

Design, development and *in vivo* pharmacokinetic evaluation of zotepine loaded solid lipid nanoparticles for enhanced oral bioavailability

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Running title: Zotepine lipid nanoparticles

ABSTRACT

The purpose of this work was to prepare and evaluate the zotepine (ZT) loaded solid lipid nanoparticles (SLNs) that might improve the oral bioavailability. ZT-SLNs were developed using homogenization method and characterized for optimal system based on physicochemical characteristics and in-vitro release. Optimized ZT-SLNs were evaluated for permeation, crystalline nature using DSC and XRD, surface morphology using SEM and physical stability. Further, pharmacokinetic (PK) studies of ZT-SLN were conducted in Wistar rats comparison with ZT-coarse suspension (ZT-CS). Optimized formulation showed Z-avg, PDI, ZP of 138.1 ± 3.2 , 0.23 ± 0.02 and -26.4 ± 1.5 mV, respectively. In-vitro release studies showed prolonged release, DSC and XRD studies revealed the conversion of ZT to amorphous form. SEM studies showed spherical shape. Permeability and PK studies showed 1.4-folds and 2.0-folds improvement in oral bioavailability, respectively in comparison with ZT-CS formulation. Therefore, the results concluded that SLNs could be considered as a new alternative delivery system for the enhancement of oral bioavailability of ZT.

Keywords: Zotepine, solid lipid nanoparticles, crystallinity, *in vitro* release, *ex vivo* permeation, oral bioavailability.

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INTRODUCTION

The amount of the drug reaching to the systemic circulation mainly depends on the rate of absorption, which in turn depends on the drug solubility¹. Oral delivery of the drugs is the most conventional and widely used technique for the administration of majority of active moieties. However, several compounds are unsuccessful and failed owing to their low absorption and low bioavailability upon oral administration². The drugs with poor oral bioavailability are unable to reach the minimum effective concentration to exhibit therapeutic action. Some of the reasons for poor bioavailability include: poor solubility; inappropriate partition coefficient as it influences the permeation of drug through lipid membrane; first-pass metabolism; P-glycoprotein (P-gp) mediated efflux; and degradation of drug in the gastrointestinal tract (GIT) due to pH of the stomach or enzymatic degradation or by chemical³.

To overcome the solubility and oral bioavailability problems of Biopharmaceutical classification system (BCS) class II and IV drugs, the research in the present scenario is focused on bioavailability improvement by using various solubility enhancement techniques like liquisolid compacts^{4,5}, micronization using nanosuspensions⁶, solid dispersions using complexation^{7, 8}, salt formation⁹. Majority of the drugs undergo first-pass effect after oral administration, there by the therapeutic concentration required in systemic circulation is lowered. However, by using the lipid based delivery systems both the solubility and first-pass effect problems are resolved and subsequently oral absorption was improved³.

Lipid based drug delivery systems are one of the approaches, which favors the lymphatic transport of oral administered drugs by escaping the first-pass effect^{10,11}. Colloidal lipid nanocarriers such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are the promising approaches in the lipid based delivery systems. SLNs are mainly developed with the solid lipid carrier, used for incorporation of drug, and lipid dispersion is stabilized by the use of biocompatible surfactants¹². SLNs offer remarkable advantages such as low toxicity, high bioavailability (pharmacokinetic and pharmacodynamic) of drugs, versatility of incorporation of hydrophilic and lipophilic drugs, and feasibility of large-scale production. SLN formulations have controlled drug release properties and provides enhanced chemical stability of drug molecules¹³. SLNs also used for drug targeting to various tissues such as liver¹⁴ and ocular¹⁵ for treatment of various diseases.

Zotepine (ZT) is an atypical anti-psychotic drug, which belongs to BCS class II category. The mechanism of action of ZT is mediated through combined dopa-

mine and serotonin antagonisms¹⁶. It has a poor oral bioavailability of about 7-13%, due to poor aqueous solubility (0.046 µg/L), highly lipophilic (log P 5.6), and also hepatic first-pass metabolism. The drug levels were also decreased in the presence of CYP enzyme^{17,18}. In oral route, 30% of the drug metabolizes into Nor-zotepine (active metabolite) and remaining 70% of drug transforms to inactive metabolites such as 3-hydroxyzotepine, 2-hydroxyzotepine and zotepine-S-oxide¹⁶. Previously, Pailla et al.,¹⁹ developed intranasal delivery of ZT for enhanced brain targeting, using nanosuspension formulation. The enhanced solubility and in vitro dissolution rate of ZT were observed and reported with self-emulsifying drug delivery systems²⁰. But, till now there are no lipid nanocarrier systems were reported for enhanced oral delivery of ZT. Hence, in this attempt ZT loaded SLNs were developed could might be improve the oral bioavailability.

