EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 4
253-259
Year: 2016

DESIGN AND DEVELOPMENT OF SOLID LIPID NANOPARTICLES (SLNs) OF ZOLMITRIPTAN FOR THE TREATMENT OF MIGRAINE

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Article Received on 24/01/2016 **Article Revised on 15/02/2016** **Article Accepted on 07/03/2016**

ABSTRACT
Solid lipid nanoparticles (SLNs) of Zolmitriptan were developed by solvent diffusion evaporation method. Precirol® ATO 5 and Tween-20 were used as solid lipid and surfactant respectively. The formulation were optimized for independent variables (types of solid lipid, types and ratio of organic solvents, concentration of surfactant, temperature of secondary phase and drug: lipid ratio) in order to achieve desired particle size with maximum percent Entrapment efficiency (%EE). Prepared SLNs were characterized by Transmission Electron Microscopy (TEM) and zeta potential measurements. To achieve our goal, twenty five formulations (SLN1 to SLN25) of SLNs were prepared and optimized. Formulation SLN21 was selected as optimized formulation and was evaluated for the independent parameters. Optimized formulation showed particle size 11.73nm, zeta potential -35.82 mV, percent Entrapment efficiency (%EE) 72.58 and 99.48% of In-vitro % drug release after 16h. The drug release data suggested that the release of the drug is sustained.

KEYWORDS: solid lipid nanoparticles, Zolmitriptan, solvent diffusion evaporation technique.

INTRODUCTION
Migraine is one of the most common headache disorders that affect 17-18% of the female and 6% of the male population. It is characterized by episodic unilateral headache attacks that are accompanied by nausea and/or photo- and phonophobia. Current migraine treatment is divided into acute care and daily preventive medication.

Diseases of the central nervous system (CNS) such as schizophrenia, meningitis, migraine, Parkinson's disease and Alzheimer's disease require delivery of the drug to the brain for treatment.

However such transport remains problematic, especially for hydrophilic drugs and large molecular weight drugs, due to the impervious nature of the endothelial membrane separating the systemic circulation and central interstitial fluid, the Blood Brain Barrier (BBB).

Zolmitriptan is a second generation triptan prescribed for patients with migraine attacks, with or without aura and cluster headaches. It selectively acts on serotonin receptors and is very effective in reducing migraine symptoms, including pain, nausea, and photo- or phonophobia.

SLNs offer unique properties such as small particle size, large surface area, high drug loading capacity and interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals.

The disadvantages associated with the liquid state of the oil droplets in tradition colloidal system are overcome by replacing the liquid state with a solid lipid which eventually get transformed into solid lipid nanoparticles.

The reasons for increasing interest in lipid based drug delivery system have many folds and it offers several advantages like;

1. Lipids enhance oral bioavailability and reduce plasma profile variability.
2. Better characterization of lipoid excipients.
3. An improved ability to address the key issues of technology transfer and manufacture scale-up.
4. SLN combine all the advantages of polymeric nanoparticles, fat emulsions and liposomes.

MATERIALS AND METHOD

Materials

Zolmitriptan IP is a gift sample from Cadila Healthcare Pvt. Ltd., Ahmedabad. Compritol® 888 ATO and Precirol® ATO 5 obtained from Gattefosse, Germany. All other excipients, chemicals and solvents are of analytical grade and were purchased commercially.

Method of preparation of solid lipid nanoparticles (SLNs)

Solid lipid nanoparticles loaded with Zolmitriptan were prepared by solvent diffusion evaporation method.

Solvent diffusion evaporation method

- Solid lipid was dispersed in organic solvent and heated at 5-10ºC temperature above the melting point of the solid lipid. In melted lipid, Zolmitriptan was dissolved with continuous stirring (primary phase/oil phase).
- An aqueous phase was separately prepared by dissolving Tween 20 in distilled water with subsequent heating to the 70-80 ºC (secondary phase).
- Now, the oil phase was quickly added into hot aqueous phase with continuous stirring. The organic solvent was allowed to get evaporated by continuous heating the suspension on hot plate.
- The resultant suspension was finally poured into same quantity of cold water (2-3^{0}C) under stirring at 1000 RPM for 5 minutes.
- The dispersion was then centrifuged at 10,000 RPM for 15 min.
- After the completion of centrifugation, SLN dispersion was obtained.

