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ARTICLE

Solid lipid nanoparticles of stearic acid for the drug delivery of paliperidone

Sacheen Kumar[a] and Jaspreet Kaur Randhawa[b†]

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ABSTRART

Paliperidone is an antipsychotic drug having poor water solubility and bioavailability. Solid lipid nanoparticles of stearic acid loaded with paliperidone was prepared to enhance the bioavailability. Spherical nanoparticles of stearic acid containing paliperidone was prepared with the surfactant, biz. Gelucire® 50/13 forming a stabilizing layer over the nanoparticles. Particle size of the SLNs was found to exponentially decrease with the increase in the surfactant concentration. Dynamic light scattering (DLS), transmission electron microscopy (TEM) and atomic force microscopy (AFM) imaging revealed that the average particle size of SLNs was 230±30 nm. Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analysis revealed that there was no chemical interaction between the ingredients of the SLNs. However, molecular dispersion of the paliperidone in the stearic acid matrix led to the reduction in crystallinity of stearic acid. The entrapment efficiency of paliperidone in the lipid was calculated as 42.4 (% w/w). The corresponding % drug loading was calculated as 4.1 (% w/w) of the total lipid content. Controlled release pattern was observed in the invitro release kinetics studies. Invitro cell culture studies against RAW 264.7 murine macrophages revealed that the paliperidone loaded SLNs have some cytotoxicity. But the observed toxicity was not concentration dependent as there was at least 60 % viability of the cells in the concentration range of 30 – 120 µg/ml.

Introduction

Paliperidone is an antipsychotic drug having many advantages over other drugs that are currently in vogue. It has faster onset of action and it offers a lot of flexibility of administration.[1] Moreover, it does not require refrigeration for safe storage.[1] Unfortunately, its poor bioavailability overshadows its advantages. Formulating paliperidone to enhance its bioavailability is actively pursued in pharmaceutical research. Among the various formulation strategies, solid lipid nanoparticles (SLN) could be promising vehicles for the delivery of paliperidone. In an earlier study, we have explored the drug entrapment and encapsulation efficiency of in a lipid Capmul® GMS 50K which is the trade name of glycerol mono stearate and is a mono-triglyceride of hydrogenated vegetable oil.[2,3] It was found that the particle size of the SLNs can be controlled by the use of a suitable surfactant as emulsifier. We have explored the use of two surfactants: sodium deoxycholate and Gelucire® 50/13. Doughnut shaped particles of size 180 to 250 nm were formed with sodium deoxycholate as the surfactant.[2] The entrapment efficiency was 55% with this surfactant[2]. The entrapment efficiency could be increased to 75 % by using Gelucire® 50/13 as the as a non-ionic surfactant.[3] The objective of our studies so far has been to prepare multi-functional SLNs. For example, sodium deoxycholate is a known blood-brain barrier enhancer. However it is cytotoxic at higher concentrations. Gelucire® 50/13 is a solubility and bioavailability enhancer having no toxicity. In continuation to our search for a suitable multi-functional SLN vehicle for the delivery of paliperidone, we have explored the nanoparticle formation of staric acid and the results are presented here.

Stearic acid has neuro-protective effects on brain due to retarded oxidative stress .[4] Hence, it will be interesting to use stearic acid as the matrix material for preparing SLNs for the delivery of

paliperidone. However, stearic acid is a crystalline solid and hence it is difficult to use it as a matrix for making SLNs.[5] Stearic acid is a saturated fatty acid having 18 carbon atoms. Polymorphic forms of stearic acid described in literature are: A form, B form, C form and E form.[6] Among the various polymorphic forms of stearic acid, C form is the most stable one. B form has hydrogen bond distance of 2.4 Å whereas C form has 2.6 Å. Stearic acid has orthorhombic packing with a molecular packing coefficient of 0.701 for B form and 0.690 for C form [7]. The melting point of stearic acid is 69.6 °C [8]. As the melting point of stearic acid is much higher than human body temperature, it could be used as a lipid matrix material in nanoparticles.[6, 9, 10] SLNs of stearic acid can be prepared by using anionic surfactants such as taurocholate. [9] However, these SLNs cannot be prepared without using a co-surfactant. Unfortunately, toxicity of anionic surfactants and co-surfactant is also high.[11] Preparing SLNs of stearic acid using non-ionic surfactant with comparative melting point to stearic acid could be a better option as these are less toxic. Gelucires are a group of basically inactive excipients derived from food grade fats and oils. These may contain either pure glycerides (mono-, di-and tri-glycerides of saturated fatty acids) or mixtures of glycerides and fatty acid esters of polyethylene glycols (mono-and di-fatty acid esters) in variable quantities. These are categorized by their hydrophile-lipophile-balance value and melting point. Required HLB value for stearic acid in water dispersion should be around 13-15. Hence, Gelucire® 50/13 was selected as a stabilizer due to its HLB value of 13. Its melting point is also above the body temperature.

In this study, we have used stearic acid as a lipid matrix for the delivery of paliperidone. SLNs were preparation with Gelucire® 50/13 as surfactant without using a cosurfactant. Invitro drug release profile was studied and toxicity of the SLNs was verified. The results of our synthesis and detailed characterization are discussed in the present paper.

