

## Original Research Article

### **Frovatriptan Succinate Loaded Lipid Nanoparticles: Formulation, Evaluation, Stability Study and Shelf Life Determination**

#### **ABSTRACT**

**Aims:** The aim of the research work was to prepare and optimize the Frovatriptan Succinate (FVN) loaded solid lipid nanoparticles.

**Methods:** SLNs were developed by solvent emulsification diffusion technique and evaluated for particle size, PDI, zeta potential, *in-vitro* drug release, and finally stability study was conducted for the detection of shelf life.

**Results:** The optimized formulation exhibited particle size, PDI, and zeta potential  $122.85 \pm 9.24$  nm, 0.129 and  $-25.85$  mV, respectively. *In-vitro* drug release study exhibited biphasic drug release pattern. Initially (in first two hrs) the drug was release in fast manor i.e. burst release ( $32.36 \pm 7.28$  %). It might be due to the presence of drug on the surface of SLNs. After 2 hrs of study, the release pattern became sustained up to 24 hrs. The total amount of drug release in 24 h was found to be  $91.29 \pm 8.26$ %. Various kinetic models were applied to evaluate the release pattern of the drug form the formulation. Higuchi model was found to be the best fitted with the  $R^2$  value of 0.9482. The release mechanism was found to be the Fickian type with the release exponent (n) value of 0.4386. Finally, stability study was conducted. The formulation was found to be the stable under the studied conditions. The shelf life of the formulation was found to be 1.77 years. **Conclusion:** Finally, it could be concluded that, the SLNs are the suitable carrier for the delivery of FVN.

**Keywords:** Frovatriptan succinate, Emulsification diffusion technique, Solid lipid nanoparticles, Stability, etc.

## 1. INTRODUCTION

Solid lipid nanoparticles (SLNs) were firstly introduced in 1991 as an alternate to other carriers such as nanoemulsion and liposomes. They are prepared by encapsulating a drug in a lipid core matrix using lipids of decent biological compatibility that are easily degradable, and have low toxicity [1]. They are used to encapsulate both hydrophilic as well as lipophilic drug(s) in biocompatible lipid core of either single lipid or combination of lipids like Compritol 888 ATO, Precirol ATO 5, glyceryl monostearate, palmitic acid, stearic acid, etc, and stabilized by surfactant which is present at the outer shell. Compared to conventional carriers, SLNs possess many advantages such as high physical stability, sustained drug release and the possibility of large-scale production with or without the use of organic solvents [2]. SLNs exhibited lower cytotoxicity, higher drug loading capacity, and easy production [3]. SLNs contain solid lipid, emulsifier and water as general ingredients. The term lipid is used in a broad sense and includes triglycerides, partial glycerides, fatty acids, steroids and waxes (e.g. cetyl palmitate). Used solid lipids and surfactants (surfactants) have GRAS (generally recognized as safe) feature and do not show toxic effects in *in-vivo* [4].

Migraine is a neurological disorder noticeably more prevalent than Alzheimer's, Parkinson's disease, and epilepsy combined [5]. It is characterized by throbbing headache attacks of unilateral or bilateral location with mild-to-intense pain. Frovatriptan (FVN), a second-generation triptan derivative, is given for the management of acute attacks of migraine with or without aura especially in females with menstrual migraine. It is 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors agonist and displaying a long half-life (26 hrs). The drug acts by constraining excessive dilation of extra cerebral and intracranial arteries, thus relieving the pain and other symptoms of migraine headache i.e. nausea, photophobia, and phonophobia [6,7]. It is currently available in the market as fast dissolving film and film-coated tablet. Existing dosage forms display limitations like slow onset of action, low bioavailability (10-30%), and adverse effects like coronary vasospasm, sensation of pain, chest tightness, and numbness in fingers. The limitations of FVN are the reduced bioavailability due to the high first-pass metabolism and it takes a long time (4 hrs) to relieve the pain after oral administration which is too long. According to International Headache Society (IHS) guidelines for the management of migraine, the pain-free response should be achieved within 2 hrs following administration of dosage form [8,9]. Therefore, in order to

overcome the limitations associated with oral tablet dosage form of FVN, SLNs containing FVN were design .

Therefore, the aim of the study was the development and evaluation of FVN-loaded SLNs. SLNs were developed by the solvent emulsification diffusion technique and characterized for particle size, entrapment efficiency, polydispersity index (PDI), zeta potential to select the optimized formulation. Structural analysis, differential scanning calorimetry (DSC), and an *in vitro* release study were performed on optimized formulation. Finally, stability study was conducted on optimized formulation (FVN-SLN6).