OPTIMIZATION OF FORMULATION PARAMETERS:

Type of solid lipid

SLN were formulated by solvent diffusion evaporation method using three different solid lipids i.e. Compritol® 888 ATO, Precirol® ATO 5 and Dynasan® 116.

Table 5.1: Optimization of type of solid lipid

Batch No.	Solid Lipid
SLN 1	Compritol® 888 ATO
SLN 2	Precirol® ATO 5
SLN 3	Dynasan® 116

Note: For all 3 batches Drug:lipid ratio (1:10), concentration of Tween 20 (2% v/v) and acetone as an organic phase were kept constant.

Type of organic phase

Zolmitriptan loaded SLN were formulated by solvent diffusion evaporation method using three different organic solvents i.e. Acetone, IPA and Ethanol.

Table 5.2: Optimization of organic phase

Batch No.	Organic Phase
SLN 4	Acetone
SLN 5	IPA
SLN 6	Ethanol

Note: For all 3 batches Precirol® ATO 5 as a lipid, Drug:lipid ratio (1:10), Concentration of Tween 20 (2% v/v) and were kept constant.

Ratio of organic solvent

Based on previous study, Acetone and Ethanol was selected as optimized organic solvents. They were further optimized for their suitable ratio.

Table 5.3: Ratio optimization of organic phase

Batch No.	Ratio of Acetone: Ethanol
SLN 7	1:1
SLN 8	1:2
SLN 9	1:3
SLN 10	2:1
SLN 11	3:1

Note: For all 5 batches Precirol® ATO 5 as a lipid, Drug:lipid ratio (1:10) and concentration of Tween 20 (2% v/v) were kept constant.

Concentration of Tween 20

The secondary phase was further optimized with various concentration of Tween 20.

Table 5.4: Optimization of concentration of Tween 20

Batch No.	Concentration of Tween 20 (% v/v)
SLN 12	0.5
SLN 13	2.0
SLN 14	4.0

Note: For all 3 batches Precirol® ATO 5 as a lipid, Drug:lipid ratio (1:10) and Acetone:Ethanol (1:1) as an organic phase were kept constant.

Temperature optimization for secondary phase

As mentioned in preparation method, secondary phase containing aqueous phase with Tween 20, which was optimized for its temperature. The secondary phase was kept at three different temperatures to analyze its effect on the formulation of SLN.

Table 5.5: Optimization of temperature for secondary phase

Batch No.	Temperature
SLN 15	0^{0}C
SLN 16	25^{0}C
SLN 17	50^{0}C
SLN 18	70^{0}C
SLN 19	80^{0}C

Note: For all 5 batches Precirol® ATO 5 as a lipid, Drug:lipid ratio (1:10), Acetone:Ethanol (1:1) as an organic phase and concentration of Tween 20 (2% v/v) were kept constant.

Optimization of drug: lipid ratio

Drug to lipid ratio was optimized for better entrapment of drug and for higher stability of formulation. For optimization different ratio of drug to lipid was selected and analyzed for particle size, zeta potential and % Entrapment Efficiency (%EE). Different drug: lipid ratio was mentioned in Table 5.6.

Table 5.6: Optimization of drug: lipid ratio

Batch No.	Drug: lipid ratio
SLN 20	1:2.5
SLN 21	1:5
SLN 22	1:10
SLN 23	1:15
SLN 24	1:20
SLN 25	1:25

Note: For all 6 batches Precirol® ATO 5 as a lipid, Acetone: Ethanol (1:1) as an organic phase, concentration of Tween 20 (2% v/v) and temperature of secondary phase (70^0C) were kept constant.