Results and Discussion

SLNs prepared in dispersion form appeared milky white, whose viscosity was found to increase with increasing the concentration of Gelucire® 50/13. Particle size of SLNs needs to be controlled by optimizing the surfactant concentration and drug loading. Hence, detailed study on the effect of concentration of surfactant and drug loading on particle size was undertaken.

Variation in particle size of SLNs with the surfactant concentration

Dynamic light scattering was primarily used to study the effect of changing the concentration of surfactant on the size of SLNs. The amount of Gelucire® 50/13 in the preparation recipe was varied systematically. A plot of z-average particle size versus amount of Gelucire® 50/13 is shown in Figure 1. It is clearly evident from the plot that the size of stearic acid SLNs (SASLNs) was highly dependent upon the Gelucire® 50/13 concentration in the SLN formulation. The z-avrage particle size decreased significantly from 1700 to 200 nm with the increase in Gelucire® 50/13 content from 200 to 800 mg. Such a strong dependency of particle size of stearic acid nanoparticles on the surfactant concentration is due to the lack of self-emulsifying property for stearic acid. In our previous study we have used Gelucire® 50/13 as a stabilizer for preparing SLNs of Capmul® GMS 50K.[3] The particle size of Capmul® GMS-50K also decreased with increasing concentration of surfactant. The effect of surfactant concentration on the particle size of Capmul® GMS 50K SLNs followed the hyperbolic fit.[3] However, for SASLNs, the concentration of surfactant versus particle size curve followed the exponential fit as shown in Figure 1. The stabilization effect of Gelucire® 50/13 has been quite drastic on the size of SASLNs than on Capmul® GMS 50K SLNs. But, SASLNs needed higher concentration of Gelucire® 50/13 to attain the size around 200 nm.

Tcholakova et. al. have reported that the size of nanoparticles varies with the surfactant content.[12] SLNs are stabilized by the adsorption of surfactants on the surface of SLNs forming a monolayer. This adsorption depends upon the chemical nature of the lipid matrix and the stabilizer used. Gelucire® 50/13 acts as a non-ionic stabilizer and it forms monolayer on the surface of SLNs through van der Waal's interaction. Whereas, the adsorption of ionic surfactants depends on the electrical charge on the surface of SLNs[13]. Ethylene oxide groups present in the

structure of Gelucire® 50/13 play a key role in the stabilization of SASLNs.

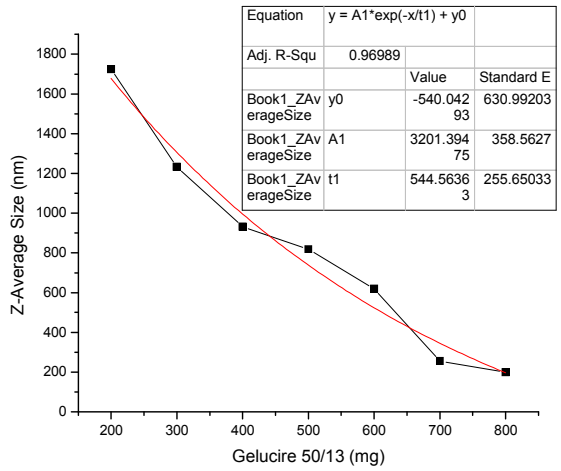

Figure 1. Z-average particle size of stearic acid SLNs decreases with increment in Gelucire® 50/13 content.

Variation in particle size of SLNs with the drug loading

Z-average particle size of SASLNs was found to increase after it was loaded with Paliperidone. The particle size distribution of drug loaded and unloaded SASLNs is shown in Figure 2. The z-average particle size of SLNs without Paliperidone loading was found to be 200 nm with pdi 0.43. On loading Paliperidone in SASLNs, the z-average particle size was increased slightly to 211 nm with pdi 0.39.

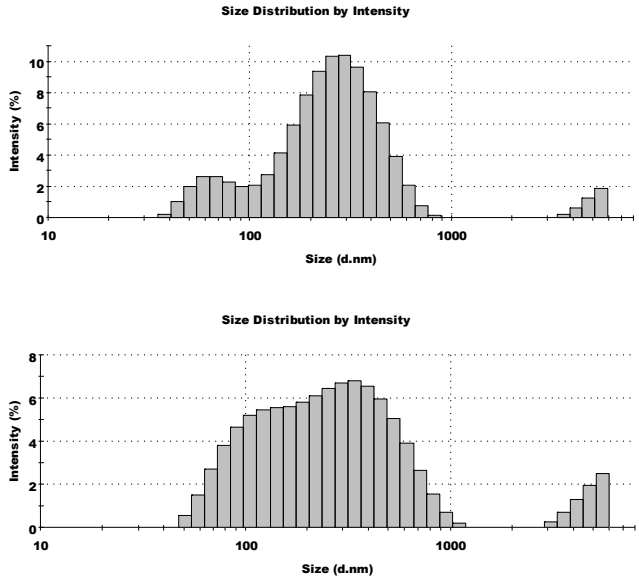

Figure 2: Particle size distribution of SASLNs: (a) before loading paliperidone and (b) after loading paliperidone.