The objective of the present investigation was aimed to develop and characterize ZT loaded SLNs for enhanced oral delivery. Accordingly, ZT-SLNs were prepared using know methods and evaluated for an optimized formulation based on the physical and chemical parameters, in vitro release studies. Further, optimized formulation also characterized for solid state characterization for crystallinity, SEM for surface morphology and *ex vivo* permeation studies. *In vivo* performance of optimized ZT-SLN were observed by pharmacokinetic (PK) studies in male Wistar rats, comparison with ZT coarse suspension (ZT-CS) as control.

METHODOLOGY

Materials

Zotepine was a kind gifted sample from Symed labs, Hyderabad, India. Stearic acid (SA), glyceryl monostearate (GMS), Dynasan-118, Dyansan-116 and Dynasan-114 were purchased from Sigma-Aldrich, Hyderabad, India. Compritol 888 ATO was gift sample from Neuheit Pharma Technologies Private Ltd., Hyderabad. Soylecithin was a gifted sample from Lipoid, Ludwigshafen, Germany. Captex355, Captex200 were gift samples from Abitec corporation. All other chemicals were of analytical grade and solvents were of HPLC grade.

Methods

Solubility studies

ZT solubility in various solid lipids (stearic acid, glyceryl monostearate (GMS), Compritol ATO 888, Precirol ATO5, Dyansan-114, Dyansan-116 and Dynasan-118) was determined by using shaking method. Solid lipids were heated to above 5°C of their melting points. An excess amount of ZT was added and was

continuously stirred on gyrator shaker at 180 rpm for 48 h. The supernatant was collected and filtered through 0.45 μ membrane filter after centrifugation²¹. Necessary dilutions were made to the filtrate with methanol and estimated the drug concentration using UV-Visible spectrophotometer (SL-159, Elico, Hyderabad, India) at λ_{max} of 261 nm. Simultaneously, solubility of ZT in release media such as distilled water, 0.1N HCl (pH 1.2), phosphate buffer of pH 6.8 and pH 7.4.

Preparation of solid lipid nano particles (SLNs)

ZT-SLNs were prepared using homogenization-probe sonication method, based on film hydration method²². Required amounts of ZT, solid lipid (SA, GMS, Compritol ATO888), and soy lecithin were dissolved in 10 mL mixture of chloroform and methanol (1:1) in a round bottom flask. Organic solvents were removed using rota evaporator (Heidolph, Germany). Drug embedded lipid layer was molten by heating at 5°C above the melting point of the solid lipid. Aqueous phase was prepared by dissolving Poloxamer188 in double distilled water and heated to the same temperature of oil phase. Hot aqueous phase was added to oil phase. Homogenization was carried out at 12,000 rpm for 5mins to form pre-emulsion. The obtained emulsion was ultra-sonicated using Probe sonicator with 12T probe tip (Bandelin, Germany) for 20 mins. ZT-SLNs were obtained upon cooling to room temperature. The composition of the SLNs are given in Table 1.

Table 1: Composition of zotepine loaded solid lipid nanoparticles and control formulation

Ingredients (%w/v)	ZT-SLN1	ZT-SLN2	ZT-SLN3	ZT-SLN4	ZT-SLN5	ZT-SLN6	ZT-CS
Zotepine (mg)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glyceryl monostearate	1.0	-	-	2.0	-	-	-
Stearic acid	-	1.0	-	-	2.0	-	-
Compritol ATO 888	-	-	1.0	-	-	2.0	-
Soylecithin	1.0	1.0	1.0	1.0	1.0	1.0	-
Poloxamer 188	1.5	1.5	1.5	1.5	1.5	1.5	-
Na CMC*	-	-	-	-	-	-	0.05
Water (mL)	QS 10	QS 10					

*Na CMC – sodium carboxy methyl cellulose

Preparation of ZT coarse suspension (ZT-CS)

ZT-CS formulation was prepared by taking about 100 mg of sodium carboxy methyl cellulose (suspending agent) in a mortar and triturated for 15 mins. Then, 10 mg of ZT was added to it and together triturated. To it, 10 mL of double distilled water was added and again triturated for about 5 min to obtain ZT-CS (1 mg/mL). This was used as control formulation.

Characterization of ZT-SLNs

Measurement of particle size, PDI and zeta potential (ZP)

The mean size as Z-avg (nm), PDI and ZP of ZT-SLNs were measured by using photon correlation spectroscopy, using a Malvern Zetasizer (Nano ZS90, UK). About 100 μ L of prepared SLN formulations were diluted to 5 mL with double distilled water to get optimum Kilo Counts Per Second (Kcps) of 50-200 for measurements in triplicate at 25°C²³.

Drug content and entrapment efficiency (EE)

About 0.1 mL of prepared ZT-SLN formulation was taken and diluted with chloroform: methanol (1:1) and drug content of the diluted samples was estimated by HPLC-method. The EE (%) in ZT-SLN formulation was calculated by estimating the concentration of the free ZT in the aqueous phase of an undiluted formulation, using an ultrafiltration method centrivot tubes (Sartorius, Germany). The aqueous medium was separated by ultra-filtration using centrivot tubes (Sartorius, Germany) which consisting a filter membrane (M.Wt.20,000 Daltons) at the base of the sample recovery chamber. Centrifugation was carried at 5000 rpm for 30 min²⁴. The SLN along with the encapsulated drug remained in outer chamber and aqueous phase moved into sample recovery chamber through filter membrane. The amount of zotepine in aqueous phase was estimated by HPLC method. The EE was calculated by using the equation 1:

$$\% EE = \left[\frac{D_i - D_f}{D_i} \right] \times 100 \quad (1)$$

Where, D_i is the total ZT content and D_f is the free ZT present in the aqueous phase.