CHARACTERIZATION OF SLN DISPERSION

Particle size

The size of optimized batch of Zolmitriptan loaded SLN was measured by Zetatrac (Microtrac U2552).

Zeta potential

Zeta potential is a parameter which is highly useful for assessment of the physical stability of colloidal dispersions. Surfaces of particles in suspension develop a charge due to adsorption of ions or ionization of surface group and the charge is correspondingly dependent on both the surface chemistry and the environment of the particles. The surface charge generates a potential around the particle, which is high near the surface and decays with distance into the suspending medium. The zeta potential can be measured by determination of the movement velocity of the particles in an electric field (electrophoresis measurements). In the present work, the SLN dispersion was analyzed by Zetatrac (Microtrac U2552).

% Entrapment Efficiency

10 ml of Zolmitriptan loaded SLN was centrifuged at 5000 rpm for 15 minutes to separate the lipid and aqueous phase. Supernatant was than analyzed for drug content by UV-VIS spectroscopy at 223.0 nm. It expresses amount of free drug, which remains unentrapped in formulation. The entrapment efficacy was calculated by following equation:

$$\% \text{ Entrapment efficiency} = \frac{Wa - Ws}{Wa} \times 100$$

Where,

Wa = Amount of drug added into formulation,

Ws = Amount of drug (free) present in supernatant after centrifugation

In vitro drug release study of SLN dispersion

For in-vitro release study, the drug release from the optimized batch of Zolmitriptan loaded SLN dispersion was performed in Phosphate Buffer Solution (pH 7.4) using dialysis membrane. A dialysis membrane (Himedia, Mumbai) weight cut off between 12000 Da was used. The membrane was soaked in distilled water for 12 hours before the release study. 10 ml (equivalent to 5 mg Zolmitriptan) SLN dispersion was placed in dialysis membrane tied at both ends to form dialysis bags and these dialysis bags were subsequently placed in flasks containing 250 mL dissolution medium at 100rpm at 37±2°C. Aliquots of the dissolution medium were withdrawn at each time interval and the same volume of fresh dissolution medium was added to the flask to maintain a constant volume. Drug concentration in the dissolution medium was determined.

Instrumental analysis

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is useful since it allows particles much smaller than 1 µm to be observed. Solid lipid nanoparticles were dispersed in distilled water and drop of dispersed SLN was incubated on carbon coated copper grid. This copper grid was fixed into sample holder and placed in vacuum chamber of transmission electron microscope (Philips Tech 20) and observed under low vacuum.

Fourier Transform Infrared Spectroscopy (FTIR) study

IR spectroscopy is one of the most powerful analytical techniques which offer the possibility of chemical interaction. Infra-red spectra of pure drug (Zolmitriptan) and Zolmitriptan loaded SLN dispersion were scanned by using FTIR in spectral region of 4000 to 400 cm^{-1}.

RESULT & DISCUSSION

Preformulation study

Table 7.1: Preformulation parameters of Zolmitriptan

Drug	Melting Point	Log P	Log Kp
Zolmitriptan	142-146 ºC	1.6 ±0.011	-5.31 ± 0.018

Compatibility study of drug and excipient

Differential Scanning Calorimetry (DSC) study

DSC thermograms of Zolmitriptan and final optimized formulation were shown in figure 7.1 below.

The DSC curve of Zolmitriptan showed a melting endotherm at 144.00^0C and DSC curve of Precirol® ATO 5 showed melting endotherm at 56.21^0C. Both endotherm is near around its melting point. i.e. 142-146^0C and 54-58^0C for Zolmitriptan and Precirol® ATO 5 respectively. Moreover, DSC thermogram of physical mixture (Zolmitriptan + Precirol® ATO 5) was observed to be 145.56^0C and 56.79^0C respectively, indicative of no significant difference in melting endotherm with respect to melting endotherm of individual ingredient. This proved the compatibility of drug with other ingredients.