Morphology of the SLNs

Particle size and shape of the SLNs was studied using TEM and AFM Imaging. Some representative TEM images are shown in Figure 3. Average particle size was calculated from a number of TEM images taken from a range of regions and was 230±30 nm. Particle size of SLNs calculated from TEM imaging was thus similar to the DLS results. It can be seen that the SLNs are having spherical morphology. TEM images showed a core shell structure of SLNs. All particles had a dark corona in TEM images with light interior. The dark corona could be due to the Gelucire® 50/13 covering around the Paliperidone loaded stearic acid. Phospho-tungustic acid staining was used to visualize the lipid nanoparticles using TEM. The PTA molecules appears to be staining the Gelucire® 50/13 shell better than the stearic acid core and hence the contrast.[14] Relatively lesser staining of the core of SLNs may be due to the similar chemical nature of stearic acid and PTA.

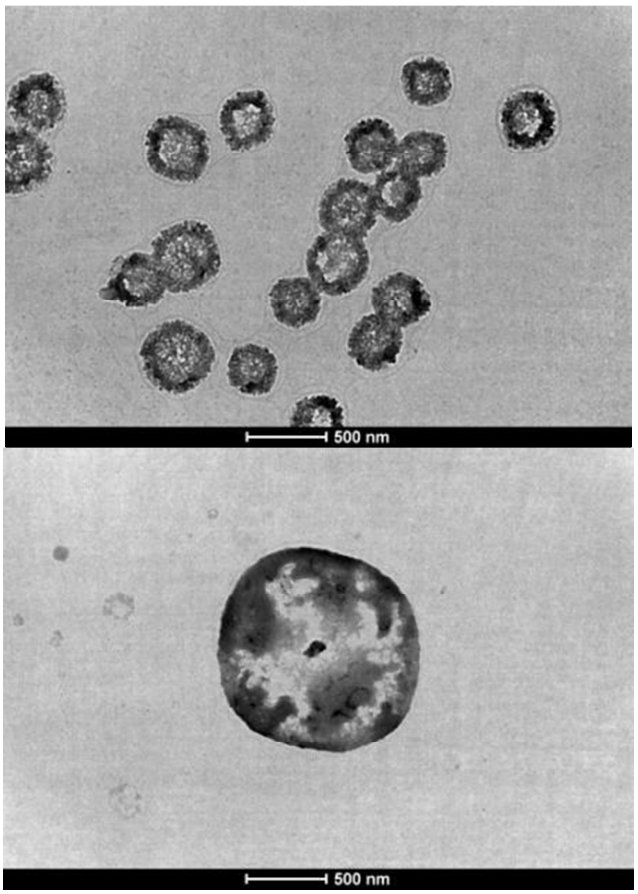

Figure 3: TEM images of stearic acid SLNs loaded with paliperidone prepared by ultasonic homogenization after negatively staining with PTA.

Three dimensional morphology of SASLNs was studied using AFM imaging. The non-contact mode was used to observe the size and shape of SLNs. Representative AFM images are shown in Figure 4. Elliptical shaped particles having a size in the range of 180 to 240 nm were observed in AFM. This is in contrast to the spherical shapes that we observed in TEM images. Elongation of the spherical particles as appeared in the AFM images may be an artifact. The spherical particles may be getting elongated due to the very soft nature of the particles when it is probed by the AFM tip.

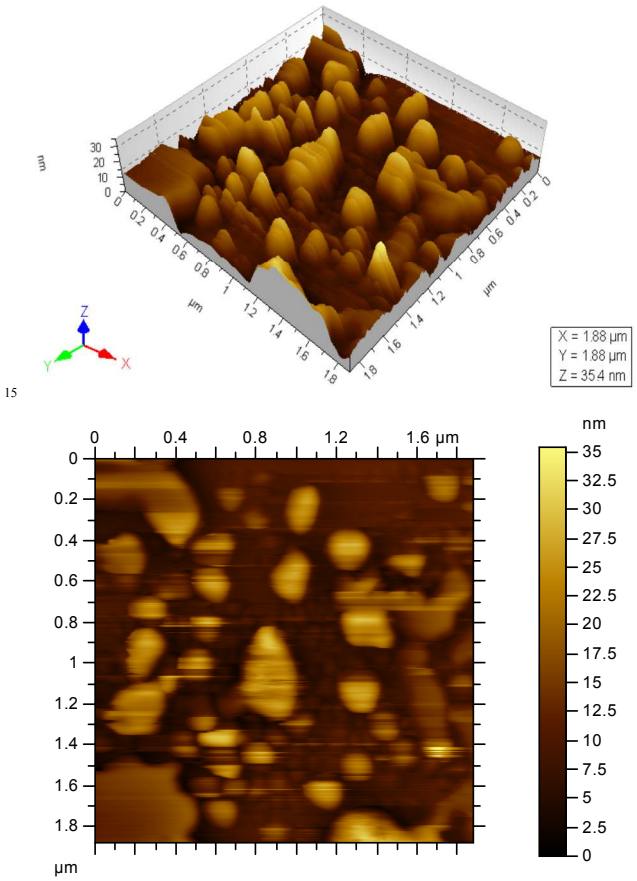

Figure 4: AFM images of stearic acid SLNs prepared by ultrasonic homogenization.

