. The nanoparticles were stabilized by 1.5% wt/vol of poloxamer 407. OL indicates olanzapine; SLN, solid lipid nanoparticle; GMS, glyceryl monostearate; PRE, Precirol ATO 5; GTS, glyceryl tristearate; WE 85, Witepsol E85.

Characterization of SLN Formulations

DSC

The DSC curve of OL (Figure 1) showed a melting endotherm at 193.05°C. The peak intensity corresponding to the melting of OL decreased in the thermograms of OL-loaded GMS, PRE, and WE 85 SLNs (Figure 1). These results indicate that only a small fraction of the drug substance existed in the crystalline state. The melting endotherm was completely absent in the thermograms of OL-loaded GTS SLNs (Figure 1), which indicates that OL was completely solubilized inside the lipid matrix of the GTS SLNs.

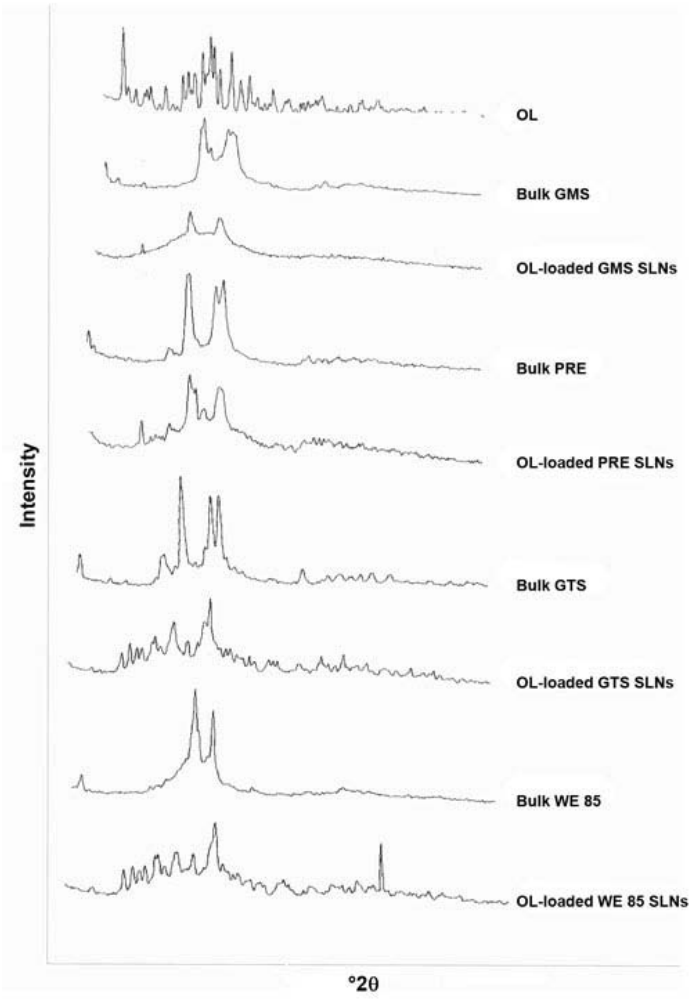

Figure 2. X-ray diffraction pattern of OL and the OL-loaded SLN formulations. OL indicates olanzapine; SLN, solid lipid nanoparticle; GMS, glyceryl monostearate; PRE, Precirol ATO 5; GTS, glyceryl tristearate; WE 85, Witepsol E85.

Reduction in the melting point and enthalpy of the melting endotherm was observed when the lipid was formulated as SLNs (Table 1). Incorporation of OL inside the lipid matrix results in an increase in the number of defects in the lipid crystal lattice, and hence causes a decrease in the melting point of the lipid in the final SLN formulations. Freitas and Muller also observed that the crystallization behavior of Compritol SLNs differed distinctly from that of the bulk lipid.[14] Small particle size of SLNs leads to high surface energy, which creates an energetically suboptimal state causing a decrease in the melting point.

pXRD Patterns

The pXRD pattern of OL (Figure 2) shows a principal peak at angle 8.535° 2θ. All 4 lipids had a principal peak at around the same °2θ (19.300 °2θ). The principal peak of OL was absent in all 4 OL-loaded SLN formulations (Figure 2); furthermore, the principal peak of the lipid did not shift but had a reduced intensity. This may be attributed to the incorporation of OL between parts of the crystal lattice of the lipid, leading to a change in the crystallinity of the OL-loaded SLNs. These values complement the DSC data and clearly indicate the possible change in crystallinity of the lipid after OL incorporation and formulation as nanoparticles.