***In vitro* drug release studies**

The *in vitro* drug release study of ZT-SLN and ZT-CS formulation was performed using dialysis method. Dialysis membrane (Hi media, Hyderabad, India) having average pore size 2.4 nm and molecular weight cut-off between 12,000-14,000 Daltons were used for the release studies²⁵. Dialysis membrane was soaked overnight in double distilled water prior to the study. Release studies were carried out for first 2 h in 0.1N HCl, followed by pH6.8 phosphate buffer for 48 h by open tube method. Dialysis membrane was tied to open tube which acts as a donor compartment. Temperature of receptor compartment was maintained at $37 \pm 0.5^\circ\text{C}$. Two mL sample was withdrawn at regular time points of 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, and 48 h from receiver compartment and replenished with same volume of fresh buffer. The collected samples were suitably diluted and analyzed by UV-Visible spectrophotometer.

***Ex vivo* studies by normal sac method**

Ex vivo studies were performed for optimized ZT-SLN formulation by using evert-sac method²⁶. The studies were conducted with prior approval of Institutional Animal Ethical Committee (IAEC/01/UCPSC/KU/2018). Evert-sac studies were carried out in order to investigate the permeation behavior of the control formulation and optimized ZT-SLN formulation (1 mL) across the small intestine. In this study, male Wistar rats of 200 ± 30 g ($n=3$) were taken and subjected to overnight fasting. The rats were sacrificed by cervical dislocation technique and jejunum of 4 cm were isolated, flushed with saline solution and transferred into oxygenated Krebs's ringer solution. The one end of the sac was tied with thread and filled with the optimized ZT-SLN or ZT-CS as control (1 mL) formulations and the other end was tied. The segment was immersed in 100 mL in Krebs's ringer solution and the medium was oxygenated using aerator. At regular time intervals (0, 15, 30, 45, 60, 90 and 120 min) samples were withdrawn from beaker and analyzed for drug content²⁷. The permeability coefficient of the SLN and control formulation was calculated from the slope of linear portion, by plotting the percentage drug permeated vs time²⁸.

Lyophilization of ZT-SLNs

The optimized ZT-SLN formulation was subjected to lyophilization. The SLN formulation was prepared, using 10% w/w of trehalose dihydrate as cryoprotectant. Prepared ZT-SLN formulation was kept in -80°C freezer for overnight and subjected to lyophilization (Lyodel, Chennai, India) with applied vacuum^{29, 30}. Lyophilized formulation was subjected to particle size analysis, drug content, EE and *in vitro* release before and after lyophilization.

Solid-state characterization

Differential Scanning Calorimetry (DSC)

DSC experiments were through using Perkin Elmer DSC 4000 (USA) model to find out the presence of any interaction between drug and the excipients and also to check the alterations in crystallinity of the drug. About 8 mg of the pure drug, physical mixture and optimized lyophilized ZT-SLN formulation samples were taken in aluminium pans, using dry nitrogen as purging gas. The heating rate was 10°C/min and the obtained thermograms were observed for any type of interaction³¹.

X-ray diffraction studies

Crystalline nature of Powder X-ray diffractometer (XRD-6000, Shimadzu, Japan) was used for diffraction studies (Arun et al., 2017). Powder XRD studies were performed on the samples by exposing them to nickel filtered CuK α radiation (40 kV, 30 mA) and scanned from 2° to 70°, 2 θ at a step size of 0.045° and step time of 0.5 s. Samples used for PXRD analysis were pure ZT, pure lipid and lyophilized ZT loaded solid lipid nanoparticles³².

Surface morphology by scanning electron microscopy (SEM)

The morphology of pure ZT and freeze-dried ZT-SLN formulation were investigated by scanning electron microscope (SEM S-3700, Hitachi, Japan). The samples were fixed on a brass stub and were coated by thin layer of gold to make electrically conductive²⁵. SEM images were recorded at various magnification levels.

Bioavailability study

Study design and sampling schedule

Healthy male Wistar rats (weighing 210±30 g) were used for the PK study. The animals were fasted overnight and had free access to water. The studies were conducted with prior approval of Institutional Animal Ethical Committee (IAEC/01/UCPSC /KU/2018). The animals were divided into two groups (each with n=6) and were orally administered with ZT-CS and optimized ZT-SLN formulation at a dose of 2.2 mg/kg body weight. Both the formulations were administered orally with the help of sterilized rat oral feeding tube. At predetermined time intervals, 0.5 mL of blood samples were collected at predetermined (0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h) time intervals by retro-orbital venous plexus puncture. The blood samples were allowed to clot and centrifuged for 15 mins at 3000 rpm. The serum was separated and transferred into clean micro centrifuge tubes and stored at - 20 °C until analysis.