Crystal structure of SLNs

It is important to analyse the crystal structure of the SLNs. This is because; a comparative study on the crystallinity of the individual constituents with the SLNs can be very useful to understand the compatibility of the constituents. XRD patterns of the SASLNs and the individual constituents are reported in Figure 5. Two characteristic peaks were found for stearic acid and the drug loaded SLNs as well as the SLNs without the drug. These peaks correspond to triclinic subcell packing of the lipid. Presence of these peaks in the SLNs clearly established that the crystal structure of stearic acid was retained in the SLNs. Percentage crystallinity of the stearic acid in the SLNs was calculated and reported in Table 1. Relative crystallinity of stearic acid in the SLNs was calculated by assuming the raw-stearic acid to be 100 % crystalline and the data is also reported in Table 1. The data clearly showed that the crystallinity of stearic acid was lost to a great extent when they were melted and re-precipitated as nanoparticles. The crystallinity was lowered even without the

drug loading. SASLNs without paliperidone had 31% lower crystallinity than the parent lipid matrix material. Drug loading lowered the crystallinity by 47% than the parent lipid matrix material (stearic acid).

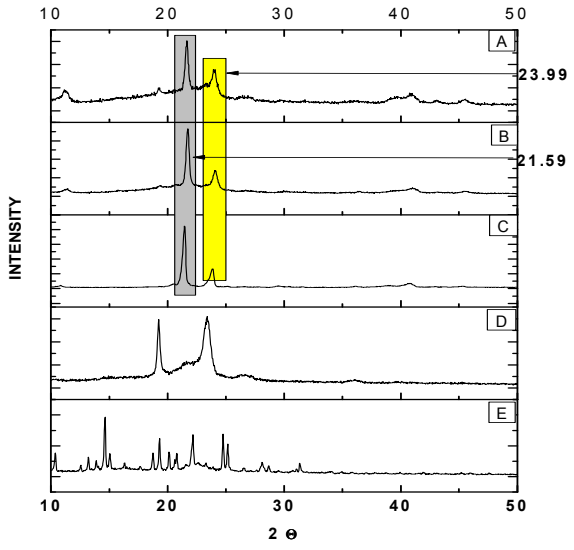

Figure 5: XRD patterns of (A) SLNs loaded with Paliperidone, (B) SLNs without Paliperidone, (C) raw-stearic acid, (D) raw-Gelucire® 50/13, and (E) raw-paliperidone.

Table 1 Absolute and relative crystallinity of stearic acid in the SLNs compared to raw-stearic acid

Sr. No.	Sample	Absolute Crystallinity (%)	Relative Crystallinity (%)
1	Stearic acid	84	100
2	SASLNs without paliperidone	58	69
3	SASLNs with paliperidone	44	55

The raw paliperidone showed a number of intense peaks in its XRD pattern [15]. The most important result was the absence of characteristic peaks corresponding to paliperidone in the drug loaded SASLNs. This clearly confirmed that paliperidone did not crystallize in the lipid matrix. Instead, the paliperidone molecules were dispersed evenly in the stearic acid matrix. Raw-Gelucire® 50/13 had an intense peak at 2θ=19.1° and another one at 23.4^0. These peaks were absent in the SLNs. This showed that the native crystallinity of Gelucire® 50/13 is lost while adsorbing on the stearic acid nanoparticles. The monolayer formation thus warrant change in the native conformation of the surfactant.

Chemical composition of the SLNs

FTIR spectra of the SLNs and the individual constituents in the SLNs were recorded to study the compatibility and chemical composition of the SLNs and the data is shown in Figure 6. FTIR spectrum of stearic acid has peaks at 689 cm^{-1} (O-C=O), dual peaks at 938 cm^{-1} and 960 cm^{-1} (OH bending), 1110 cm^{-1} (skeletal vibration), 1186 cm^{-1} to 1258 cm^{-1} (CH_2 wagging), 1298 cm^{-1} (OH bending), 1410 cm^{-1} (αCH_2 deformation), 1462 cm^{-1} and 1472 cm^{-1}(CH_2 scissoring) and 1705 cm^{-1}(C=O stretching)[16]. Strong bands near 1700, 1435, 1300, 940, and 690 cm^{-1} have been ascribed to vibrations of the carboxyl groups. Similar pattern was also observed in the SASLNs. Thus, the FTIR results confirmed that there was no chemical interaction of ingredients. Distinct peaks characteristic to paliperidone was absent in the FTIR data of paliperidone loaded SASLNs. This is due to the overlapping of the characteristic peaks of paliperidone with other constituents.

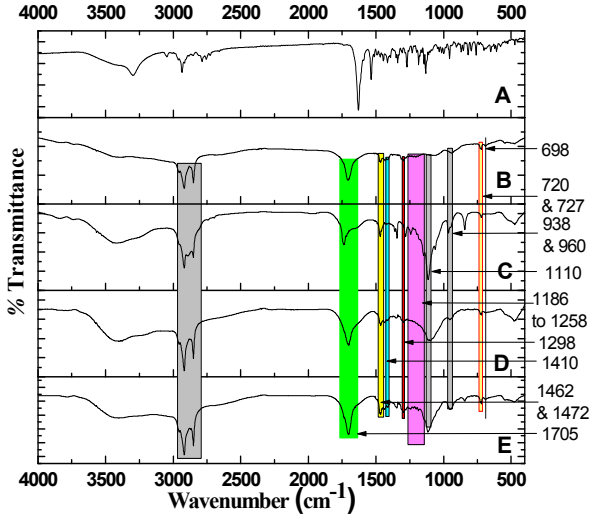

Figure 6: FTIR spectra of (A) paliperidone, (B) stearic acid, (C) Gelucire® 50/13, (D) SASLNs without paliperidone and (E) SASLNs with paliperidone.