## **2.MATERIALS AND METHODS**

### **2.1 Chemicals and other Materials required**

FVN was procured from Spectrum Labs (Roorkee, India). Compritol ATO 888, Glyceryl monostearate (GMS), Precirol ATO 5 were obtained as a gift sample from Gattefosse, Witten (Germany), whereas Stearic acid, Palmitic acid, and Tween 80 along with all the other chemicals were purchased from Sigma-Aldrich, New Delhi (India). All other solvents & chemicals used were of analytical grade. Water was distilled and filtered before use through a 0.22 µm membrane filter.

### **2.2.Method**

#### **2.2.1 Selection of Excipients**

Solubility of FVN in melted lipid is one of the most important factors for the determination of encapsulation efficiency of the drug in the lipid. However, equilibrium solubility studies cannot be carried out in this case. Hence, we used a modified method to identify the solid lipid having better solubilization potential for drug [10,12]. Glyceryl monostearate, compritol ATO 888, precirol ATO 5, stearic acid and palmitic acid were screened for their potential to solubilize FVN.

FVN (20 mg) was taken in screw capped vial. The solid lipids were separately heated above their melting point. These lipid melts were gradually added in portions to the vial containing FVN with continuous stirring using vertex mixer and maintaining the same temperature (above the melting point of lipid). The end point of the solubility was the formation of clear, pale yellow solution of molten lipid [12]. The amount of molten lipid required to solubilize the FVN was noted visually. The experiments were perform in triplicate (n= 3).

### 2.2.2 Preparation of FVN Loaded SLNs

In an initial laboratory study, various factors like lipid concentration (3% w/w), surfactant concentration (Tween 80, 2 % w/v), methanol (3 % v/v) as the solvent for drug and lipid, homogenization time (40 min), stirring time (2 hrs) and stirring speed (3000 rpm), sonication time 5 min were fixed and their influence on particle size, entrapment efficiency were observed. Factors like lipid concentration, surfactant concentration, stirring speed and stirring time were further optimized. All of the experiments were performed in triplicate and the average±SD taken for the presentation of result.

**Table 1:** Composition of various batches of FVN-SLNs

Formulation code	Variables				
	Drug (mg)	Lipid amount (drug:lipid ratio)	Surfactant % (w/v)	Stirring time (h)	Stirring Speed (rpm)
FVN-SLN1	50	200 (1:4)	2.0	2.0	3000
FVN-SLN2	50	250 (1:5)	2.0	2.0	3000
FVN-SLN3	50	250 (1:5)	2.50	2.0	3000
FVN-SLN4	50	250 (1:5)	2.0	2.5	3000
FVN-SLN5	50	250 (1:5)	2.5	2.5	3000
FVN-SLN6	50	250 (1:5)	2.5	2.5	3500

FVN loaded SLNs were prepared by solvent emulsification diffusion technique (Table 1). To develop the lipid phase, lipid (250 mg) was melted to 70 °C. The drug (50 mg) was incorporated in the lipid phase. The aqueous phase containing Tween 80 (2.5 % v/v) was heated to the same temperature. The aqueous phase was then dispersed in the melted lipid phase, while the temperature was maintained at 80°C. The mixture was then homogenized (Remi Instruments Pvt. Ltd, India) for 30 min at 3,000 rpm to form primary emulsion (o/w). The above emulsion was poured into 80 ml of ice-cold water (2-3°C) containing surfactant (tween 80, 2.5 %) and stirred to get the SLNs. The stirring was continued (2.5 h) at 3,500 rpm to get SLNs dispersion. The SLN dispersion was then sonicated for 5 min (1 cycle, 100 % amplitude, Bandelin sonoplus, Germany) to obtain the SLN dispersion of uniform size.

### 2.2.3 Evaluation of FVN-SLNs Formulation

**Particle Size, Poly-Dispersibility (PDI), Zeta Potential, Morphological Study:** The formulated SLNs were analyzed for particle size, PDI as well as zeta potential using Zeta Sizer (Nano ZS, Malvern Instruments, UK) which is based in Dynamic Light-Scattering Measurements (DLS) in a Zeta sizer. SLNs were irradiated with a laser to the middle of the cell region at a fixed detection array of 90 ranges and variations in the intensities of the dispersed light were analyzed.