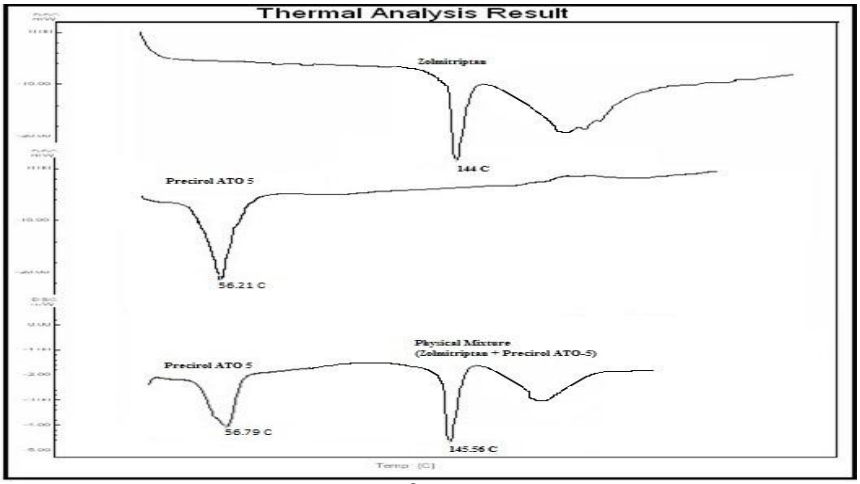

Figure 7.1: DSC thermogram of Zolmitriptan, Precirol® ATO 5 and Physical mixture Zolmitriptan and Precirol® ATO 5

Optimization of formulation parameters

Type of solid lipid

Different lipids like Compritol® 888 ATO, Precirol® ATO 5 and Dynasan® 116 were evaluated for the formulation of SLN. Based on the smallest particle size, Precirol® ATO 5 was selected as best suited solid lipid for SLN formulation.

Table 7.2: Selection of type of lipid

Batch No.	Solid Lipid	Particle Size (nm)	% Entrapment Efficiency±S.D.
SLN 1	Compritol® 888 ATO	224.2	66.15±0.781
SLN 2	Precirol® ATO 5	105.2	68.27±0.586
SLN 3	Dynasan® 116	247.4	63.94±0.966

Type of organic phase

The organic solvents namely Acetone, Isopropyl alcohol (IPA), Ethanol were selected on the basis of particle size of SLN. Results indicate lipid precipitation when IPA was used as an organic solvent. Acetone and Ethanol resulted into nano-sized particles, therefore they were chosen for further development.

Table 7.3: Selection of organic phase

Batch No.	Organic Phase	Particle Size (nm)	% Entrapment Efficiency± S.D.
SLN 4	Acetone	105.2	65.59±0.675
SLN 5	IPA	Lipid ppt	-
SLN 6	Ethanol	114.8	65.07±1.021

Ratio of organic solvent

Acetone and Ethanol selected in earlier stage had been subjected to finalize their appropriate ratio. These solvents were combined in different ratio like 1:1, 1:2, 1:3, 2:1, 3:1 and tested for characterization.

The results suggest the suitability of 1:1 ratio of Acetone: Ethanol as organic solvent in batch SLN 7 whereas batch with 1:3 & 3:1 ratio (SLN 9 & 11) exhibited precipitation on storage. The other two batches SLN 8 & SLN 10 with 1:2 & 2:1 ratio respectively had exhibited higher particle size as compared to SLN 7.

Table 7.4: Selection of ratio of organic solvent

Batch No.	Ratio of Acetone: Ethanol	Particle Size (nm)	% Entrapment Efficiency ± S.D.
SLN 7	1:1	105.2	69.78±0.629
SLN 8	1:2	224.2	68.53±0.854
SLN 9	1:3	Precipitation	-
SLN 10	2:1	247.3	64.91±0.712
SLN 11	3:1	Precipitation	61.46±1.064

Concentration of Tween 20

Table 7.5: Optimization of concentration of Tween 20

Batch No.	Concentration of Tween 20 (% v/v)	Particle size (nm)	Zeta potential (mv)
SLN 12	0.5	11.73	-12.54
SLN 13	2.0	90.45	-34.18
SLN 14	4.0	560.83	-39.92

The results of above batches suggest that with increase in concentration of Tween 20 in the formulation, value of zeta potential tends to become smaller which might be due to stearic stabilization provided by Tween 20.

