

## DESIGN AND OPTIMIZATION OF SOLID LIPID NANOPARTICLES (SLNs) OF ZOLMITRIPTAN FOR THE MANAGEMENT OF MIGRAINE

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### ABSTRACT

Solid lipid nanoparticles (SLNs) of zolmitriptan were produced by solvent emulsification-diffusion technique. Soya lecithin and poloxamer 188 were used as surfactants and stabilizers of the particles. The formulations were optimized for independent variables (amount of stearic acid, amount of lecithin and homogenization time) in order to achieve desired particle size with maximum percent entrapment efficiency (% EE). Prepared SLNs were characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM) and zeta potential measurements. To achieve our goal, eight formulations (F<sub>1</sub>-F<sub>8</sub>) of SLNs were prepared by solvent injection technique and optimized by 2<sup>3</sup> full-factorial design. The responses of the design were analyzed using Minitab 15. On the basis of software analysis, formulation F<sub>8</sub> was selected as optimized formulation and was evaluated for the independent parameters. Optimized formulation showed particle size of 340nm, percent entrapment efficiency (EE) of 81.36 and 79.11% of *in-vitro* drug release after 24h. The release kinetics of the optimized formulation best fitted the Higuchi model.

**Key words:** solid lipid nanoparticles, zolmitriptan, solvent emulsification-diffusion technique, *in-vitro* release.

### INTRODUCTION

Migraine is a common and frequently disabling headache disorder characterized by recurrent episodic attacks of moderate to severe headache variably accompanied by neurological, gastrointestinal and/or autonomic symptoms (Goadsby *et al.*, 2002). There are two major forms of migraine: migraine without aura and migraine with aura. The aura consists of focal neurologic symptoms (usually visual symptoms) that precede or accompany headache, and it appears in 15-25% of migraine sufferers. Migraine affects approximately 18% of women and 6% of men in western countries, and 20-40% of patients experience an attack frequency of greater than one per month (Belvis *et al.*, 2009).

Zolmitriptan, 4S-4-({3-[2-(dimethylamino)ethyl]-1H-indol-5-yl} methyl)-1, 3-oxazolidin -2-one, is a second generation triptan prescribed for patients with migraine attacks, with or without an aura, and cluster headaches. It has a selective action on serotonin (5-HT<sub>1B/1D</sub>) receptors and is very effective in reducing

migraine symptoms, including pain, nausea, and photo-or phonophobia (Gayathri, *et al.*, 2000). Patients with migraine generally suffer from nausea and vomiting; oral treatment can therefore be inconvenient or could fail (Kolsure, *et al.*, 2012). The inherent shortcomings of conventional drug delivery and the potential of nanoparticles as drug delivery systems have offered tremendous scope for researchers in this field and are fast moving from concept to reality.

Nanoparticles based on solid lipids have been proposed as a promising alternative to colloidal drug delivery system, polymeric nanoparticles, and liposomes. Compared to traditional carriers, the solid lipid nanoparticles (SLNs) combine the advantages of polymeric nanoparticles and o/w fat emulsions for drug administration such as good tolerability, lower cytotoxicity (Muller, *et al.*, 1996), higher bioavailability by oral administration (Yang, *et al.*, 1999), and increase in the drug stability (Mehnert and Mader, 2001). Other advantages of lipid excipients, such as biodegradability and

cost effectiveness (Mukherjee, *et al.*, 2007), promote their use as novel drug carriers. Consisting of physiological and biodegradable lipids, lipid nanoparticles are suitable for the incorporation of lipophilic, hydrophilic, and poorly water-soluble drugs within the lipid matrix in considerable amounts (Bunjes, *et al.*, 2001). Lipidic carriers used to prepare SLNs can be highly purified lipids such as tristearin or tripalmitin, hard fats such as stearic acid or behenic acid, waxes such as cetylpalmitate, and acylglycerol mixtures such as compritol or glycerylmonostearate (GMS) (Souto, 2006).