HPLC method

HPLC analysis was conducted with C₁₈ column (250 mm X 4.6 mm; 5 μm). Mobile phase consisting of 45: 55 v/v ratio of acetonitrile and phosphate buffer adjusted to pH 4.7 at a flow rate of 1 mL/min at 261 nm with UV detector was used for detection of peaks.

Extraction procedure from rat serum sample

To 100 μL of serum, 100 μL of internal standard (Ramipril, 2 μg/mL), 300 μL of methanol was added. After vortex mixing for 5 min at room temperature, the samples were centrifuged at 5000 rpm for 15 min. After centrifugation the whole organic layer was separated and transferred into another eppendorf tube. Finally, a volume of 20 μL was injected into HPLC system.

Estimation of Pharmacokinetic parameters and statistical significance

Kinetic 2000 software (version 5.0, USA) was used for the estimation of PK parameters like C_{max}, T_{max}, AUC, t_{1/2} and MRT and the values were expressed in mean ± SD. One-way ANOVA was performed using Graph pad prism software (version 5.03, San Diego, CA, USA) for statistical comparison of data at p value of less than 0.05.

Physical stability studies

An optimized ZT-SLN formulation was stored at room temperature and refrigerated temperature conditions over two months to assess the physical and chemical stability. Z-avg, PDI, ZP, assay and EE of the samples were observed at predetermined time intervals and checked for statistically significance.

RESULTS AND DISCUSSION

Solubility studies

Solubility of ZT was studied in various solid lipids. SA, GMS, Compritol ATO 888 were selected as solid lipids for SLN formulations development. In order to determine the effect of mono and mixed glycerides on the SLN formulation SA, GMS and Compritol ATO 888 was selected. Order of solubility of ZT in solid lipids were GMS, SA, Dynasan-118, Dynasan-114 (showed nearly same solubility) > Compritol ATO888 > Dynasan-112 > Precirol ATO5. The solubility of ZT was decreased with increased pH of dissolution media and indicates pH dependent solubility. The order of solubility in release media as follows: water > 0.1N HCl > pH 6.8 phosphate buffer > pH 7.4 phosphate buffer.

Characterization of ZT-SLNs

ZT-SLNs formulations were prepared using three different lipids such as GMS, SA and Compritol ATO 888, each at 1 and 2% w/v, respectively. Homogenization followed by probe sonication method used for the preparation of ZT-SLNs. The homogenization time and probe sonication time used at 12000 rpm for 5 mins and 20 mins at 40% amplitude, respectively. The conditions were optimized based on the earlier reported methods²³.

Measurement of particle size, PDI and ZP

Prepared ZT-SLNs were characterized for particle size, PDI and ZP, using Zeta-sizer and reported in Table 2. Particle size, PDI and ZP of the ZT-SLNs formulations (ZT-SLN1 to ZT-SLN6) were ranged from 137.3±3.5 to 340.4±3.0 nm, 0.22±0.01 to 0.54±0.08, -10.6±0.3 to -27.4±2.5 mV, respectively. From the results, as the concentration of lipids increased from 1% w/v to 2% w/v, increased in particle size and PDI, decreased in ZP were observed. This might be due to the changes in the orientation of the drug and presence of the void spaces of lipid and drug. PDI of the formulations (SLN1-SLN5) were below 0.5, except for formulations prepared with Compritol ATO® 888. The PDI value of up to 0.5 were considered to be homogeneous dispersion and was evidenced from the earlier reports.^[15] ZP of the ZT-SLNs were crucial factor the stability assessment. In this case, the combination of surfactants imparts the electrostatic repulsion and steric stabilization as well. The presence of Poloxamer 188 in the development of SLNs could be favored for the maintenance of this ZP³³. In general, ZP with ±30 mV could be considered as stable dispersion system. Formulations prepared with GMS showed above -25 mV and indicates stability. But, formulations prepared with SA had below -20 mV of ZP.

Table 2: Physicochemical characteristics - size, PDI, Zeta potential, entrapment efficiency and assay of zotepine loaded solid lipid nanoparticle formulations (mean ± SD, n=3)

Formulation	Size (nm)	PDI	ZP (mV)	Assay (mg)	EE (%)
ZT-SLN1	137.3±3.5	0.22±0.01	-26.7±1.7	9.9±0.4	95.1±2.1
ZT-SLN2	154.5±4.7	0.26±0.06	-27.4±2.5	9.2±0.4	90.4±3.2
ZT-SLN3	187.3±2.8	0.46±0.05	-10.6±0.3	9.1±0.5	85.7±2.6
ZT-SLN4	253.7±5.8	0.34±0.03	-18.4±2.4	8.7±0.4	88.9±2.5
ZT-SLN5	340.4±3.0	0.29±0.07	-29.4±2.1	8.9±0.1	92.6±1.6
ZT-SLN6	303.3±2.1	0.54±0.08	-25.6 ± 1.0	8.8±0.4	94.8±3.5

Drug content and entrapment efficiency

All the formulations were analyzed for EE and total drug content by HPLC (Table 2). EE of the all the formulations (ZT-SLN1 to ZT-SLN6) were ranged from 85.7 ± 2.6 to $95.1 \pm 2.1\%$. As the concentration of lipid increases, EE of the ZT-SLNs were decreased, but not significant difference. This is due to the presence of less orientation of drug molecule in the lipid matrix. Among all the developed formulations ZT-SLN1 formulation is having the high EE. Drug content of all the formulations were in the range of 8.7 ± 0.4 to 9.9 ± 0.4 mg.