Temperature optimization of secondary phase

The temperature of secondary phase was optimized by formulating the SLN batches at different temperature as per Table 7.6

Table 7.6: Optimization of temperature for secondary phase

Batch No.	Temperature	Particle size (nm)
SLN 15	0^0C	Lump formation
SLN 16	25^0C	589
SLN 17	50^0C	260
SLN 18	70^0C	105
SLN 19	80^0C	105

From the result it was concluded that with increase in the temperature particle size reduced. But at temperature 70^0C and 80^0C, no difference in particle size was noted. SLN 15 at 0^0C temperature formed lump. Hence, 70^0C was considered as an optimized temperature for secondary phase.

Optimization of Drug: Lipid ratio

Table 7.7: Optimization of drug: lipid ratio

Batch No.	Drug: lipid ratio	% Entrapment Efficiency ± S.D.	Particle size (nm)	Zeta potential (mV)
SLN 20	1:2.5	63.51±1.198	10.32	-24.19
SLN 21	**1:5**	**72.58±0.872**	**11.73**	**-35.82**
SLN 22	1:10	70.65±1.035	65.12	-32.15
SLN 23	1:15	68.81±0.886	110.5	-28.93
SLN 24	1:20	60.82±0.689	269.6	-14.86
SLN 25	1:25	58.47±1.047	699	-12.04

The results of all six batches were mentioned above. As we increased drug: lipid ratio, particle size of SLN also increased. This might be attributed to the larger lipid matrix formed with increased lipid concentration. Apart from particle size, zeta potential was also measured and it was an indicative of stability. Moreover, SLN 21 batch with drug: lipid ratio 1:5 exhibited particle size 11.73 nm and zeta potential value -35.82 mV.

Characterization of SLN Dispersion of optimized batch (Batch SLN21)

Particle size

Particle size of optimized formulation was found to be 11.73nm**.**

Zeta potential

Zeta potential of optimized SLN formulation was -35.82 mV. Tween 20 provides a stearic stability form maintaining the stability of SLN.

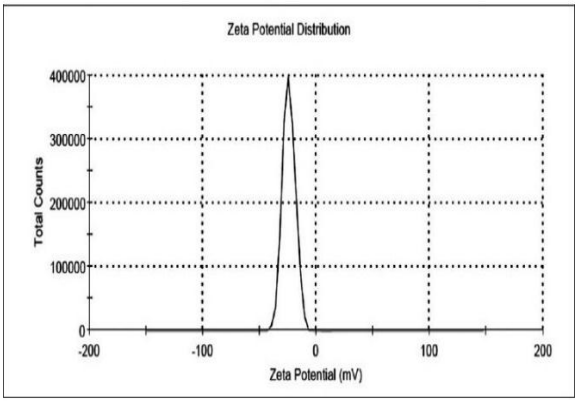

Figure 7.9: Zeta potential of optimized batch

Zeta Potential (mV)	Zeta Deviation (mV)	Conductivity (mS/cm)
-35.82	4.60	0.0493

Preferably value of zeta potential should be ≥ ±30 mv for stability. In our formulation it was -35.82, which meant

it complied with requirement of zeta potential for stability.

% Entrapment efficiency

Percentage Entrapment Efficiency was determined by measuring weight of free drug present in SLN. Percentage entrapment efficiency for optimized SLN formulation was found to be 72.58±0.872.

In vitro drug release study of SLN dispersion

Results obtained after in-vitro drug release study using dialysis bag, showed sustained release of drug from SLN dispersion as 99% drug was released in 16 hours from SLN dispersion, whereas in case of conventional ZOMIG® Tablet, 99% of drug was released within 1 hr.