The surface morphology of optimized SLNs was studied using Transmission electron microscope (TEM) (Oxford, Wycombe, UK) and Cryo-Electron Microscope (Gatan Alto 2500 Cryo transfer system, Pleasanton, CA, USA). To perform the TEM observation, SLNs dispersion (approx. 10  $\mu$ l) were dropped on a 300 mesh copper grid coated with carbon film, allowing **sitting until** air-dried. After complete drying, the sample was stained with 2% w/v **phosphotungstic acid** solution several times and dried at room temperature. Digital micrograph and soft imaging viewer software were used to perform the image capture and analysis.

**Determination of Entrapment Efficiency (%):** A fixed quantity of FVN-SLNs dispersion (10 mL) was centrifuged (Remi Instruments, Pvt. Ltd, India) at 18,000 rpm for 20 min at 20 °C. The supernatant was analyzed spectrophotometrically at  $\lambda_{\text{max}}$  244.5 nm (Shimadzu 1800, Japan) for determination of un-encapsulated drug [13]. The entrapment efficiency (%) were calculated by using equation 1.

$$EE (\%) = \frac{(\text{Total amount of drug} - \text{amount of Free drug})}{\text{Total amount of drug}} \times 100 \dots \dots (1)$$

**DSC Analysis:** The thermogram of drug, lipid and optimized FVN-SLNs were recorded with DSC (Pyris 6 DSC Perkin Elmer, CT, USA) under an inert atmosphere. Sufficient amount of samples (5 mg) were loaded into an aluminium pan and an empty aluminium pan was used as a reference. Samples were heated at a scanning rate of 10 °C/min over a temperature range of 40–300 °C and the thermogram were recorded [14].

**In Vitro Release and Release Kinetic Studies:** *In vitro* release studies from FVN-SLNs were carried out to evaluate the release of drug from the optimized formulation and comparing it with the pure drug dispersion. It was performed by dialysis bag diffusion technique employing a dialysis membrane (Himedia, molecular weight 12,000-14,000 Daltons) [15]. Before the study, the membrane was activated by dipping in phosphate buffer for overnight. An accurate amount of FVN - SLNs and FVN dispersion (FVN-dispersion) each containing the drug equivalent to 2.5 mg was transferred to dialysis bag and sealed at both ends. The sealed bag was then suspended in a beaker containing 200 mL of phosphate buffer (pH 7.4) and stirred at a constant speed (50 rpm) at 37±0.5 °C. Aliquots (5 mL) were withdrawn at predetermined time intervals up to 24 hrs from receiver compartment (beaker) and replaced with an equal volume of fresh medium to maintain sink condition. The samples were analyzed spectrophotometrically at  $\lambda_{\text{max}}$  of 244.5 nm.

*In vitro* release data of optimized formulation was fitted to zero order, first order and Higuchi release model [15]. To find out the mechanism of drug release, data was fitted in Korsmeyer–Peppas model ( $M_t/M_\infty = Kt^n$ ) and value of n (exponent) was determined [15].

#### 2.2.4 Stability Studies and Shelf Life Determination

The stability studies were conducted to determine the effect of the presence of formulation additives on the stability of drug and also to determine the physical stability of the prepared lipid nanoparticles formulation under conditions of storage temperature and relative humidity (RH) [15]. The storage conditions used for stability testing were 4±2 °C (refrigerator), 25±2 °C /60 ±5 % RH, 40±2 °C /75 ±5 % RH in stability chamber (Hicon instruments, N. Delhi). The sample was withdrawn after a period of 0 (initial), 30, 90 and 180 days and examined particles size, PDI, zeta potential, entrapment efficiency, drug content etc. For the determination of shelf life, additional study was conducted the 30 °C. The samples were withdrawn at a distinct time point and examined for the drug content. Amount of drug remaining was determined at each time interval. Logarithm of percent drug remaining versus time (days) was plotted. The degradation rate constant 'K' was determined from the slope of the lines at each elevated temperature using the following equation (2).

$$\text{Slope} = \frac{-K}{2.303} \dots \dots \dots (2)$$

Arrhenius Plot was constructed between log K and 1/T to determine the shelf life of optimized SLNs. By extrapolation method, K value at 4 0C was determined (K4) .The shelf life (T 0.9) at 4 0C was calculated with the help of following equation (3).

$$\text{Shelf lfe } (t_{90} = \frac{0.105}{K4} \dots \dots \dots (3)$$

### 2.2.5 Statistical Analysis

Data were expressed as mean ± standard deviation. Differences were considered statistically significant with p < 0.05. Student t test was used for the comparision between two group and one was ANOVA was used for more than two group.

