DESIGN AND DEVELOPMENT OF ZOLMITRIPTAN LOADED NANOSTRUCTURED LIPID CARRIERS (NLC) FOR TREATMENT OF MIGRAINE

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Abstract: The aim of this study was to prepare Zolmitriptan Loaded Nanostructured Lipid Carrier (NLC) for the treatment of migraine. NLC of Zolmitriptan were prepared by Solvent Diffusion Evaporation method. Miglyol ® 812 were selected as liquid lipid material and Precirol® ATO 5 were selected as solid Lipid material. In conclusion, NLC with small particle size and high % entrapment efficiency can be obtained by this method.

I. INTRODUCTION

- Zolmitriptan is currently available as a conventional tablet and nasal spray (2.5 mg and 5.0 mg per dose)
- Its half-life is 3 hours.
- Currently, Zolmitriptan is available in conventional tablet and in nasal spray form in Indian market. Hence, the development for the novel nanoparticulate drug delivery were intended. On the other hand, nanoparticulate system possess a number of advantages.
- Many patients suffer from relapse of pain for longer time after taking conventional tablet, so overcome this problem by first giving them conventional tablet after that NLC of Zolmitriptan, hence it will relieve pain for longer time (12 hours)
- Also its half -life is 3 hours so require 5.0 mg for relief of migraine pain (3 to 4 times in a day). So it's a perfect candidate for NLC.
- A clear advantage of the use of lipid particles as drug carrier systems is the fact that the matrix is composed of physiological components or physiologically related components, i.e. excipients with Generally Recognized as Safe (GRAS) status for oral administration, which decreases the danger of acute and chronic toxicity.

II. METHOD OF PREPARATION OF NANOSTRUCTURED LIPID CARRIER (NLC)

Nanostructured Lipid Carrier loaded with Zolmitriptan was prepared by solvent diffusion evaporation method.

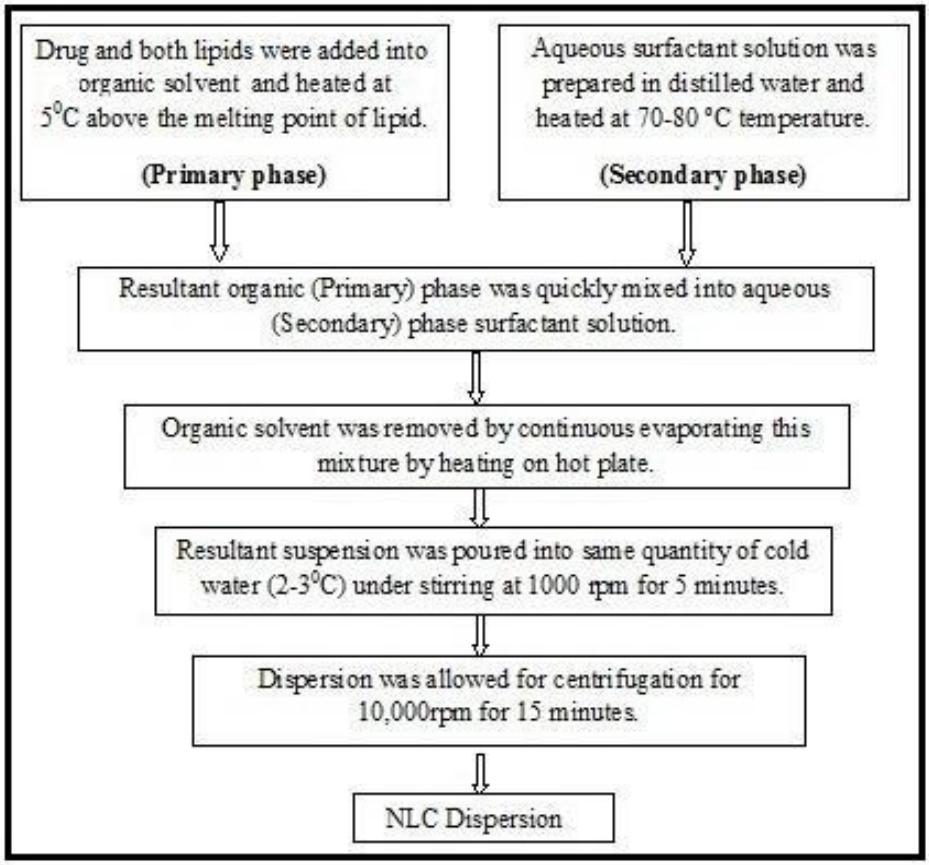

Figure.1 method of preparation of NLC of Zolmitriptan

III. MATERIALS

1. Zolmitriptan
2. Precirol® ATO 5
3. Miglyol® 812
4. Capryol® 90
5. Transcutol® HP
6. Lipophile WL 1349
7. Lauroglycol® 90