Thermal response of SASLNs: DLS and DSC studies

Stability of SLNs varies greatly with temperature. Hence it is important to study the thermal response of the SLNs. Thermal response of the SLNs was studied using both DLS and DSC. Variation of z-average particle size with increasing temperature was clearly observed in the plot of particle size versus temperature (Figure 7).The particle size initially varied randomly

when the temperature was raised upto 46 ^{0}C. Particle size measurement using DLS is based on the scattering of light, assuming the particles to be hard spheres. Particles may change the shape on raising the temperature and this will affect the particle size as measured by DLS. When the temperature was increased further, particle size decreased significantly. This is due to melting of the surface of nanoparticles and the dispersion of agglomerated particles.

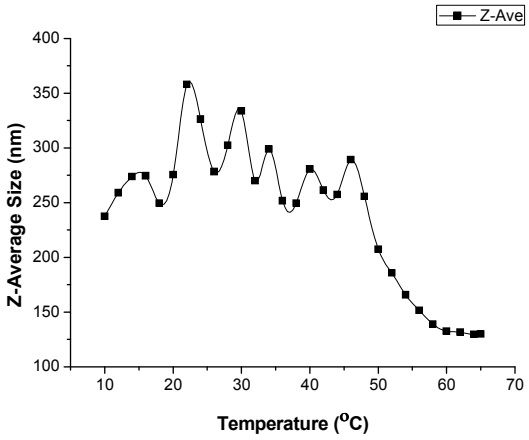

Figure 7 Plot of Z-average particle size that was measured using DLS versus temperature.

Thermal response of the SASLNs was studied by DSC also and was compared with the thermal response of the raw-ingredients. The DSC thermal curves are shown in Figure 8. The corresponding phenomenological data is given in Table 2. The pure components had single endotherms corresponding to their melting points. Gelucire® 50/13 has the lowest melting point of 40 ^{0}C. Melting point of stearic acid and paliperidone was 57 and 173 ^{0}C respectively. Thermal curve of SASLNs loaded with drug showed two endotherms corresponding to the melting of the surfactant and the lipid. This showed that the surfactant melted first followed by the lipid. A physical mixture of the ingredients also showed similar thermal response to the SASLNs. This confirmed that there was no interaction between the ingredients in the SASLNs. Melting peak of paliperidone was not observed in the thermal curve of SASLNs. This is because paliperidone was not crystallised in the lipid matrix as revealed by XRD. Additionally, paliperidone gets solubilized in the molten lipid matrix.

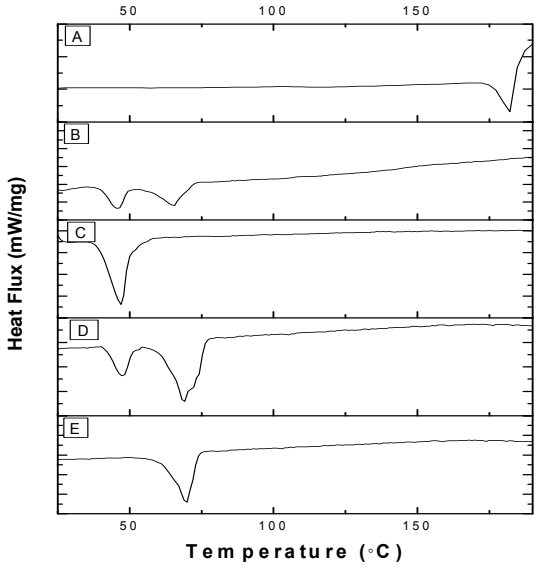

Figure 8: DSC thermal curves of (A) Paliperidone, (B) physical mixture of ingradients, (C) Gelucire® 50/13, (D) SLNs loaded with paliperidone and (E) stearic Acid

Table 2. Phenomenological data for the thermal response of the SASLNs, the raw-ingredients and a physical mixture of ingredients recorded using DSC.

Sr. No.	Sample	Onset of melting (°C)	Peak melting temperature (°C)
1	Paliperidone	173	182
2	Stearic Acid	56	70
3	Gelucire® 50/13	40	46
4	SLNs loaded with paliperidone	41	47, 69
5	Physical mixture of ingredients	40	45, 66

Entrapment efficiency and % drug loading

Entrapment efficiency of paliperidone was determined by UV-visible spectroscopy. Paliperidone has good solubility in 0.1 N HCl. Hence, 0.1 N HCl was used to disrupt the SLNs and solubilize paliperidone. The entrapment efficiency of paliperidone in the lipid was calculated to be 42.4 (% w/w). The corresponding % drug loading was calculated to be 4.1 (% w/w) of the total lipid content. Entrapment efficiency and % drug loading of SASLNs was lower than the entrapment efficiency of Capmul® GMS 50K due to the high crystallinity of stearic acid. However, higher amount of SASLN loaded with paliperidone can be administered due to the lower toxicity of stearic acid and Gelucire® 50/13 compared to sodium deoxycholate.