In vitro dissolution studies

All the prepared SLNs were subjected to *in vitro* release studies, using dialysis method. 0.1N HCl pH 1.2 followed by pH 6.8 phosphate buffer for the release studies. *In vitro* release of ZT from SLN formulations were observed to 69 ± 2.1 to $82.9 \pm 3.6\%$ range, over a 48 h (Figure 1). However, formulations prepared with 1% and 2% w/v lipid concentrations were not significantly influence the drug release behavior from the SLN formulations. This could be due to the high EE (more than 90%) and also free drug availability in the outer lipid phase was very less. Formulations prepared with GMS, SA and Compritol ATO888 showed 83.3 ± 2.4 and $75.7 \pm 3.2\%$ (from SLN1 and SLN2), 76.8 ± 1.9 and $73.6 \pm 2.3\%$ (from SLN3 and SLN4), $71.5 \pm 1.2\%$ and $68.5 \pm 1.8\%$ (from SLN5 and SLN6), respectively. The crystal lattice of the lipid matrix might be the reason for difference in the drug release from ZT-SLNs. Monoglyceride formulations (GMS and SA) showed slightly better release then mixed glyceride (Compritol ATO®888) formulations. The drug release from the ZT-CS formulation showed $50.2 \pm 2.2\%$ in 120 min (data not showed). In all the prepared formulations, ZT-SLN1 formulation prepared with GMS was having less particle size and PDI, stable ZP, with high EE, acceptable drug content and also showed maximum drug release in 48 h, comparison with other formulations. Therefore, ZT-SLN1 formulation was considered as an optimized formulation and selected for further studies.

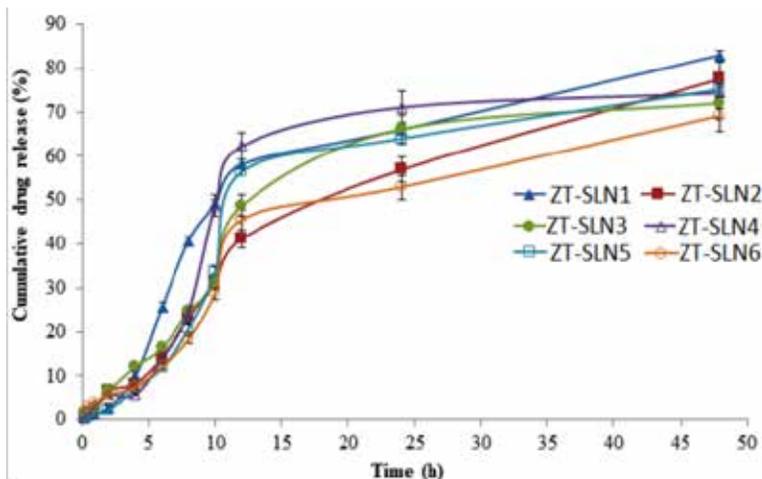


Figure 1: In vitro release profiles of zotepine from ZT-SLN_s (mean \pm SD, n=6)

Ex vivo permeation studies

Ex vivo permeation studies were performed for optimized ZT-SLN₁ formulation in comparison with ZT-CS formulation through rat intestine using everted sac perfusion method. The duration of the study was 120 min and represented in Figure 2. From the results, the % of ZT permeation from ZT-SLN₁ and ZT-CS formulation was $18.8 \pm 1.6\%$ and $28.3 \pm 2.2\%$, respectively. Statistically significance difference ($p < 0.05$) was observed from the ZT-SLN₁ compared with ZT-CS formulation. The prolonged release of the ZT from the ZT-SLN formulation was observed from the *in vitro* release and *ex vivo* permeation studies. Furthermore, the permeability rate coefficient of the ZT-SLN₁ formulation was calculated from the slope of linear portion by plotting percentage drug permeation against time. From the results, permeability rate of the ZT-SLN₁ and ZT-CS formulation was 0.19 and 0.13 ml/min. cm², respectively. About 1.4-folds enhancement in the permeation of ZT-SLN₁ was observed compared with ZT-CS formulation.