IV. OPTIMIZATION OF FORMULATION PARAMETERS

Solubility study for selection of liquid lipid

Solubility of Zolmitriptan plays a precious role while selecting the liquid lipid candidates. An excessive amount of Zolmitriptan was added individually to each sample of 5 ml liquid lipid (Capryol® 90, Transcutol® HP, Miglyol® 812). Both parts were blended for 5-10 minutes using blender. At that point, keep specimen in undisturbed condition for at least 24 hrs. duration of time. After 24 hrs time period, centrifuge each specimen sample by using equipment centrifuge at 1000 rpm speed and for duration of 15 minutes. Collect the supernatant solution and analyze it under Ultraviolet- Visible spectrophotometer (Model: UV-1800; Make: Shimadzu Corporation, Japan) for Zolmitriptan content at 283 nm wavelength.

Batch no.	solid lipid
NLC 1	Migyol® 812
NLC 2	Lipophile WL 1349
NLC 3	Lauroglycol® 90

Table 1: Selection of liquid lipid

- **TYPE OF SOLID LIPID**

Batch no.	solid lipid
NLC 4	Compritol® 888 ATO
NLC 5	Dynasan® 116
NLC 6	Precirol® ATO 5

Table 2: Optimization of type of solid lipid

Note: Constants for all 3 batches are:

- Liquid lipid: Myglyol® 812
- Solid lipid: Precirol ® ATO 5
- Solid lipid: liquid lipid ratio (1:10)
- Concentration of Polysorbate 80 (2 %v/v)
- Organic Phase: Acetone

- **OPTIMIZATION OF RATIO OF SOLID LIPID: LIQUID LIPID**

Batch no.	Ratio of solid lipid: liquid lipid
NLC 7	1:1
NLC 8	1:2
NLC 9	1:3
NLC 10	2:1
NLC 11	3:1

Table 3: Optimization of ratio of solid lipid: liquid lipid

Note: Constants for all 3 batches are:

- Liquid lipid: Miglyol® 812
- Solid lipid: Precirol ® ATO 5
- Solid lipid: liquid lipid ratio (1:10)
- Concentration of Polysorbate 80 (2 %v/v)
- Organic Phase: Acetone

- **TYPE OF ORGANIC PHASE**

Batch no.	Organic Phase
NLC 12	Acetone
NLC 13	Chloroform
NLC 14	Ethanol

Table.4: Optimization of organic phase

Note: Constants for all 3 batches are:
- Liquid lipid: Miglyol® 812
- Solid lipid: Precirol ® ATO 5
- Solid lipid: liquid lipid ratio (1:10)
- Concentration of Polysorbate 80 (2 %v/v)

- **RATIO OF ORGANIC SOLVENT**

Batch No.	Ratio Acetone: Ethanol
NLC 15	1:1
NLC 16	1:2
NLC 17	2:1
NLC 18	3:1

Table 5: Optimization of ratio of organic solvent

Note: Constants for all 4 batches are:
- Liquid lipid: Myglyol® 812
- Solid lipid: Precirol ® ATO 5
- Solid lipid: liquid lipid ratio (3:1)
- Drug: Lipid Ratio: (1:10)
- Concentration of Polysorbate 80 (2 %v/v)
- Organic Phase: Acetone

- **CONCENTRATION OF POLYSORBATE 80**

Batch No.	Concentration of Polysorbate 80 (% v/v)
NLC 19	0.5
NLC 20	1.0
NLC 21	1.5
NLC 22	2.0
NLC 23	2.5

Table 6: Optimization of concentration of Polysorbate 80

Note: Constants for all 5 batches are:
- Liquid lipid: Myglyol® 812
- Solid lipid: Precirol ® ATO 5
- Solid lipid: liquid lipid ratio (3:1)
- Drug: Lipid Ratio: (1:10)
- Concentration of Polysorbate 80 (2 %v/v)
- Organic Phase: Acetone

- **TEMPERATURE OPTIMIZATION FOR SECONDARY PHASE**

2° phase containing Polysorbate-80 were evaluated and temperature was optimized as specified in preparation method. The secondary phase were allowed to keep on three unique temperature conditions to show effect on NLCs formulation.