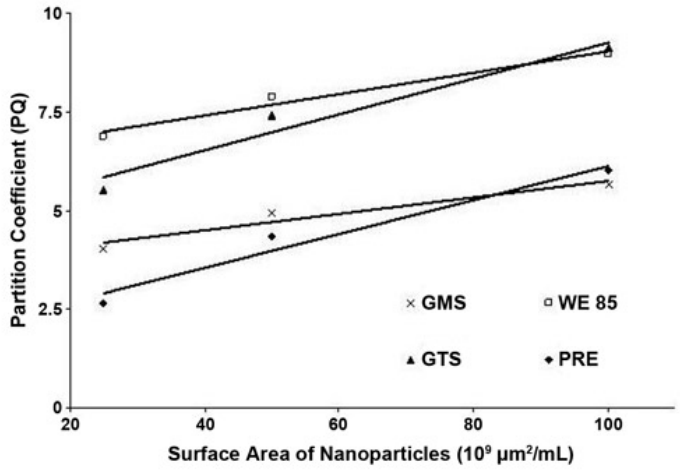

Figure 4. Plot of the partition coefficient vs the total surface area of the nanoparticles. GMS, glyceryl monostearate; GTS, glyceryl tristearate; WE 85, Witepsol E85; PRE, Precirol ATO 5.

Determination of In Vitro Steric Stability by Electrolyte Flocculation Test

The SLN dispersions showed a gradual increase in flocculation as the concentration of electrolyte (sodium sulfate) was increased. All 4 SLN dispersions showed sharp flocculation (increase in the absorbance value) when the concentration of sodium sulfate was above 0.6M. The results are given in Figure 3.

Coating the particulate systems with hydrophilic surfactants provides steric stability by rendering a hydrophilic surface, which in turn reduces the binding of serum opsonins and cells of the reticuloendothelial system.[15] Excess electrolyte concentrations can distort this steric barrier around the particle. This results in flocculation of the particles, with a corresponding increase in optical turbidity of the particle dispersion, which can be measured by the absorbance of the dispersion at 400 nm. During a study on the effect of various concentrations of poloxamer 407 on the in vitro steric stability of etoposide-loaded GMS SLNs and PRE SLNs, Reddy and Murthy observed nanoparticle agglomeration on addition of 0.4 M sodium sulfate. The authors explained that this behavior was due to dehydration of the polyoxypropylene and polyoxyethylene during emulsification and high-pressure homogenization.[16]

Determination of Surface Hydrophobicity by Rose Bengal Adsorption Method

The slopes—that is, the surface hydrophobicity—obtained were as follows: GTS SLNs (0.0457) > PRE SLNs (0.0432)