Invitro drug release kinetics

Dialysis bag method was used for the release kinetics study. Data was collected and computed for cumulative release after 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 h by keeping the same experimental parameters for all the samples in PBS (6.8 pH) and HCl (0.1N HCl). Data was plotted between time and % cumulative release of paliperidone from SASLNs. The drug release profiles in HCl and PBS buffer (pH 6.8) are shown in Figure 9. Initial burst release of paliperidone was observed in 0.1 N HCl and PBS (6.8 pH) upto 8 h. Rate of release was slowed down thereafter.

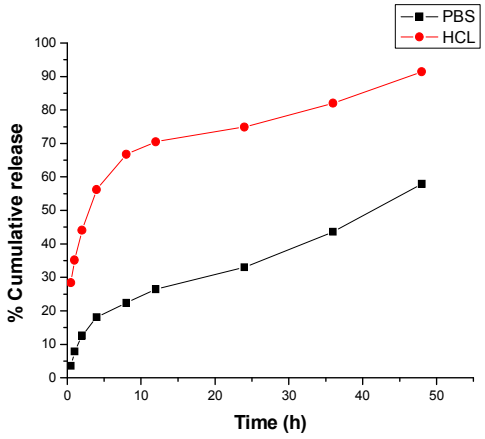

Figure 9: Invitro drug release profile of paliperidone from SASLNs in acidic (0.1 N HCl) and neutral (PBS, pH 6.8) conditions. The release profile was studies by dialysis bag method.

This burst effect may be due to the release of paliperidone from the outer layer of lipid matrix as well as the surfactant shell of the nanoparticles. The quantity of paliperidone released in the burst release phase was much higher in acidic pH than in neutral pH. This is because of the higher solubility of paliperidone in acidic medium. It is known that paliperidone acts as a mild base in acidic medium and hence has enhanced solubility in acidic pH.[18] Its solubility drops as the pH goes up as it behaves as a neutral molecule in higher pH media.[18] Release rate after the 8 h was found to be slow because the drug has to cross the lipid matrix and release is limited by diffusion. Erosion of the lipid matrix was found to negligible as the rate of drug release was almost a constant after the burst release phase. Hence, these observations support the pH dependant, diffusion controlled, extended release pattern of Paliperidone from the SASLNs.

In-Vitro Cell Culture Study

The toxicity of SASLNs on living cells was studied in vitro on macrophages cell line Raw 264.7. Viability of cells after the treatment with SASLNs was analysed and the plot of %viability versus concentration of SASLN is shown in Figure 10. The concentration of SASLN was varied in the range of 30-120 µg/ml to check the effect of SLNs on the cells. Interestingly, the viability of macrophage cells was lowered considerably even at a low concentration of SLNs. The concentration of SASLNs did not increase the toxicity considerably.

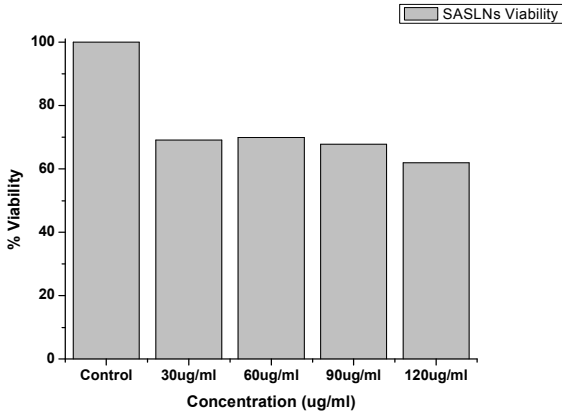

Figure 10 Cell viability of RAW 264.7 murine macrophages after the treatment with SASLNs

Paliperidone is a commercially available drug and hence clinically proven to be safe. Stearic acid is produced from vegetable oils and is a naturally occurring chemical in fats and oils. It is in the list of chemicals that are generally recognized as safe (GRAS) by food and drug administration (FDA).[19] Gelucire 50/13 also is a commonly used excipient that has been used for preparing floating lipid beads and was tested invivo in humans, yet no toxicity was reported.[20] It appears that the observed toxicity of paliperidone loaded SASLNs is due to the typical morphology and nature of the surface of the SLNs. It has to be noted that Scholer et al. observed marked cytotoxic effects when murine peritoneal macrophages cells were incubated with SLN

consisting of stearic acid or dimethyl-dioctadecylammonium bromide at concentrations of 0.01%.[21] Whereas, SLN consisting of triglycerides, cetylpalmitate or paraffin did not exert major cytotoxic effects at the same concentrations. They observed a remarkable effect of concentration of SLNs as the stearic acid SLNs were non-toxic at lower concentrations (0.001 %). There was no effect of particle size and hence they concluded that the products of enzymatic degradation of the lipid matrix such as free fatty acid could be the cause of apparent toxicity. However, there was no apparent effect of concentration of SASLNs on the toxicity in the present study. Also, the cell viability even at high concentration of SASLNs loaded with paliperidone was at least 60 % whereas almost 100 % cell death was observed by Scholer et al. However, there is a growing opinion that characteristics of nanoparticles such as size, charge and surface properties will influence their pharmacokinetics after oral administration.[22] Hence, further studies are required to thoroughly understand the observed toxicity of paliperdone loaded SASLNs.