In the present study, stearic acid has been used to formulate SLNs. The selection of stearic acid as its lipid matrix is made from physiological lipids that decrease the danger of acute and chronic toxicity in epidemiologic and clinical studies. Stearic acid was associated with lowered LDL cholesterol in comparison with other saturated fatty acids indicating it less unhealthy than other saturated fatty acids.

## MATERIAL AND METHODS

Zolmitriptan was a gift from Zyclus Cadila Health Care Limited, Ahmedabad. Stearic acid (Molar mass 284.48g mol<sup>-1</sup>, melting point 69.6°C, density 0.84g/cm<sup>3</sup> at 70°C) was purchased from Clearsynth Labs, Andheri, Mumbai, India. Soya lecithin (Epikuron, 200) was purchased from LeciImpex, Indore, M.P. Poloxamer F 68 (molecular weight 12 kDa) was purchased from Balaji Drugs, Surat. Dialysis bag (molecular weight cut off (MWCO) 12-14kDa; pore size 2.4nm) was supplied by Hi Media, Mumbai, India. Other chemicals used were of analytical grade.

### Experimental design of SLNs

In this study, a 2<sup>3</sup> full-factorial experimental design was used to optimize SLNs. In this '2' indicates levels i.e. higher and lower and 'n' is factor i.e. concentration of stearic acid, concentration of soya lecithin and time for homogenization. Eight formulations of SLNs (F<sub>1</sub> to F<sub>8</sub>) were designed. In order to optimize, the amount of stearic acid (X<sub>1</sub>), amount of lecithin (X<sub>2</sub>) and homogenization time (X<sub>3</sub>) were selected as independent variables. Each factor was set at a high level and low level. The actual values and coded values of different variables are given in table I. The particle size

and % entrapment efficiency (EE) were taken as response parameters.

### Preparation of SLNs

#### Solvent emulsification-diffusion technique

Solid lipid nanoparticles of zolmitriptan were prepared by the solvent emulsification-diffusion technique. The solvent (ethyl acetate) and water were mutually saturated for 10min at room temperature in order to generate equilibrium between the two liquids. Heating up to 70°C was required to solubilize the lipid, the saturation step was performed at this temperature. Typically, specified amount of lipid, 10mg of drug zolmitriptan were dissolved in 10mL of water-saturated solvent and this organic phase (internal phase) was emulsified with 20mL of the solvent-saturated aqueous solution containing 10% w/v of stabilizer (dispersion medium) using a mechanical stirrer at 3000rpm for 15min. After formation of an oil in-water emulsion, 80mL of water (dilution medium) was added to the system in order to allow solvent diffusion into the continuous phase thus causing the aggregation of the lipid in nanoparticles. When heating was required to dissolve the lipid, both phases were maintained at 70°C and the diffusion step was performed either at RT or at the temperature under which the lipid was dissolved. Throughout the process constant stirring was maintained. Thereafter, the dispersion was centrifuged to 10,000rpm for 30min at 10°C in Remi cooling centrifuge (Model C-24BL, VCAO-779, Vasai, India), and aggregates were purified by dialysis bag and resuspended to 10mL distilled water containing 4% poloxamer F68 (by weight) as stabilizer with stirring at 1,000rpm for 10min.

#### Purification of zolmitriptan-loaded SLNs

Purification of zolmitriptan-loaded SLNs was done by using dialysis technique. Sedimented soft pellet was taken in the dialysis bag and sealed at both ends. The dialysis bag was then immersed into 100mL of distilled water containing 0.2% w/v sodium lauryl sulphate and stirred at 100 rpm for 30min. Five milliliter of sample was withdrawn at different time intervals of 5, 10, 15, and 30min. The samples were diluted appropriately and analyzed for amount of drug by UV/

visible spectrophotometer (JASCO V-30 UV Spectrophotometer) at 283nm (Shah and Pathak, 2010).