## 3. RESULTS AND DISCUSSION

Excipients used for the formulation development should be pharmaceutically acceptable, non-irritant and non-sensitizing in nature. They should be generally regarded as safe (GRAS). For the SLNs **development**, selection of suitable lipid and surfactant is important. Solubility of drug in the lipid is a determinant of encapsulation efficiency of the lipid nanoparticles. It is expected that high lipid solubility can result in high encapsulation efficiency[16].

Result of solubility study designated that amongst all lipids, GMS effectively solubilized the FVN. The applicability of Glyceryl monostearate in various drug delivery had been already reported previously **[10]**.

### 3.1 Preparation of SLNs

Various batches of SLNs were prepared by **solvent emulsification diffusion technique** according to table 1 and various observed responses are given in table 2.

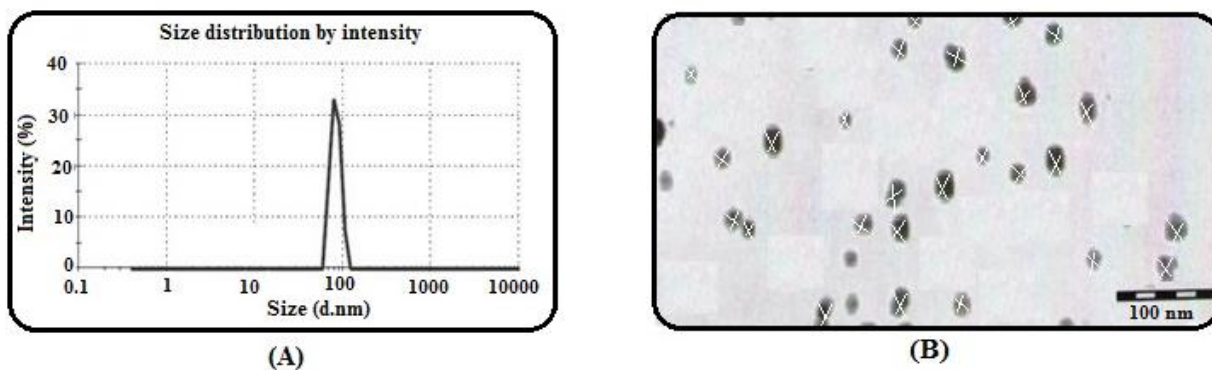
### 3.2 Evaluation of Optimized Formulation

Optimized FVN -SLN formulation (FVN-SLN**6**) was evaluated for different parameters with following results:

### 3.2.1 Particle Size, Zeta Potential and Morphological Study

Particle size of nanoparticle is an important for the drug delivery system. Here, particle size was found to be  $122.85 \pm 9.24$  nm optimized formulation exhibited PDI 0.129 indicating the almost uniform distribution of particle size (Fig. 1A). Zeta potential indicates the degree of surface charge present on the particle. Zeta potential (+ 30 mV to -30 mV) value should be high for the proper stability of the system. In this case, the value of zeta potential was found to be -25.85 mV suggested the good stability of the system. TEM image exhibiting **roughly spherical** surface of the particle (Fig. 1B). The result of TEM image result was found to be in good agreement with particle size analysis

**Effect of Formulation Variables on Particle Size:** particle size was increased on the increasing the lipid (Table 2, FVN-SLN1 and FVN-SLN2). This might be due to the insufficiency of surfactant to compete the emulsification but on increasing the surfactant, the particle size was decreased [17]. It might be due to **availability** of sufficient surfactant to the proper emulsification (Table 2, FVN-SLN1 and FVN-SLN3)



**Fig. 1:** (A) Particle size distribution curve (B) TEM image of optimized formulation

### 3.2.2 Drug Loading (%) and Entrapment Efficiency (%)

The entrapment efficiency and drug loading of optimized formulation (FVN-SLN6) were found to be  $78.73 \pm 4.65\%$  and  $32.37 \pm \%$  respectively (Table 2).

**Effect of formulation variables on Entrapment efficiency:** if the surfactant is sufficient, the entrapment efficiency increase with increasing the lipid (Table 2, FVN-SLN1 and FVN-SLN3).

This might be due to availability of surfactant ensure the solubility of drug by reducing the interfacial tension [17]. On increasing the surfactant, the entrapment efficiency is increased (Table 2, FVN-SLN2 and FVN-SLN3). The stirring time and duration also exhibiting the same effect but it was not much significant like other factors.