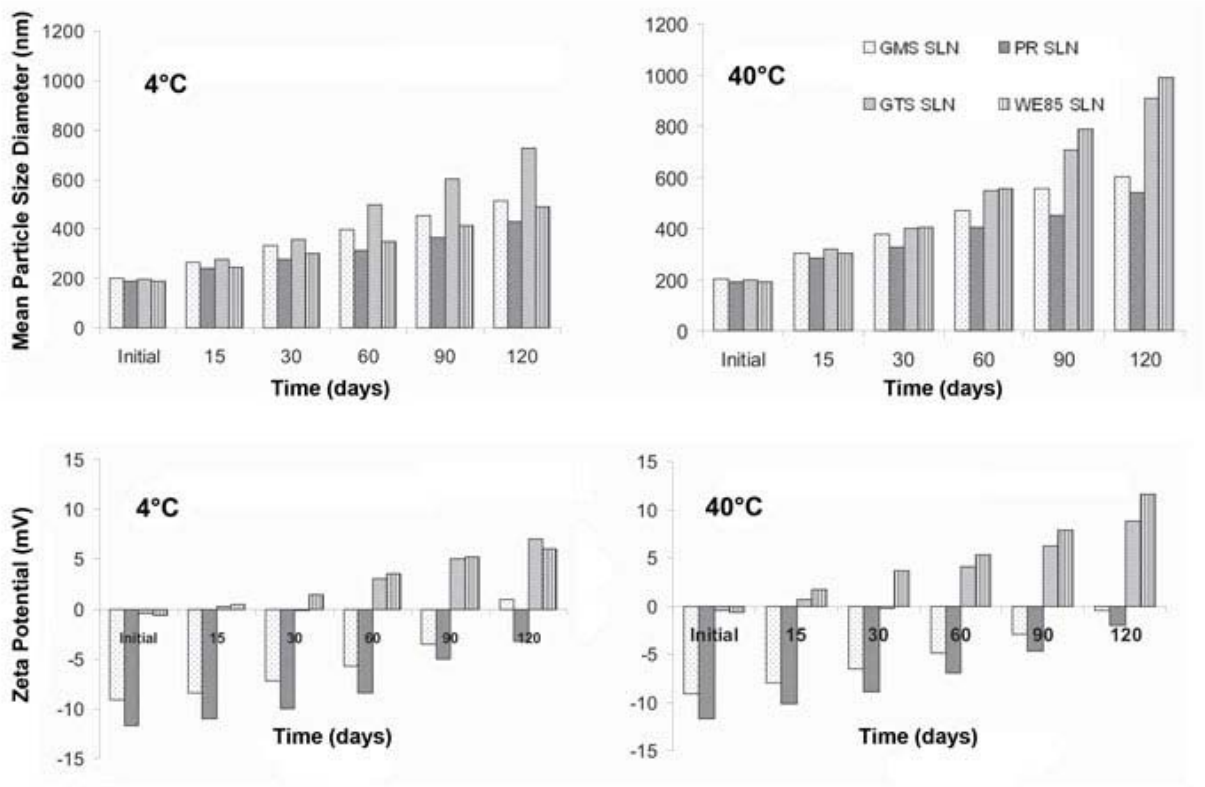

Figure 5. Mean particle diameter and zeta potential values of the OL-loaded SLN dispersions stored at 4°C and 40°C after 15, 30, 60, 90, and 120 days. OL indicates olanzapine; SLN, solid lipid nanoparticle.

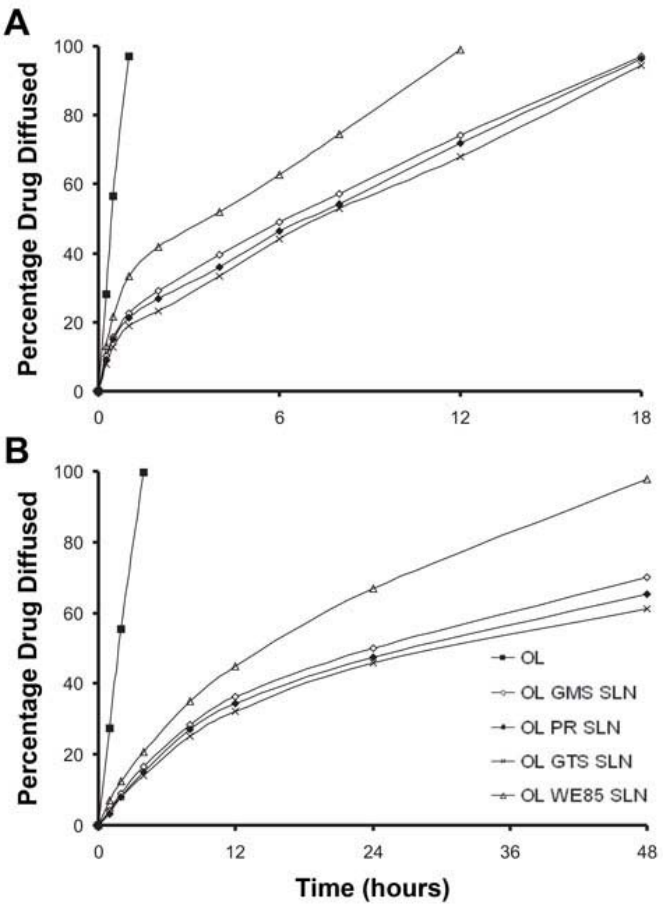

Figure 6. In vitro release of OL from plain OL solution and the OL-loaded SLN dispersions in 0.1 N HCl (A) and pH 7.4 PB (B). OL indicates olanzapine; SLN, solid lipid nanoparticle; PB, phosphate buffer.