### Characterization of zolmitriptan SLNs

#### Transmission electron microscopy

Structure of the SLNs were studied using Morgagni 268D transmission electron microscopy (TEM) FEI, Netherland operating at 70KV and capable of point to point resolution. Combination of bright field imaging at increasing magnification and diffraction modes were used to reveal the form and size of SLNs. In order to perform the TEM observations, a drop of SLN dispersion was applied on carbon coated grid with 2% phosphotungstic acid (PTA) and was left for 30sec. The dried coated grid was taken on a slide and covered with a cover slip. The slide was observed under the electron microscope (Garcia-Fuentes *et al.*, 2003).

#### Atomic force microscopy (AFM)

By using AFM one can not only image the surface in atomic resolution but also measure the force at nano-newton scale. The sample preparation for AFM is simple and quick and it allows the material to be preserved in its original form. For AFM study, one drop of formulation was spread out homogenously on separate mica piece (5mm × 5mm) and the sample was then allowed to dry at room temperature for one day before imaging at a scan speed of 2Hz (Dubes *et al.*, 2003).

#### Entrapment efficiency

Entrapment efficiency (EE) of SLNs was determined by centrifugation method with rotor at 10,000rpm for 30min. The supernatant was collected. The quantity of drug present in the supernatant was determined by the UV Spectrophotometer. The entrapment efficiency was calculated using the following equation.

$$EE(\%) = (W_{\text{initial}} - W_{\text{free}}) / W_{\text{initial}} \times 100$$

where,  $W_{\text{initial}}$  is the total amount of zolmitriptan used in preparation of solid lipid nanoparticles and  $W_{\text{free}}$  is the amount of zolmitriptan detected in the supernatant, respectively (Bhaskar *et al.*, 2009, Sarmiento *et al.*, 2007).

#### Drug loading capacity

The drug loading (DL) capacity of Zolmitriptan was also calculated using the formula:

$$DL(\%) = (W_{\text{initial}} - W_{\text{free}}) / W_{\text{lipid}} \times 100$$

where,  $W_{\text{initial}}$  is the total amount of zolmitriptan used in preparation of solid lipid nanoparticles,  $W_{\text{free}}$  is the amount of zolmitriptan detected in the supernatant, and  $W_{\text{lipid}}$  is the amount of lipid in the preparation of solid lipid nanoparticles, respectively (Lv *et al.*, 2009).

#### Particle size distribution, zeta potential and polydispersity index (PDI)

The zeta potential and size distribution of SLNs were measured in triplicates in multimodal mode (Doijad *et al.*, 2008). The particle size distribution and polydispersity index were determined using computerized Malvern instrument particle size analyser v2.0 (Malvern UK) and zeta potential was determined by using Malvern zetasizer inspection system v2.2 (Malvern UK) at 25°C. Prior to the measurement, SLNs were diluted with distilled water and the measurements were taken in triplicate (n=3).

#### In-vitro release

*In vitro* release was evaluated by using a dialysis membrane diffusion technique (Reddy *et al.*, 2005). The drug release from Zolmitriptan SLNs (ZOL-SLNs) was performed in PBS (pH 7.4) using dialysis membrane. A dialysis membrane (Himedia, Mumbai) weight cut off between 12.000 and 14.000 was used. The membrane was soaked in distilled water for 12 hours before the release study. Two mL ZOL-SLNs suspension was placed in dialysis membrane tied at both ends to form dialysis bags and these dialysis bags were subsequently placed in flasks containing 50mL dissolution medium at 100rpm at 37°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. The absorbance was used to calculate concentration using calibration curve. The experiments were performed in triplicate.

Table I. Formulations of zolmitriptan SLNs

Code	Experiment	Factor A	Factor B	Factor C	Particle size (nm)	Entrapment efficiency (%)
F1	1	-	-	-	410	57.12
F2	A	+	-	-	450	76.42
F3	B	-	+	-	320	58.36
F4	Ab	+	+	-	390	75.48
F5	C	-	-	+	360	60.14
F6	Ac	+	-	+	430	77.81
F7	Bc	-	+	+	250	59.16
F8	Abc	+	+	+	340	81.36

*Coded values*

Factor A = Amount of stearic acid (low level 200mg, high level 600mg)

Factor B = Amount of soya lecithin (low level 200mg, high level 800mg)

Factor C = Stirring time (low level 15min, high level 30min)

- denotes lower and + denotes higher limit, respectively.