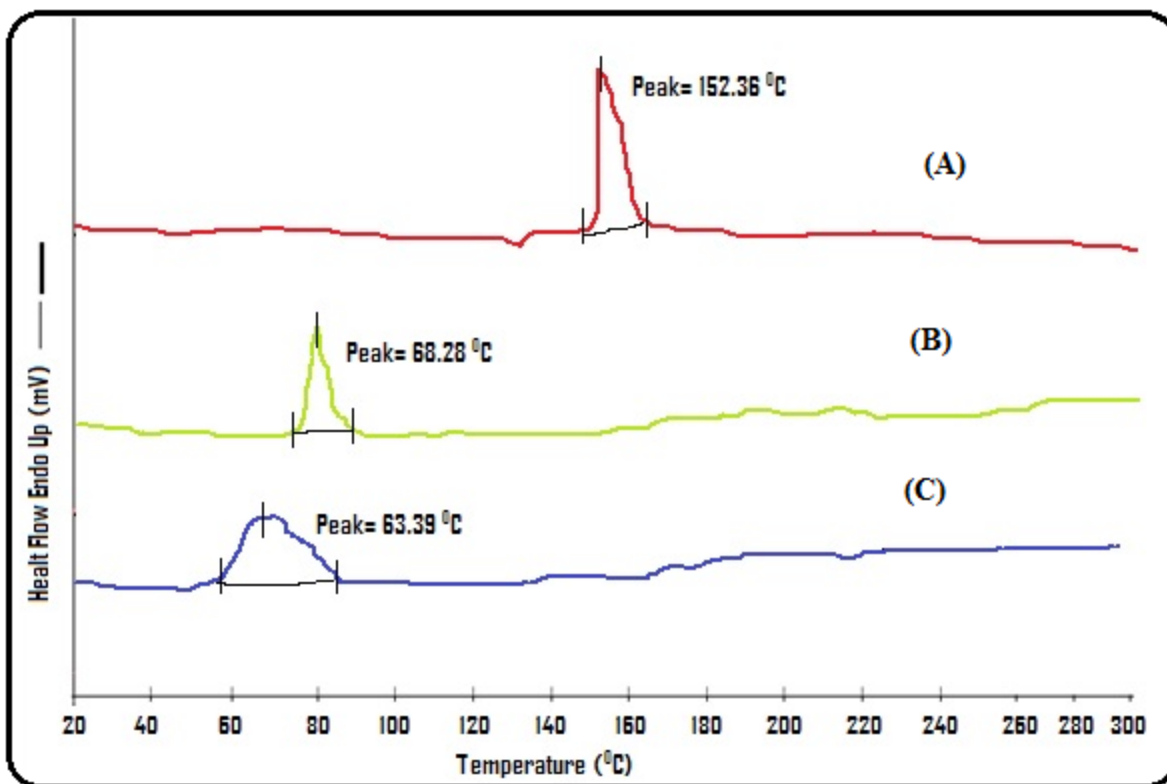
**Table 2:** Observed responses of various batches

Formulation code	Particle size (nm)*	PDI	Zeta potential (mV)	Entrapment efficiency (%)*
FVN-SLN1	208.98±12.37	0.274	-25.3	75.73±3.27
FVN-SLN2	264.77±15.91	0.429	-21.62	71.61±2.39
FVN-SLN3	145.03±11.35	0.239	-27.26	77.73±4.41
FVN-SLN4	163.92±12.47	0.185	-22.62	74.24±1.98
FVN-SLN5	135.79± 12.48	0.144	-24.73	77.63±2.48
FVN-SLN6	122.85±9.24	0.129	-25.85	78.73±4.65

\*Values are expressed as mean ± SD, n=3

### 3.2.3 DSC Analysis

The DSC thermogram of drug (FVN) showed a melting peak of 152.36 °C while lipid (Glyceryl behenate) and optimized DPL-SLNs showed at 68.28 °C and 63.39 °C respectively (Fig. 2). The thermogram of drug incorporated SLNs did not show the melting peak of crystalline DPL around 152.36 °C, pinpointing complete solubilization of drug inside the lipid matrix and being in amorphous form. Similar finding were observed by Tayade and Kale [18]. Decline in melting peak of lipid in SLNs by 4.89 °C suggested its possible existence in **amorphous** form. Similar findings were observed by Bunjes and Unruh [19].