> WE 85 SLNs (0.0271) > GMS SLNs (0.0207). Results are shown in Figure 4. Gessner et al reported that a decrease in surface hydrophobicity is accompanied by a decrease in the quantitative amount of adsorbed proteins.[11] However, the functional groups present on the particle surface also significantly affect the protein adsorption. Poloxamer block copolymers are synthetic copolymers of ethylene oxide (EO; hydrophilic part) and propylene oxide (PO; hydrophobic part) with a molecular weight of around 11 500. Poloxamer 407 has 64% EO and 36% PO, with a hydrophilic-lipophilic balance value of 18 to 23. Carstensen et al observed that the hydrophobicity of nanoparticles decreased with an increase in the polyethylene oxide chain length of the poloxamer grade used for surface stabilization.[17]

Stability Studies

The particle sizes of SLNs prepared from the different lipids immediately after production did not differ significantly. However, their stabilities at different temperatures varied. Particle size growth was observed to be a function of storage temperature, with a gradual increase in size observed with increasing temperature. Mean particle size of the OL-loaded GTS SLNs stored at 40°C increased from 198 nm to 908 nm in 120 days, whereas the OL-loaded GTS SLNs stored at 4°C increased from 198 nm to 728 nm (Figure 5). Another observation was formation of a rigid gel in the stability samples of OL-loaded GMS SLNs stored at 4°C after 15 days. Samples kept at 40°C did not gel. Furthermore, a clear drug phase separation was observed in OL-loaded GTS SLN formulations. A thin layer of solid was found to sediment at the base of the glass vial.

Drug expulsion from the lipid matrix is a common problem during storage of SLN dispersions. Similar gelling and drug phase separation was also observed by Westesen et al during their study of the stability of phospholipid/tyloxapol trimyristin, Witepsol H42, and Witepsol H35 nanoparticles.[3] The authors observed that the temperature of gel formation depends mainly on the lipid matrix of the colloidal lipid emusions, and could be prevented by using a cosurfactant.[3] Freshly prepared lipid dispersions are characterized by a less ordered crystal lattice when compared with the bulk lipid. The lipid tends to crystallize to more perfect crystalline β-modifications, which ultimately lead to drug expulsion.[18] Jenning and Gohla observed that the SLNs made from wax (cetyl palmitate and beeswax) were more stable when compared with glyceride nanoparticles, which showed particle growth and aggregation.[19]

The zeta potential was found to increase with longer duration of storage and higher temperature in the case of GTS SLNs

Table 2. Comparative t_{25}, t_{50}, and t_{90} (time taken for 25%, 50%, and 90% of OL to Be Released) of OL Solution and the SLNs (Medium: 0.1 N HCl)*

	Time (mins) (mean ± SD)				
Parameter	OL Solution	GMS SLNs	PRE SLNs	GTS SLNs	WE 85 SLNs
t_{25}	18.24 ± 1.09	69.01 ± 1.39	76.12 ± 1.80	115.0 ± 2.48	42.19 ± 1.31
t_{50}	35.42 ± 1.26	358.1 ± 3.44	415.1 ± 4.06	442.9 ± 3.35	237.1 ± 2.75
t_{90}	61.06 ± 1.44	946.5 ± 7.99	967.1 ± 6.12	1026 ± 9.53	541.4 ± 4.76

*OL indicates olanzapine; SLNs, solid lipid nanoparticles; GMS, glyceryl monostearate; PRE, Precirol ATO 5; GTS, glyceryl tristearate; WE 85, Witepsol E85.