Batch No.	Temperature (ºC)
NLC 24	60
NLC 25	70
NLC 26	80

Table 7: Optimization of temperature for secondary phase

Note: Constants for all 3 batches are:
- Liquid lipid: Miglyol® 812
- Solid lipid: Precirol ® ATO 5
- Solid lipid: liquid lipid ratio (3:1)

- Drug: Lipid Ratio: (1:10)
- Concentration of Polysorbate 80 (1.5 %v/v)
- Organic Phase: Acetone: Ethanol (1:1)

- **OPTIMIZATION OF DRUG: LIPID RATIO**

In order to achieve higher drug entrapment and good stability, optimization of ratio of drug substance to lipid candidate was identified. Henceforth, different proportion of drug substance to lipid candidate was chosen and analyzed to find various parameters like particle size, zeta potential and Percentage Entrapment Efficiency etc. Different batches indicating ratio of drug: lipid was mentioned below:

Batch No.	Drug: lipid ratio
NLC 27	1:2
NLC 28	1:3
NLC 29	1:4
NLC 30	1:5

Table 8: Optimization of drug: ratio

Note: Constants for all 3 batches are:

- Liquid lipid: Myglyol® 812
- Solid lipid: Precirol ® ATO 5
- Solid lipid: liquid lipid ratio (3:1)
- Drug: Lipid Ratio: (1:10)
- Concentration of Polysorbate 80 (2 %v/v)
- Temperature of secondary phase (70º C)

V. FORMULATION PARAMETER OPTIMIZATION:

- **Solubility study for selection of liquid lipid**

To achieve good formulation parameters of NLCs, solubility study is required to be performed for the selection of liquid lipid in the formulation of Zolmitriptan loaded NLCs. The solubility in different liquid lipid are mentioned below:

Batch No.	Liquid lipid	Solubility (mg/ml)			
		I	II	III	IV
NLC 1	**Migyol® 812**	**1.338**	**1.335**	**1.338**	**1.338**
NLC 2	Lipophile WL 1349	0.634	0.633	0.634	0.636
NLC 3	Lauroglycol® 90	0.852	0.855	0.851	0.852

Table: 9 Solubility of Zolmitriptan in liquid lipid

- **Effect of different types of solid lipid**

Different lipids namely Compritol® 888 ATO, Dynasan® 116 and Precirol® ATO 5 were used to optimize the solid lipid. Amongst all, Precirol® ATO-5 provided optimistic result with lowest particle size 90.29 nm compared to others. Therefore, preferably Precirol® ATO-5 is selected for further parameter optimization.

Batch no.	Solid Lipid	Particle size (nm)	% Entrapment Efficiency± S.D
NLC 4	Compritol® 888 ATO	176.54	63.10±0.66
NLC 5	**Precirol® ATO 5**	**88.92**	**67.98±0.87**
NLC 6	Dynasan® 116	180.43	62.33±0.59

- **Optimization of ratio of solid lipid: liquid lipid**

  Liquid lipid and Solid lipid were optimized for its ratio and evaluated for percentage entrapment efficiency and particle size.

Batch no.	Ratio of Solid Lipid: Liquid Lipid	Particle size (nm)	% Entrapment Efficiency± S.D
NLC 7	1:1	136.54	63.90±0.69
NLC 8	1:2	168.92	65.98±0.77
NLC 9	1:3	100.43	52.33±0.09
NLC 10	2:1	86.57	63.10±0.36
NLC 11	**3:1**	**71.98**	**70.01±0.79**

Table 10: Selection of ratio of solid lipid: liquid lipid

- **Type of organic phase**

Batch no.	Organic Phase	Particle size (nm)	% Entrapment Efficiency± S.D
NLC 12	Acetone	73.54	65.10±0.76
NLC 13	Chloroform	128.92	57.98±0.44
NLC 14	Ethanol	76.93	66.33±0.50

Table 11: Selection of organic phase

As the starting with selecting 3 different organic solvents. These 3 solvents include Ethanol, Chloroform and Acetone. By performing the formulation process, chloroform was found to be non-useful as it shows higher particle size compared to others. On the other hand, acetone offered particle size of 73.59nm and Ethanol made it of 78.12nm. Both Ethanol and Acetone shows almost same entrapment efficiency and particle size. Hence, both organic phase was used for further proceedings.