**Fig. 2:** DSC thermogram of (a) Frovatriptan (b) Glyceryl monostearate (c) Optimized FVN-SLN6

### 3.2.4 *In Vitro* Release and Release Kinetic Studies

Optimized formulation (FVN-SLN6) was subjected for *in vitro* release study. The dissolution profile from FVN-SLN6 indicated an initial burst release, followed by slow release. The initial burst release may be attributed to the presence of free drug on the external phase and adsorbed drug onto the surface of particles, while the slow release may be owed to the encapsulated drug within the lipid matrix [20,21]. The optimized DPL-SLNs showed initial burst release of  $32.36 \pm 7.28$  % after 2 hrs and thereafter, it exhibited sustained drug release with maximum % cumulative drug release of  $91.29 \pm 8.26$  % in 24 hrs. For optimized formulation (FVN-SLN6), Higuchi model was found to be the best fitted model (Table 3) with the highest value of correlation coefficient ( $R^2 = 0.9482$ ). The value of release exponent “n” was found to be 0.4386, which appears to indicate diffusion controlled release mechanism, so-called Fickian diffusion.

**Table 3:** Release kinetic models for optimized DPL-SLN formulation

Optimized FVN-SLNs	Zero order		First order		Higuchi Model		Korsmeyer-Peppas	
	R <sup>2</sup>	K0 (hr <sup>-1</sup> )	R <sup>2</sup>	K0 (hr <sup>-1</sup> )	R <sup>2</sup>	K0 (hr <sup>-1</sup> )	R <sup>2</sup>	n value
FVN-SLN6	0.7345	3.5045	0.9396	0.0448	0.9482	20.011	0.9178	0.4386

### 3.3 Stability Studies and Shelf Life Determination

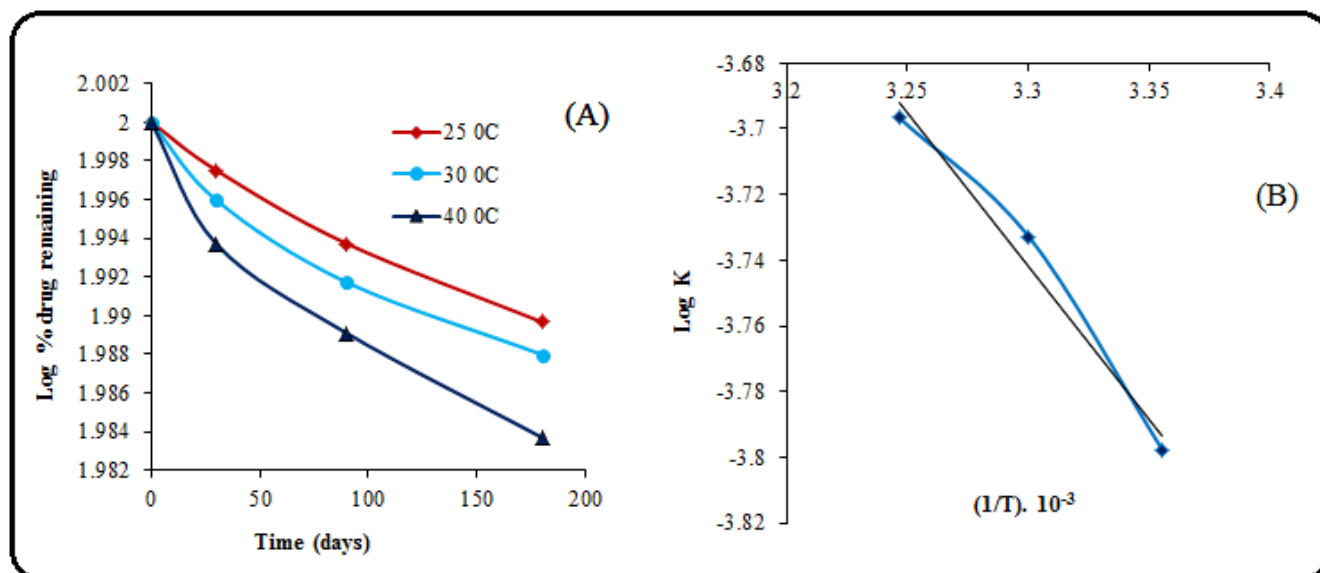
As shown in Table 4, Optimized formulation (FVN-SLN6) did not show any significant ( $P < 0.05$ ) change in particle size of formulation when it was stored at  $4 \pm 2$  °C (refrigerator) and  $25 \pm 2$  °C /  $60 \pm 5\%$  RH up to six months. On the other hand, the particle size increased significantly ( $P < 0.001$ ) when it was stored at  $40 \pm 2$  °C /  $75 \pm 5\%$  RH due to aggregation. The average particle size after 6 months at  $40 \pm 2$  °C /  $75 \pm 5\%$  RH was found to be  $1628.26 \pm 145.28$  nm while the PDI was 0.662. Zeta potential plays an important role in physical stability. There was no significant change observed in zeta potential of SLNs formulation when they were stored at  $4 \pm 2$  °C (refrigerator) and  $25 \pm 2$  °C /  $60 \pm 5\%$  RH up to six months but a significant drop in zeta potential at  $40 \pm 2$  °C /  $75 \pm 5\%$  RH ( $P < 0.001$ ) was found to be a function of time and temperature. This might be due to the fact that at high temperature & relative humidity, the outer surfactant coating get dissolved leading to aggregation of lipid nanoparticles. The entrapment efficiency (%) and drug loading (%) were also reduced with time but no significance difference ( $P < 0.05$ ) was observed [15,22].