Table 7.9: In-vitro % drug release of SLN* dispersion *(n=3)

Time (hr.)	SLN Dispersion ± S.D.	ZOMIG® Tablet
0.5	5.92±0.856	86.13±0.617
1	13.34±0.562	99.96±0.592
2	24.15±0.657	-
3	38.70±0.642	-
4	49.34±0.786	-
6	63.88±0.735	-
8	74.13±0.857	-
10	81.37±0.625	-
12	89.64±0.814	-
14	96.75±0.873	-
16	99.48±0.951	-

Instrumental analysis

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy imaging of optimized batch of Zolmitriptan loaded SLN dispersion is shown in figure 7.11. TEM imaging of Zolmitriptan loaded SLN dispersion exhibit a spherical shape of solid lipid nanoparticles with very narrow size distribution. The particle size from TEM is also in accordance with that of particle size analysis.

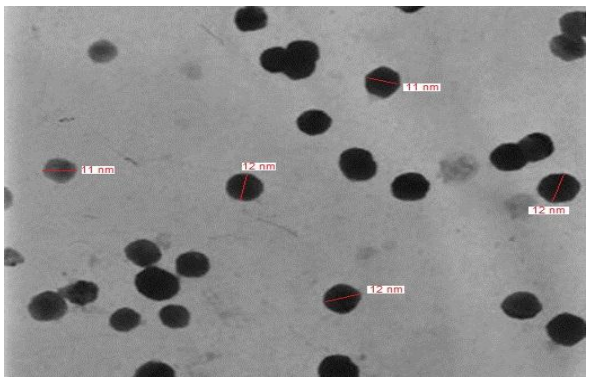

***Figure 7.11: TEM imaging of Zolmitriptan loaded SLN* dispersion**

Fourier Transform Infrared Spectroscopy (FTIR) study

The FTIR spectrum provides useful information about the conformation of the molecules and formation mechanism. Infra-red spectra of Zolmitriptan pure and Zolmitriptan loaded SLN dispersion formulation were scanned and presented below in figure 7.12.

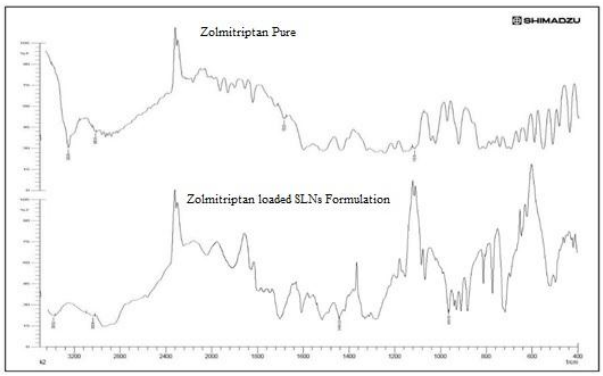

Figure 7.12: FTIR spectra of Zolmitriptan pure and Zolmitriptan loaded SLN dispersion

In this study, FTIR technique was employed to check the physical and chemical interaction between drug and excipients used in the formulation. Infrared (IR) spectra of Zolmitriptan pure and Zolmitriptan loaded SLN dispersion were analyzed. From the figure 7.12 it was observed that there were no changes in main peaks of IR spectra for the mixture of drug and excipients, which indicated that no physical interactions existed.

CONCLUSION

In the present investigation an attempt was made to enhance the availability of Zolmitriptan for the treatment of migraine by preparing Zolmitriptan loaded SLN dispersion. The SLN dispersion was prepared by solvent diffusion evaporation technique using Precirol® ATO 5 as lipid matrix. The prepared SLN dispersion were characterized for various parameters such as particle size, zeta potential, % entrapment efficiency. Formulation was also characterized for Transmission Electron Microscopy to determine shape and size of particles. Thus, SLN dispersion also have the potential to localize the drug at the site and could be useful for site-specific delivery of drug.

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