- **Ratio of organic solvent**

Selection of appropriate ratio of Acetone and Ethanol was done in this stage. Different proportions of Acetone to Ethanol like 1:1, 1:2, 2:1, 3:1 was selected and evaluated for particle size and entrapment efficiency.

Batch No.	Ratio of Acetone: Ethanol	Particle Size (nm)	% Entrapment Efficiency±S.D.
NLC 15	1:1	**70.72**	**70.03±2.01**
NLC 16	1:2	107.09	67.39±0.80
NLC 17	2:1	123.90	64.97±0.16
NLC 18	3:1	172.01	62.29±1.09

Table 12: Optimization of ratio of Acetone: Ethanol

The result of 1:1 ratio of acetone: ethanol shown smaller particle size of 70.72 nm and 70.03 entrapment efficiency. Thus, acetone: ethanol with ratio of 1:1 is selected for further development.

- **Concentration of Polysorbate 80**

Batch No.	Polysorbate 80 (% v/v)	Particle size (nm)	Zeta potential (mv)
NLC 19	0.5	64.01	-10.32
NLC 20	1.0	71.02	-21.88
NLC 21	**1.5**	**59.76**	**-36.48**
NLC 22	2.0	71.15	-34.15
NLC 23	2.5	69.67	-35.34

Table 13: Optimization of concentration of Polysorbate 80

As we increase the concentration of Polysorbate -80 (%v/v), particle size and zeta potential level got increased. But, amongst all 1.5% Polysorbate -80 shown lowest particle size i.e. 59.76 nm and effective value of zeta potential. Hence, 1.5% Polysorbate -80 is selected as an optimized batch.

- **Temperature optimization of secondary phase**

Different temperature condition i.e. 60°C, 70°C and 80°C were used to optimize the temperature of secondary phase.

Batch No.	Temperature	Particle size (nm)
NLC 24	60°C	52.83
NLC 25	**70°C**	**38.89**
NLC 26	80°C	38.01

Table 14: Optimization of temperature for secondary phase

- **Optimization of drug: Lipid ratio**

Batch No.	Drug: lipid	Percent Entrapment Efficiency ± S.D.	Particle size (nm)	Zeta potential (mV)
NLC 27	1:2	61.04±0.48	11.98	-16.74
NLC 28	**1:3**	**75.86±0.19**	**11.19**	**-38.40**
NLC 29	1:4	68.81±0.41	11.46	-29.41
NLC 30	1:5	73.15±0.96	11.57	-36.08

Table 15: Optimization of drug: lipid ratio

The results of different batches for drug to lipid ratio optimization was described in above table. Effect of enhance in lipid level clearly indicated increase in particle size of NLCs. At the same time, as we increase the lipid concentration % entrapment efficiency decreases and zeta potential was also found to be reduced. Amongst all batches, drug to lipid ratio 1:3 shows best optimistic result with % entrapment efficiency 75.86 and 11.19 nm particle size and -38.60 mV zeta potential.

- **Optimized formula:**

Based on the trials carried out, optimized formula was mentioned below:

Method	Solvent Diffusion evaporation
Liquid Lipid	Miglyol® 812
Solid Lipid	Precirol® ATO 5
Liquid Lipid: Solid Lipid	1:3
Drug: Lipid	1:3
Solvent	Acetone: Ethanol
Solvent Ratio	1:1
Polysorbate conc. (% v/v)	1.5 %
Temperature of Secondary Phase	70 ºC

Table 16: Optimized formula for Zolmitriptan loaded NLC dispersion

VI. Characterization of NLC Dispersion

- **Particle size**

Bioavailability of drug can be increased by achieving lesser particle size as it offers more penetration of drug via skin barrier. By performing particle size analysis on Zetatrac (Model: Microtrac-U2552, Make: Metrohm), particle size was found to be 11.19 ± 0.86 nm.