**Statistical analysis**

Statistical analysis was performed with Minitab 15 software. Results were expressed as mean  $\pm$  standard deviation (SD). Statistical significance was determined using analysis of variance (ANOVA) with  $P \leq 0.05$  as a minimal level of significance.

**RESULTS AND DISCUSSION****Formulation considerations**

SLNs were prepared by solvent emulsification-diffusion technique that relies on the rapid diffusion of the solvent across the solvent-lipid interface with the aqueous phase. Thus, the diffusion rate of the organic solvent through the interface seems to be a critical parameter for particle size determination (Schubert *et al.*, 2003). The major problem with the formulation of SLNs is its separation. Owing to their small size and low density of lipids, SLNs present difficulty in settling upon centrifugation. To overcome this problem, in the present study, the pH of the dispersion was reduced to 2-3 to adjust the zeta potential for aggregation of SLNs (Hu *et al.*, 2002) and facilitate centrifugation and consequently separation.

A possibility of presence of zolmitriptan particles in the sediment of zolmitriptan-loaded SLNs was explored. It is suggested that these particles can potentially interfere in the *in vitro* and *in vivo* behavior of zolmitriptan-loaded SLNs. Therefore, free drug particles were

removed from the sediment of SLNs by purification by dialysis technique. Dialysis technique was considered suitable, as zolmitriptan with a low molecular weight could be efficiently removed using dialysis bag made of Himedia membrane. Consequently, purification was accomplished by monitoring percent free drug removed after 5, 10, 15, and 30min, respectively. Initially, the percent free drug removed increased with time and reached plateau levels by 30min. Slowest% drug release using dialysis technique after 30 min was observed by formulation F8 (2.40%) and highest % drug release was observed by formulation F4 (20.66%).

ANOVA was used to check any significant difference between the percent free drug removed at 15 and at 30 min. It was observed that although the extent of free drug in various SLN formulations was significantly different ( $p < 0.05$ ), there was no significant difference in percent free drug removed after purification time of 15 and 30min ( $p > 0.05$ ). Thus, free zolmitriptan from sediment of SLNs could be efficiently removed after purification for 15min and hence was used throughout the experiment.

**Statistical analysis of experimental data by minitab 15 software**

The results of the experimental design were analyzed using Minitab software that provided considerable useful information and

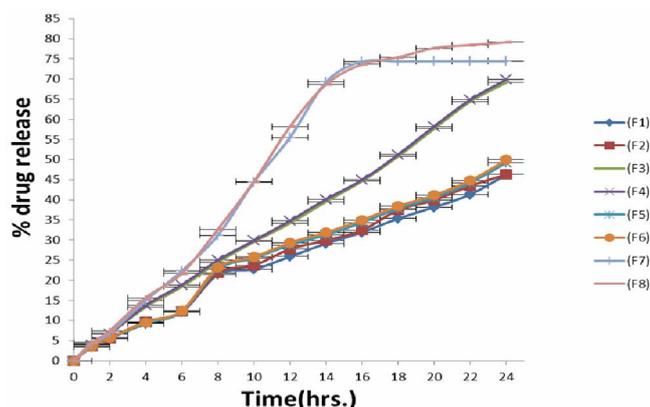
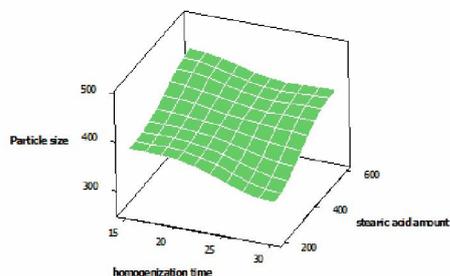