Table 3. Comparative t_{25} and t_{50} (Time Taken for 25% and 50% of OL to Be Released) of OL Solution and the SLNs (Media: PB pH 7.4)*

	Time (hrs) (mean ± SD)				
Parameter	OL Solution	GMS SLNs	PRE SLNs	GTS SLNs	WE 85 SLNs
t_{25}	1.03 ± 0.09	7.11 ± 0.17	7.49 ± 0.24	8.05 ± 0.20	5.49 ± 0.15
t_{50}	2.15 ± 0.11	23.42 ± 0.56	26.60 ± 0.67	28.79 ± 0.70	13.05 ± 0.57

*OL indicates olanzapine; SLNs, solid lipid nanoparticles; PB, phosphate buffer; GMS, glyceryl monostearate; PRE, Precirol ATO 5; GTS, glyceryl tristearate; WE 85, Witepsol E85.

and WE 85 SLNs. The changes in the zeta potential were more prominent for all the OL-loaded SLN samples stored at 40°C when compared with those stored at 4°C (Figure 5). An increase in energy input to the SLN dispersion can lead to changes in the crystalline structure of the lipid.[20] Siekmann and Westesen observed an increased β-modification during the storage of tripalmitate SLNs.[6] Crystalline reorientation can result in changes to the charges on the particle surface (Nernst potential) and subsequently the zeta potential. Furthermore, different sides of a crystal can possess a different charge density (eg, aluminum silicates like Bentone). Formation of long β-crystals can take place during 1-dimensional growth of a crystal.[21] This ultimately results in modification of the surface ratio of differently charged crystal sides and, consequently, zeta potential changes.

In Vitro Release

OL is freely soluble in 0.1 N HCl and poorly soluble in pH 7.4 PB. The 0.1 N HCl was used as the diffusion medium to discriminate the release patterns between the different SLN formulations because pH 7.4 PB was not able to do so. The release profiles indicated that SLN dispersions showed a retarded release of the drug from the lipid matrix when compared with plain OL solution. The in vitro release graphs of OL from the SLN dispersions in 0.1 N HCl and pH 7.4 PB are shown in Figure 6. Comparative t_{25}, t_{50}, and t_{90} (time taken for 25%, 50%, and 90% of OL to be released) of OL solution and the SLNs in 0.1 N HCl and pH 7.4 PB appear in Tables 2 and 3, respectively.

The release profiles of the 4 SLNs best fit into the Higuchi equation. The Higuchi equation describes the diffusion of drug from homogenous and granular matrix systems. The drug release from a matrix system is said to follow Higuchi's release kinetics if the amount of drug released is directly proportional to the square root of time. The slopes obtained from the above plot are proportional to an apparent diffusion coefficient. Excluding the burst effect by omitting the early time data points (time points up to 2 hours), linear fits were obtained indicating that release was diffusional.[22] The in vitro release kinetic constants are given in Table 4.

All the nanoparticles exhibited initial burst release followed by sustained release. The initial in vitro burst release is probably caused by the drug adsorbed on the nanoparticle surface or precipitated from the superficial lipid matrix.[16] The sustained release is probably due to diffusion of drug from the lipid matrix. GTS, the most lipophilic lipid in this study, had the highest sustained-release effect.

CONCLUSIONS

The present investigation demonstrates that partition studies of OL between the molten lipid and pH 7.4 PB gave an idea about the drug entrapment during the formulation of SLNs. Particle size of the nanoparticles can be controlled by varying process variables such as homogenization pressure and cycle number, and formulation variables such as surfactant. In vitro steric stability and surface hydrophobicity, coupled with sustained release in vitro, means that further investigation of these nanoparticles as long-circulating carriers in blood should be performed.

Table 4. Kinetic Evaluation of OL Release Data for the SLN Formulations in 0.1 N HCL and pH 7.4 PB*

	0.1 N HCL		pH 7.4 PB	
SLN Type	Higuchi (R^2)	Slope	Higuchi (R^2)	Slope
OL-loaded GMS SLNs	0.9932	22.10	0.9928	11.19
OL-loaded PRE SLNs	0.9814	21.99	0.9901	10.57
OL-loaded GTS SLNs	0.9831	21.84	0.9909	9.90
OL-loaded WE 85 SLNs	0.9884	26.47	0.9965	15.46

*OL indicates olanzapine; SLN, solid lipid nanoparticle; PB, phosphate buffer; GMS, glyceryl monostearate; PRE, Precirol ATO 5; GTS, glyceryl tristearate; WE 85, Witepsol E85.