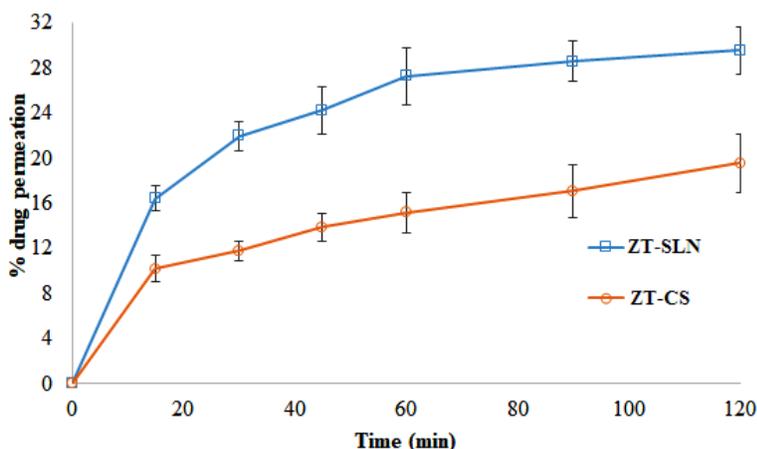


Figure 2: *Ex vivo* permeation profiles of zotepine from ZT-CS and ZT-SLN1 formulations (mean \pm SD, n=3)

Lyophilization of ZT-SLN

The crystalline nature, surface morphology and conversion of lipid nanoparticles into solid dosage forms by incorporating in capsules or compression to tablets were easy by the conversion of dispersion to solid form of the nanoparticles. This is generally achieved with either spray drying or freeze drying techniques. Optimized ZT-SLN formulation was subject to lyophilization, using 10 %w/w of trehalose as cryoprotectant. The cryoprotectant and its concentration were selected based on the previously reported methods²⁴. The lyophilized formulation was characterized for before and after lyophilization and presented in Table 3. From the results, about two to three-fold increase in the particle size and PDI of the formulation was observed after lyophilization. This could be due to the aggregation of the particles during the process of freeze-drying. But, there were no significant changes noticed in the other parameters.

Table 3: Physicochemical characteristics of optimized ZT-SLN formulation before and after lyophilization (mean \pm SD, n=3)

Condition	Size (nm)	PDI	ZP (mV)	Assay (mg)	EE (%)
Pre-lyo	145.2 \pm 2.7	0.23 \pm 0.05	-27.6 \pm 1.4	9.8 \pm 0.3	94.4 \pm 2.8
Post-lyo	288.9 \pm 5.2	0.38 \pm 0.06	-29.1 \pm 3.5	9.9 \pm 0.6	93.6 \pm 1.6

Solid state characterization

Differential Scanning Calorimetry

DSC studies were used to determine the compatibility status of the solid lipids and other excipients used in the SLN formulation, crystalline nature of the drug in the nanoformulations and was based on the fact that different lipids possessed different melting points and enthalpies. DSC thermograms of pure ZT, pure GMS, physical mixture of ZT and GMS in 1:1 ratio and lyophilized ZT-SLN1 formulation are shown in Figure 3.

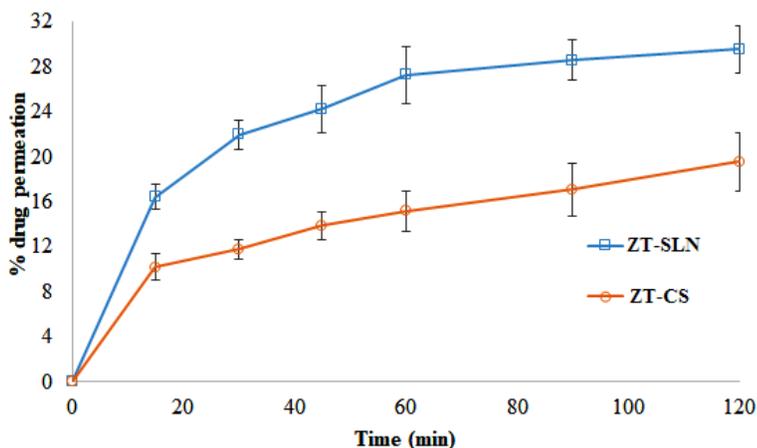


Figure 3: DSC thermograms of pure ZT (A), pure Dynasan-118 (B), physical mixture of ZT + lipid (1:1) (C) and lyophilized optimized ZT-SLN1 formulation (D)

The DSC thermogram of pure ZT showed a sharp endothermic peak at 97.76 °C and it is corresponding to reported melting point. Pure GMS showed a sharp endothermic peak at 72.06 °C. Physical mixture of ZT with GMS showed drug peaks at 98.82 °C, however with less enthalpy compared with pure ZT enthalpy. But, melting endotherm of drug was well preserved with slight changes in terms of shifting in the temperature of the melt. It is known that the quantity of material used, especially in drug-excipient mixtures, could influence the peak shape and enthalpy. Thus, these minor changes in the melting endotherm of drug could be due to the mixing of drug and excipient, which lowered the purity of each component in the mixture and this, might not necessarily indicate potential incompatibility. The absence of endotherm peak of drug in lyophilized ZT-SLN1 formulation unravels the conversion of native crystalline state of the drug to amorphous state.