Shelf life was detected by determining the amount of drug remaining after storage at elevated temperatures up to 180 days (2). Graphs were plotted between log % drug remained and time (Fig. 3A). From each slope, the degradation rate constant was determined. The Arrhenius graph was drawn between log K and  $1/T$  (Fig. 3B). By extrapolation of the Arrhenius plot, the value of K at 4 °C was determined. Finally, the shelf was determined by the fitting value in equation 3. The shelf life of the optimized formulation (FVN-SLN6) was found to be 1.77 years. Moreover, the degradation kinetics of the optimized formulation followed the first-order reaction.

**TABLE 4:** Particle size, PDI, zeta potential of FVN-SLNs after 180 days study at different conditions temperature and humidity.

Temperature (°C)/ % Relative humidity	Time (Months)	Characteristics parameters			
		Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
4±2	0	122.85 ± 13.47	0.129	-25.85	78.73±4.65
	30	123.47 ± 14.21	0.198	-24.38	76.48±2.38
	60	127.48±11.37	0.208	23.24	76.01±3.46
	90	118.61 ± 14.26	0.237	-25.98	75.82±3.76
	180	127.37 ± 14.91	0.381	-26.25	76.39±4.81
25±2/ 60 ±5	0	122.85 ± 13.47	0.129	-25.85	78.73±4.65
	30	119.26 ± 15.27	0.175	-24.38	77.37±3.27
	60	126.37 ± 20.25	0.235	-23.48	76.83±4.28
	90	116.86 ± 14.28	0.285	-26.37	79.27±5.71
	180	130.12 ± 14.29	0.427	-22.39	76.82±3.27
40±2/ 75 ±5	0	122.85 ± 13.47	0.129	-25.85	78.73±4.65
	30	627.26 ± 23.24*	0.284	-18.39*	71.28±4.92*
	60	892.82 ± 29.36	0.326	-17.94	70.72±5.28*
	90	1211.34 ± 103.9*	0.398*	-14.38*	68.38±5.21*
	180	1628.26±145.28*	0.683*	-09.26*	67.27±4.27*

All data expressed as mean ± S.D.;  $n = 3$ ;  $P < 0.05$ , \*Significantly different from initial values at  $P < 0.05$



**Fig 3.** Shelf life determination curve of optimized formulation (FVN-SLN6) (A) log % drug remained and time (A), and Arrhenius plot i.e. between log K and  $1/T$  (B). Values are taken as mean $\pm$ SD, (n=3).

#### 4. CONCLUSION

SLNs for Frovatriptan succinate was developed by the solvent emulsification and diffusion technique and successfully evaluated for the characteristics parameters. All parameters were in acceptable range. The value of PDI (0.129) and zeta potential indicating the almost uniform distribution of particle size and proper stable system, respectively. Result of *in-vitro* release study indicated that, the optimized formulation exhibited the drug release up to 24 hrs. Higuchi model was found to be the best fitted with the highest value of correlation coefficient ( $R^2=0.9482$ ). The value of release exponent “n” was found to be 0.4386, which appears to indicate diffusion controlled release mechanism, so-called Fickian diffusion. The formulation was found to be the stable under the studied condition. The shelf life of the formulation was found to be 1.77 years. Finally, it could be concluded, the SLNs are the suitable carrier for the delivery of FVN