REFERENCES

1. Heiati H, Tawashi R, Phillips NC. Drug retention and stability of solid lipid nanoparticles containing azidothymidine palmitate after autoclaving, storage and lyophilization. *J Microencapsul.* 1998;15:173–184.

2. Bunjes H, Westesen K, Koch MHJ. Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. *Int J Pharm.* 1996;129:159–173.

3. Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading and sustained release potential. *J Control Release.* 1997;48:223–236.

4. Cavalli R, Gasco MR, Morel S. Behaviour of timolol incorporated in lipospheres in the presence of a series of phosphate esters. *STP Pharma Sci.* 1992;2:514–518.

5. Mehnert W, Mader K. Solid lipid nanoparticles production, characterization and applications. *Adv Drug Deliv Rev.* 2001;47: 165–196.

6. Siekmann B, Westesen K. Submicron-sized parenteral carrier systems based on solid lipids. *Pharm Pharmacol Lett.* 1992;1:123–126.

7. Bever KA, Perry PJ. Olanzapine: a serotonin-dopamine receptor antagonist for antipsychotic therapy. *Am J Health Syst Pharm.* 1998;55:1003–1016.

8. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. *J Control Release.* 2004;95:627–638.

9. Reddy LH, Vivek K, Bakshi N, et al. Tamoxifen citrate loaded solid lipid nanoparticles (SLN™): preparation, characterization, in vitro drug release and pharmacokinetic evaluation. *Pharm Dev Technol.* 2006;11:167–177.

10. Subramanian N, Murthy RSR. Use of electrolyte induced flocculation technique for an in vitro steric stability study of steric stabilized liposome formulations. *Pharmazie.* 2003;59:74–76.

11. Gessner A, Waicz R, Lieske A, et al. Nanoparticles with decreasing surface hydrophobicities: influence on plasma protein adsorption. *Int J Pharm.* 2000;196:245–249.

12. Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. *Eur J Pharm Biopharm.* 2000;50:161–177.

13. Muller RH, Keck CM. Challenges and solutions for the delivery of biotech drugs—a review of drug nanocrystal technology and lipid nanoparticles. *J Biotechnol.* 2004;113:151–170.

14. Freitas C, Muller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase. *Eur J Pharm Sci.* 1999;47:125–132.

15. Huang SK, Martin FJ, Jay G, et al. Extravasation and transcytosis of liposomes in Kaposi's sarcoma—like dermal lesions of transgenic mice bearing HIV Tat gene. *Am J Pathol.* 1993;143:10–14.

16. Reddy LH, Murthy RSR. Etoposide-loaded nanoparticles made from glyceride lipids: formulation, characterization, in vitro drug release, and stability evaluation. *AAPS PharmSciTech [serial online].* 2005;6: Article 24.

17. Carstensen H, Muller BW, Muller RH. Adsorption of ethoxylated surfactants on nanoparticles, I: characterization by hydrophobic interaction chromatography. *Int J Pharm.* 1991;67:29–37.

18. Muller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. *Int J Pharm.* 2002;242: 121–128.

19. Jenning V, Gohla S. Comparison of wax and glyceride solid lipid nanoparticles (SLN®). *Int J Pharm.* 2000;196:219–222.

20. Freitas C, Muller RH. Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. *Int J Pharm.* 1998;168:221–229.

21. Sato K. Crystallization of fats and fatty acids. In: Garti N, Sato K, eds. *Crystallization and Polymorphism of Fats and Fatty Acids.* New York, NY: Marcel Dekker; 1988:227–266.

22. Vivek K, Harivardhan Reddy L, Murthy RSR. Comparative study of some biodegradable polymers on the entrapment efficiency, release behavior of etoposide from microspheres. *Pharm Dev Technol.* In press.