Experimental

Materials

Gelucire® 50/13 pellets were provided by Gattefosse France as a gift sample. Double distilled water from Bio-age water purification system was used in all the preparations and washing. Stearic Acid, Hydrochloric acid and potassium bromide were purchased form Fischer chemicals.

Methods

Preparation of SASLNs stabilized by Gelucire® 50/13 with and without Paliperidone

Method used for the preparation of SLNs was similar to our previous study.[2,3] Preparation of SLNs involved three steps. Exact quantity of Stearic Acid and Gelucire® 50/13 was primarily weighed and heated to 80 °C so that the whole mixture melts completely. Paliperidone was subsequently weighed and suspended in molten lipid through stirring at 80 °C. Afterwards water was added to the drug-lipid-stabilizer mixture by incessant stirring to make a pre-emulsion. The pre-emulsion was further homogenized by ultrasound homogenizer to trim down the particle size. The hot micro-emulsion was cooled slowly up to 4 °C for solidification. The solid particles were collected by centrifugation at 10000 RPM for 2 hours. The particles obtained were dried in vacuum oven at 40 °C to obtain dried samples for further analysis.

Z-average particle size measurement

Diluted samples of SLNs were analyzed using Malvern Zeta sizer Nano ZS at a detecting angle of 173^0 at room 25 ^{0}C. Z–average particle size and polydisperisity index were recorded.

Transmission electron microscopy

The Tecnai G20 S-Twin of FEI, USA was used to obtain HRTEM images at 200 kV. SLN samples were negatively stained by phospho tungstic acid. Images were obtained in dark field as well as bright field modes at different magnifications.

Atomic force microscopy

The low frequency silicon cantilever at frequency of 146 kHz to 236 kHz was used with a closed loop scanner in SPM 5500 (Agilent) system to acquire AFM images. Processing of images was done by Pico image software.

X-ray diffraction

XRD patterns of the processed powder samples was collected in the 2θ range of 10-50° with a scan step size of 2° using Cu Kα1with λ=1.54060 Å as anodic material by PanalyticalXpert Pro.

FTIR spectroscopy

FTIR spectra of dried powder samples pelletized along with KBr powder in the range 4000-400 cm^{-1} at a resolution of 1 cm^{-1} was measured by Perkin-Elmer Spectrum 65 spectrophotometer.

Differential scanning calorimetry (DSC) analysis

Small quantities of samples were analyzed by NetzschSTA 449 F1 in hermetically sealed aluminium pans for DSC measurements at the heating rate of 2 ^{0}C/min from 25 °C to 200 °C under N_2 flow at the rate of 60 ml/min.

Entrapment efficiency and percentage drug loading

The free content of paliperidone present in SLNs suspension was

solubilized with 0.1N HCl and centrifuged to separate out SLNs from the un-entrapped drug in solution/supernatant. Then the supernatant was filtered through 0.45µm filter for collecting solution free from any particle contaminant. Quantification of paliperidone was done by using UV-Visible-NIR spectrophotometer. λ_{max} at 238 nm was used to plot the calibration curve (10-50 µg/ml) for the computation of paliperidone concentration in the solution. Below mentioned formula was used to compute %EE and %DL.[17] The weight of all content was in mg in the formula and PPN refers to paliperidone.

$$\%EE = \frac{(\text{Total PPN}) - (\text{Free PPN})}{(\text{Total PPN})} \times 100$$

$$\%DL = \frac{(\text{Total PPN}) - (\text{Free PPN})}{(\text{Total PPN}) + (\text{Total Lipid}) - (\text{Free PPN})} \times 100$$

Invitro release kinetics study

In vitro drug release study was done by using submerged dialysis bag method. The solid lipid nanoparticle dispersion was placed in prewashed dialysis tubing which was sealed after filling. The dialysis sac was then dialyzed against a suitable dissolution medium (0.1N HCl and pH6.8 PBS) at 37±0.5 ^{0}C. The samples were withdrawn from the dissolution medium at suitable intervals (0.5, 1, 2, 4, 8, 12, 24h, 36 and 48h), centrifuged and analyzed for the drug content using UV-visible spectroscopy. Unknown concentration of paliperidone in the samples was found out from a calibration curve that was plotted from standard solutions of paliperidone in 0.1 N HCl.

In-Vitro cell culture assay

RAW 264.7 murine macrophages were used for in-vitro assays. The cells were cultured in DMEM containing 10 % FBS and 1% penicillin–streptomycin. DMEM and FBS were obtained from Sigma Chemicals. Antibiotic was purchased from Invitrogen. Cell cultures were maintained in flasks under standard conditions: incubation at 37 °C and 5% CO_2. All the subcultures were used prior to passage 15. Cells were routinely passaged using 0.25% trypsin/0.1% EDTA. For treatment, cells were cultured in the presence of increasing concentrations of SASLN samples. Metabolic activity was determined by MTT assay (MTT obtained from Hi-Media). Cells were plated at 90% confluence and incubated in the presence or absence of increasing concentration of SLNs. After 24 h incubation, cells were treated with MTT solution for 4 h at 37 °C in a cell culture incubator. MTT which is a tetrazolium salt is converted into insoluble formazan by mitochondrial dehydrogenases in live cells. Formazan is dissolved in DMSO (Merck) and absorbance was measured at dual wavelength of 550 nm and 630 nm on an ELISA plate spectrophotometer. The total number of viable cells relative to viable cells in untreated control was calculated.