## ETHICAL APPROVAL

Not applicable

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## REFERENCES

1. Wang W, Wang C, Zhang Y, Fu H, Gao , Zhang R. Preparation and characterization of solid lipid nanoparticles loaded with salmon calcitonin phospholipid complex. *J Drug Deliv Sci Tec.* Aug 2019; 52: 838–45.
2. Steiner D, Bunjes H. Influence of process and formulation parameters on the preparation of solid lipid nanoparticles by dual centrifugation. *Int J Pharma X.* 2021; 3: 100085.
3. Montoto S.S, Muraca G, Ruiz M.E. Solid Lipid Nanoparticles for Drug Delivery: Pharmacological and Biopharmaceutical Aspects. *Front. Mol. Biosci.* 2020; 7: 1-24, 587997.
4. Abdel Hady M, Sayed O.M, Akl M.A. Brain uptake and accumulation of new levofloxacin-doxycycline combination through the use of solid lipid nanoparticles: formulation; Optimization and in-vivo evaluation. *Colloids Surf. B Biointerfaces.* 2020; 193:111076
5. Kelman L., Review of frovatriptan in the treatment of migraine, *Neuropsychiatr.* 528 Dis. Treat. 2008; 4: 49-54.
6. Negro A, Lionetto L, Casolla B, Lala N, Simmaco M, Martelletti P. Pharmacokinetic evaluation of frovatriptan. *Expert Opin Drug Metab Toxicol.* 7 (2011) 1449-1458.

7. Balbisi EA, Frovatriptan: A review of pharmacology, pharmacokinetics and clinical potential in the treatment of menstrual migraine. *Ther. Clin. Risk Manag.* 2006;2: 303–308.
8. Singh H, Narang JK, Singla YS, Narang RS, Mishra V. TPGS stabilized sublingual films of frovatriptan for the management of menstrual migraine: Formulation, design and antioxidant activity. *J. Drug Deliv. Sci. Technol.* 2017;41: 144-156.
9. Verma H, Verma S, Prasad SB, Singh H, Sublingual delivery of frovatriptan: An indication of potential alternative route. *Int. Sch. Res. Notices.* 2014;1-9675868.
10. Yasir M, Sara UVS. Preparation and Optimization of Haloperidol loaded Solid Lipid Nanoparticles by Box-Behnken Design. *J Pharm Res.* 2013; 7 (6):551-558.
11. Shah AK, Date AA, Joshi MD, Patravale VB. Solid lipid nanoparticles of tretinoin: potential in topical delivery. *Int J Pharm.* 2007; 345(2):163-71.
12. Trotta M, Debernardi F, Caputo O. Preparation of solid lipid nanoparticles by a solvent emulsification diffusion technique. *Int J Pharm.* 2003; 257 (2):153-60.
13. Kumar R, Yasir M, Saraf SA, Gaur PK, Kumar Y, Singh AP. Glyceryl monostearate based nanoparticles of mefenamic acid: Fabrication and in vitro characterization. *Drug Invent Today* 2013; 5(3) :246 - 50.
14. Chadha R, Bhandari S. Drug-excipient compatibility screening—role of thermo-analytical and spectroscopic techniques. *J. Pharm. Biomed. Anal.* 2014; 87 (4): 82-97.
15. Yasir M, Sara UVS. Solid lipid nanoparticles for nose to brain delivery of haloperidol: in vitro drug release and pharmacokinetics evaluation. *Acta Pharm. Sin. B.* 2014; 4 (6):454–463
16. Jose S, Anju SS, Cinu TA. In vivo pharmacokinetics and biodistribution of resveratrol-loaded solid lipid nanoparticles for brain delivery. *Int J Pharm.* 2014; 474 (2): 6–13.
17. Varshosaz J, Tabbakhian M, Mohammadi MY. Formulation and optimization of solid lipid nanoparticles of buspirone hcl for enhancement of its oral bioavailability. *J Liposome Res.* 2010; 20 (4): 286-96.
18. Tayade PT, Kale RD. Encapsulation of water-insoluble drug by a crosslinking technique: effect of process and formulation variables on encapsulation efficiency, particle size, and in vitro dissolution rate. *AAPS PharmSci.* 2004; 6 (1):1-8.

19. Bunjes H, Unruh T. Characterization of lipid nanoparticles by differential scanning calorimetry, x-ray and neutron scattering. *Adv Drug Deliv Rev.* 2007; 59 (6): 379-402.
20. Ahmad I, Pandit J, Sultana Y, Mishra AK, Hazari PP, Aqil M. Optimization by design of etoposide loaded solid lipid nanoparticles for ocular delivery: characterization, pharmacokinetic and deposition study. *Mater Sci Eng C Mater Biol Appl.* 2019;100: 959–970.
21. Pandita D, Ahuja A, Velpandian T, Lather V, Dutta T, Khar RK. Characterization and in vitro assessment of paclitaxel loaded lipid nanoparticles formulated using modified solvent injection technique. *Pharmazie.* 2009; 64 (5):301–10.
22. Yasir M, Sara UVS, Chauhan I, Bisht D, Puri D, Maurya J. Donepezil base: physicochemical characterization, hplc method development, validation and its application for the determination of shelf life in developed solid lipid nanoparticles. 2020; 10(1): 48 – 62.