XRD studies

XRD studies of pure ZT, pure lipid and lyophilized ZT-SLN1 were showed in Figure 4. From the results, powder-XRD patterns of ZT showed sharp peaks at 2θ scattered angles of 10.3, 11.4, 13.5, 17.1, 18.3, 19.8, 20.3, 23.2 and 24.1 degrees, these were indicating the crystalline nature of drug. These characteristic peaks of ZT peaks were absent in the lyophilized ZT-SLN1 sample. This specified that the ZT was not in crystalline form and converted to amorphous nature, after lyophilization of optimized ZT-SLN1. This reduction in crystallinity or conversion to amorphous state of ZT from ZT-SLN was noticed in DSC analysis as well³⁴.

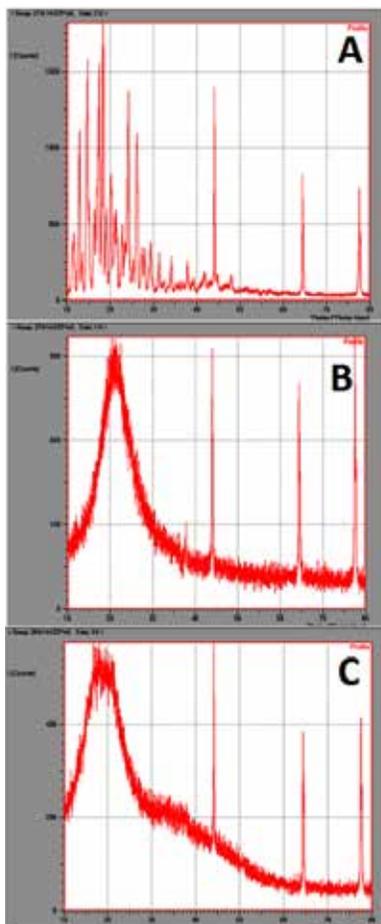


Figure 4: XRD spectra of pure ZT (A), pure Dynasan-118 (B), p and lyophilized optimized ZT-SLN1 formulation (C)

Scanning electron microscopy

The shape of the ZT before and after loading onto SLN formulation was observed using SEM method. For this purpose, surface morphology study was conducted for pure drug and lyophilized ZT-SLN formulation. The results are showed in Figure 5. SEM picture revealed that pure drug of ZT particles are possessed different shaped (cubic and nearly spherical) but highly aggregated (Figure 5A). Optimized formulation of ZT-SLN₁ particles are nearly spherical shaped and also aggregated³⁵. The results of the SEM studies also correlated with particle size of after lyophilized sample.

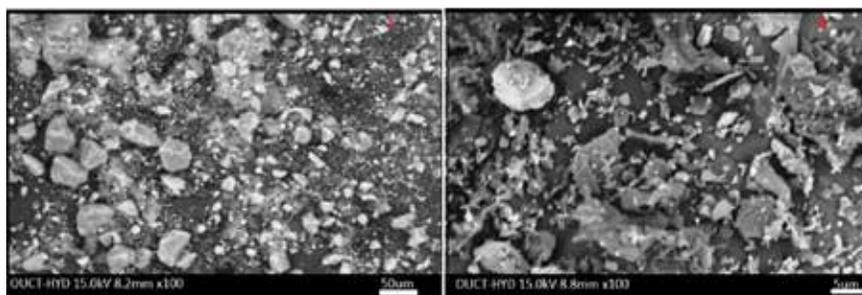


Figure 5: SEM images of pure ZT (A) and optimized ZT-SLN formulation (B)

Stability study of optimized ZT-SLN

The stability of the optimized ZT-SLN was monitored by storage at room temperature (25 °C) and refrigerated temp (4°C) for 60 days. The formulations were analyzed on 1st day, 30th day, and 60th day respectively for size, PDI, ZP, EE and drug content. The results indicated that no significant variations were noticed in all the measured parameters, and found to be stable up to two months (Table 4).

Table 4: Stability studies of optimized formulation of ZT-SLN (F1) at both room temperature (25°C) and refrigerated (4°C) for a period two of months (mean ± SD, n=3)

Time (day)	At room temperature (25°C)					At refrigerated temperature (4°C)				
	Size (nm)	PDI	ZP	EE	Assay	Size (nm)	PDI	potential	EE	Assay
1	138.1±3.2	0.23±0.02	-26.4±1.5	9.9±0.3	94.7±3.1	138.1±3.2	0.23±0.02	-26.4±1.5	9.9±0.3	94.7±3.1
30	140.5±2.7	0.23±0.04	-27.1±1.0	9.8±0.4	92.3±2.4	145.5±4.2	0.24±0.06	-25.4±1.9	9.8±0.5	91.4±2.8
60	144.3±2.7	0.24±0.05	-6.2±2.3	9.8±0.6	91.7±2.5	148.3±5.3	0.25±0.03	-24.3±1.4	9.7±0.4	90.2±2.4

PK study

Oral bioavailability study for ZT-CS and optimized ZT-SLN1 formulation was performed in male Wistar rats with $n=6$ in each group. The collected serum samples were analyzed for drug content, using HPLC method. Ramipril is used as internal standard. Retention times of both ZT and IS were 7.8 and 4.5 min, respectively. The linearity of the ZT was observed in the concentration range of 0.25-12 $\mu\text{g/mL}$. The limit of detection and limit of quantification of the ZT were 0.1 and 0.2 $\mu\text{g/mL}$, respectively. About 98.5% of extraction efficiency was observed from recovery studies.