Conclusions

Spherical SLNs with a core-shell structure having the surfactant adsorbed on the stearic acid core was prepared. The concentration of the surfactant played a crucial role in particle size reduction due to the core-shell morphology of the SASLNs, with the lipid core and surfactant shell. Drug loading increases the particle size of SASLNs only to a minor extent. FTIR, XRD and DSC analysis revealed that there was no chemical interaction between the ingredients of the SASLNs. However, molecular dispersion of the paliperidone in the stearic acid matrix led to the reduction in crystallinity of stearic acid. Drug encapsulation efficiency and % drug loading of the stearic acid matrix is lower than Capmul® GMS 50K due to the higher crystallinity of the former. However, higher amount of paliperidone loaded SASLNs can be administered due to the neuroprotective effects and lower toxicity of stearic acid. The release of paliperidone from the SASLNs was pH dependent, but diffusion controlled extended release could be achieved. The paliperidone loaded SASLNs showed some cytotoxicity against RAW 264.7 murine macrophages. Characteristics of the nanoparticles such as size, surface charge and charge could be the reason for the observed toxicity as the toxicity of SASLNs was not concentration dependent.

Notes and references

[a]*Centre for Material Science and Engineering, National Institute of Technology, Hamirpur, Himachal Pradesh, India-177005*
[b]*School of Engineering, Indian Institute of Technology Mandi, Himachal Pradesh, India.E-mail: jaspreet@iitmandi.ac.in; Tel:+91 9418085224*
† Corresponding Author

1. D. Bishara, *Neuropsychiatric Disease and Treatment*, 2010, **6**, 561-572.
2. S. Kumar and J. K. Randhawa, *Colloids and Surfaces B: Biointerfaces*, 2013, **102**, 562-568.
3. S. Kumar and J. K. Randhawa, *RSC Advances*, 2014, **4**, 30186-30192.
4. Z.-j. Wang, G.-m. Li, W.-l. Tang and M. Yin, *Acta Pharmacol Sin*, 2006, **27**, 145-150.
5. V. P. Torchilin, *Nanoparticulates as Drug Carriers*, Imperial College Press, 2006.
6. A. V. Bailey, D. Mitcham, R. A. Pittman and G. Sumrell, *J Am Oil Chem Soc*, 1972, **49**, 419-421.

7. V. Bello, G. Mattei, P. Mazzoldi, N. Vivenza, P. Gasco, J. M. Idee, C. Robic and E. Borsella, *Microscopy and Microanalysis*, 2010, **16**, 456-461.
8. P. Severino, S. C. Pinho, E. B. Souto and M. H. A. Santana, *Colloids and Surfaces B: Biointerfaces*, 2011, **86**, 125-130.
9. E. Ugazio, R. Cavalli and M. R. Gasco, *International Journal of Pharmaceutics*, 2002, **241**, 341-344.
10. H. Yuan, J. Chen, Y.-Z. Du, F.-Q. Hu, S. Zeng and H.-L. Zhao, *Colloids and Surfaces B: Biointerfaces*, 2007, **58**, 157-164.
11. F. M. Menger and A. R. Elrington, *Journal of the American Chemical Society*, 1991, **113**, 9621-9624.
12. S. Tcholakova, N. D. Denkov and T. Danner, *Langmuir*, 2004, **20**, 7444-7458.
13. M. J. Schick, *Nonionic Surfactants: Physical Chemistry*, Taylor & Francis, 1987.
14. J. Ayache, L. Beaunier, J. Boumendil, G. Ehret and D. Laub, *Sample Preparation Handbook for Transmission Electron Microscopy: Methodology*, Springer, 2010.
15. J. R. R. Ravi, R. M. Pulla, K. S. B. R. Adibhatla and C. N. Venkaiah, PCT/IN2009/000077.
16. F. Kimura, J. Umemura and T. Takenaka, *Langmuir*, 1986, **2**, 96-101.
17. A. E. Yassin, M. K. Anwer, H. A. Mowafy, I. M. El-Bagory, M. A. Bayomi and I. A. Alsarra, *International journal of medical sciences*, 2010, **7**, 398-408.
18. A. Aleksovski, M. Luštrik, R. Šibanc and R. Dreu, European Journal of Pharmaceutical Sciences (2015), doi: http://dx.doi.org/10.1016/j.ejps.2015.03.010.
19. Code of Federal Regulations, Title 21, Volume 3, 2014, 21CFR184.1090.
20. S. M. Abouelatta, A. A. Aboelwafa, R. M. Khalil, O. N. ElGazayerly, *European Journal of Pharmaceutics and Biopharmaceutics* 2015, **89** 82–92.
21. N. Scholer, H. Hahn, R.H. Muller, O. Liesenfeld, *International Journal of Pharmaceutics* 2002, **231**, 167–176.
22. A., Francisca, *Expert Opinion on Drug Metabolism & Toxicology*, 2015, **11(3)** 381-393.