Figure 1. *In-vitro* drug release of prepared SLN formulations

Surface Plot of Particle size vs stearic acid amo, homogenization t



Contour Plot of Particle size vs stearic acid amo, homogenization t

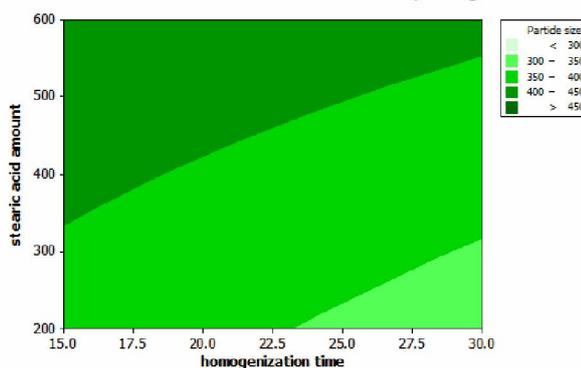


Figure 2. Response surface plots showing particle size as dependent variables plotted against two independent parameters (stearic acid and homogenization time) taking one parameter (lecithin amount) as constant of the prepared SLN formulations.

reaffirmed the utility of statistical design for conduct of experiments. The selected independent variables like the amount of stearic acid, amount of lecithin, and stirring time significantly influenced the particle size, % EE, and % cumulative drug release (CDR) that is very much evident from the results in table I. The *in vitro* drug release profiles of F<sub>1</sub> to F<sub>8</sub> are shown in figure 1.

Based on the results obtained for particle size, % EE, the response polynomial coefficients were determined in order to evaluate each response. Each response coefficient was studied for its statistical significance. The non-significant response coefficients were deleted, and the following significant polynomial response equation(s) for particle size, % EE, were generated.

**Particle size**

$$\text{Particle size (nm)} = 546 + 0.215 \text{ Stearic acid (mg)} - 0.136 \text{ Lecithin (mg)} - 3.93 \text{ Stirring time (min)}$$

$$S = 10.8167 \quad R\text{-Sq} = 99.0\% \quad R\text{-Sq(adj)} = 98.2\%$$

**Entrapment efficiency (EE)**

$$\text{EE (\%)} = 47.0 + 0.0444 \text{ Stearic acid (mg)} - 0.000347 \text{ Lecithin (mg)} + 0.0978 \text{ Stirring time (min)}$$

$$S = 0.836324 \quad R\text{-Sq} = 99.6\% \quad R\text{-Sq(adj)} = 99.2\%$$

These equations were utilized for validation of the experimental design. The response surface plots generated using polynomial equations represent simultaneous effect of any two variables on response parameter taking one variable at constant level.

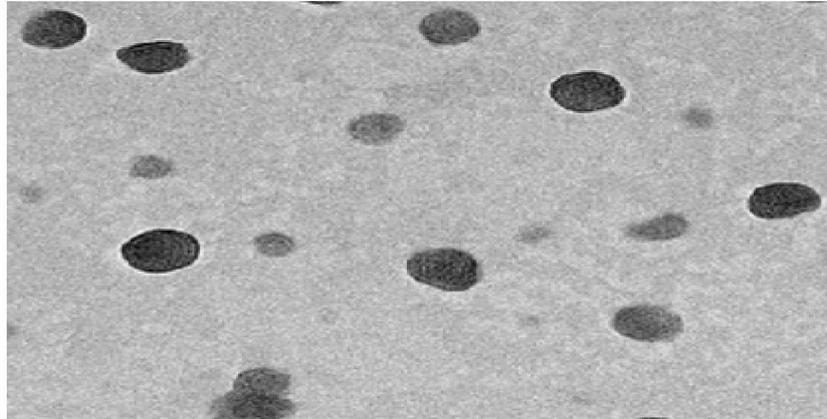


Figure 3. Transmission electron microscopy (TEM), with magnification 150 KX.