The PK parameters of ZT from testing formulations were calculated using Kinetica 2000 software with non-compartmental model. Mean serum concentration vs time profiles of ZT-CS and ZT-SLN1 formulation after oral administration of single dose are shown in Figure 6. PK parameters like $\text{AUC}_{\text{total}}$, C_{max} , T_{max} , MRT and $t_{1/2}$ of the formulations were calculated and shown in Table 5. A significant difference observed in C_{max} and MRT of the F1 formulation, indicates the prolonged release of the ZT. AUC is the major indicator of the bioavailability of the formulations. From the results, AUC values of ZT-CS and ZT-SLN1 formulations were found to be 18.2 ± 1.3 and 37.1 ± 2.2 $\mu\text{g/mL.h}$, respectively and were statistically significant ($p < 0.05$). From the results, about 2.0-folds improvement in the bioavailability observed from ZT-SLN1 formulation in comparison with ZT-CS formulation. The improved oral bioavailability of SLNs of ZT could be due to the contribution of individual and/or combined effects of nanosized particles of the SLNs. The effective surface would influence the adhesion to GIT. Consequently, there is increased contact time of the SLN particles³⁶⁻⁴². In addition, the soylcithin and poloxamer could alter the permeability characters of the GI membrane. The fatty acid chains present in the lipids of SLNs improve the uptake by lymphatic transport. This lymphatic transport minimizes the first-pass effect of the drug⁴³⁻⁴⁷.

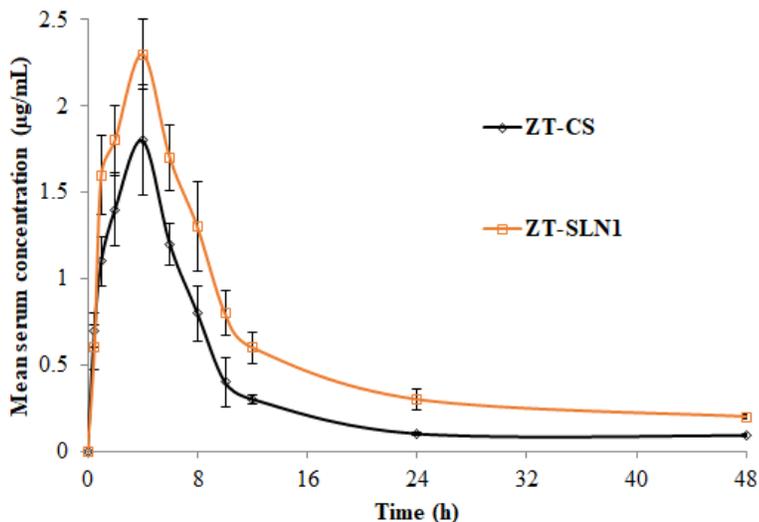


Figure 6: Mean serum concentration – time profiles of zotepine from ZT-CS and optimized ZT-SLN1 formulation after oral administration in rats (mean \pm SD, n=6)

Table 5: Pharmacokinetic parameters of zotepine from zotepine coarse suspension (ZT-CS) and zotepine solid lipid nanoparticle (ZT-SLN1) formulations after oral administration in rats (mean \pm SD, n=6)

Parameter	ZT-CS	ZT-SLN1
C_{max} ($\mu\text{g/mL}$)	1.8 \pm 0.3	2.3 \pm 0.2*
T_{max} (h)	4	4
AUC _{total} ($\mu\text{g/mL}\cdot\text{h}$)	18.2 \pm 1.3	37.1 \pm 2.2*
$t_{1/2}$ (h)	18.6 \pm 1.2	18.5 \pm 2.1
MRT (h)	17.7 \pm 2.6	23.8 \pm 1.8*

*indicates statistically significant at $p < 0.05$ in comparison with ZT-CS.

ZT loaded solid lipid nanoparticles were successfully developed for the improved oral delivery. The ZT-SLNs were prepared with well-known reported method and characterized for an optimal system. DSC and XRD studies revealed the conversion to amorphous form of ZT in SLN formulation. *In vitro* and *ex vivo* permeation studies confirms the sustained release of the ZT from SLN formulation comparison with coarse suspension as control. ZT-SLN formulation stable for two months at storage conditions. Pharmacokinetic study in male Wistar rats revealed that there was 2.0-fold improvement for SLN when compared to coarse suspension. The overall results indicated that the lipid based delivery systems considerably improved the bioavailability of ZT.

Disclosure statement

The authors declare no conflict of interest in this study.

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