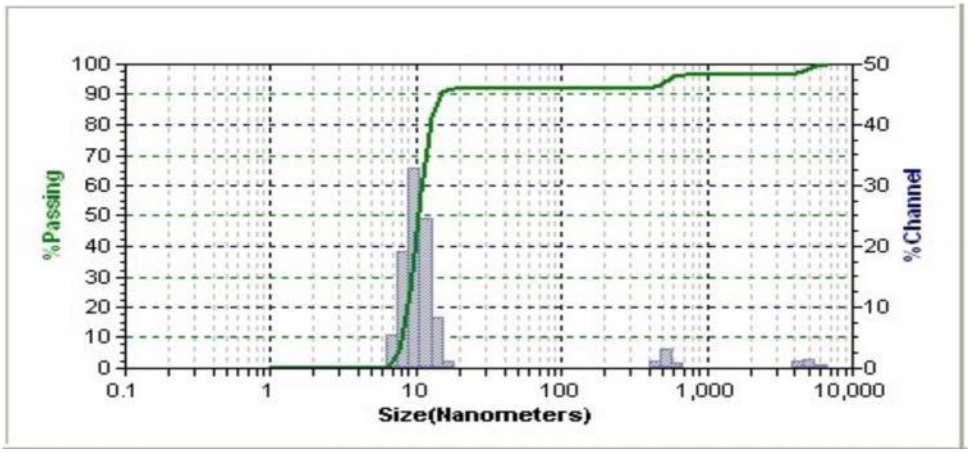

Figure 2 : Particle size of optimized batch of NLC

- **Zeta potential**

Optimized batch of NLCs was found to be offering value of Zeta Potential as -38.40mV.

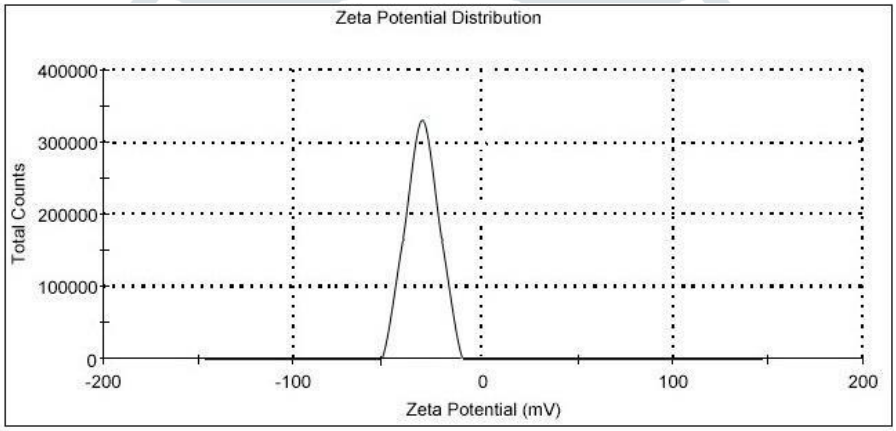

Figure 6.3: Zeta potential of optimized batch of NLC

For stability of any formulation, value of Zeta Potential requires preferably to be more than ±30 mv. Our optimized batch can be considered stable as we achieved value of Zeta Potential -38.40mV.

- **% Entrapment efficiency**

By performing analysis of % entrapment efficiency determination, optimized batch of formulation was offered 75.86±0.19 value. The resultant value of % entrapment efficiency is high and it is because of drug nature is more lipophilic. So, it shows higher affinity toward lipid barrier.

- **In vitro drug release study of NLC dispersion**

By analyzing the result, it is understood that optimized formulation shows prolonged release profile i.e. for the release of 88% drug it required 12 hours' time duration.

Time (hr)	NLC Dispersion ± S.D.
0.5	5.62±0.964
1	13.98±0.878
2	24.49±0.810
3	38.98±1.021
4	49.97±0.913
6	63.94±0.941
8	75.19±0.568

10	80.33±1.103
12	88.99±0.591
14	96.19±0.901
16	98.99±0.826
17	99.79±0.862

Table 16: In-vitro % drug release of NLC dispersion (n=3)

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