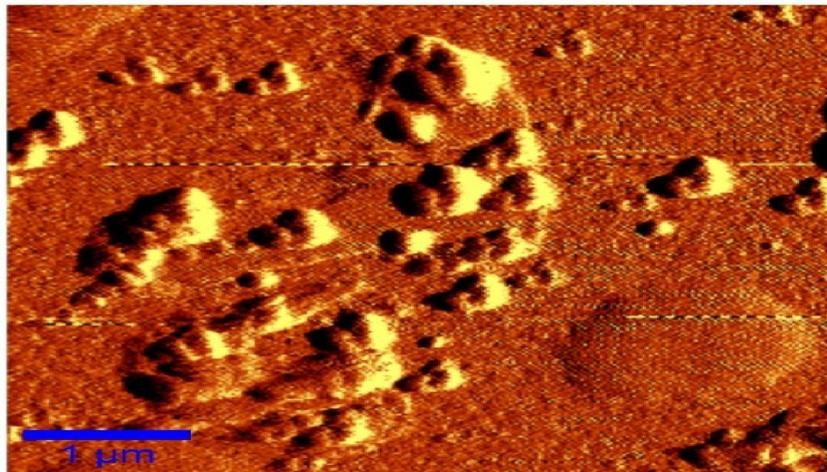


Figure 4. Atomic force microscopy (AFM) images of SLNs on 1 $\mu$ m scale.

On carefully observing these plots, the qualitative effect of each variable on each response parameter can be visualized. Figure 2 shows response surface plots with particle size as dependent variables plotted against two independent parameters (stearic acid and homogenization time) taking one parameter (lecithin amount) as constant.

Response graph shows the variation in independent variables strongly affect the dependent variable. The wavy surface show more variations in dependent variable on increasing or decreasing the value of independent variable and almost straight surface denote less variation in dependent variable for same condition.

The release kinetics was evaluated by fitting the data into first order ( $r^2 = 0.7421$ ), zero order ( $r^2 = 0.9428$ ), Higuchi ( $r^2 = 0.9747$ ) and Peppas equations ( $r^2 = 0.9330$ ). Based on the results, the release of zolmitriptan from SLNs best-fitted Higuchi equation ( $r^2 = 0.9747$ ) and the possible mechanisms for the drug release from F<sub>8</sub> might be diffusion of the drug from the matrix and matrix erosion resulting from degradation of lipids. A similar report has been made by other researchers as well (Souto, 2006 and Lai *et al.*, 2009). Thus, the *in-vitro* release data indicated that the optimized SLNs were capable of sustaining the release of zolmitriptan.

### Selection of optimized formulation

Optimized formulation (F<sub>8</sub>) was selected on the basis of small particle size, higher entrapment efficiency, and higher *in-vitro* cumulative drug release after 24 h. The optimized formulation was then subjected to Zeta potential, TEM and AFM.

### Zeta potential and Polydispersity Index (PDI)

The zeta potential was determined to be  $-35.3 \pm 0.02$  mV (n=3). The polydispersity index of  $0.131 \pm 0.12$  (n=3) indicated a polydisperse colloidal dispersion.

### Transmission Electron Microscopy (TEM)

TEM showed that the particles had round and uniform shapes. The mean diameters of SLNs were in the range of approximately 260-290nm (Figure 3).

### Atomic force microscopy

The AFM tapping mode images of ZOL-SLNs were observed. Both the planar and three-dimensional images are presented. The imaging showed that ZOL-SLNs had a spherical shape. The obtained images showed a clear topography of particles (Figure 4).

### CONCLUSION

The present investigation demonstrated that particle size of the nanoparticles can be controlled by varying process variables such as homogenization time, amount of stearic acid used and the amount of lecithin. Solid lipid nanoparticles of zolmitriptan were successfully developed to yield an optimized formulation with least nanometric particle size and highest possible entrapment efficiency that could sustain the release of drug for over 24h. Use of 2<sup>3</sup> full-factorial design enabled to develop an acceptable formulation using minimum raw materials and in minimum time.

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