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Original Article

Development and evaluation of solid lipid nanoparticles containing anti-migraine drug

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ABSTRACT

The purpose of this research was to investigate novel particulate carrier system such as solid lipid nanoparticles for enhanced permeation of hydrophilic and lipophilic drug. A drug carrier of colloidal lipid particles with improved drug loading capacity and enhanced storage stability was investigated. A new type of solid lipid nanoparticles (SLN) has been developed by incorporating tripalmitine containing oils in the solid core of said particle. The hot high pressure homogenization technique was used as production methods for SLNs. The drug has poor bioavailability due to hepatic first-pass metabolism and low permeability into the brain due to efflux by P-glycoprotein's, the present investigation aimed to prepare solid lipid nanoparticles by tripalmitine as lipid material, egg lecithin and Poloxamer 188 as emulsifying agent, Naratriptan is provide nose-to-brain delivery for brain targeting and sustained release. The drug-excipient study was performed by DSC and FTIR. The Solid Lipid Nanoparticles was evaluated and optimized for various parameters such as particle size, polydispersity index, zeta potential, encapsulation efficiency, *in vitro* drug release and stability studies were found to be 110±1.4nm, 0.22±0.02, –16.21±4.02, 95.20±0.1 and 98 % drug release respectively. SLNs formulation was subjected to stability study over a period of 3 month.

Keywords: Naratriptan, Solid lipid Nanoparticles, migraine, hot high pressure homogenization.

INTRODUCTION

Nanoparticles (NPs) are capable of transport across the blood–brain barrier and could be qualified carriers for delivering Hydrophilic and lipophilic drug into the CNS (1,2).The Nanoparticles delivery system possibly will reduce the side effects of active pharmaceutical ingredient with high biocompatibility to normal tissue. For example, solid lipid nanoparticles (SLNs) were commonly encountering drug carriers composed mainly of biodegradable and biocompatible lipids and stabilizing surfactant (3). SLNs are composed of physiological and compatible lipids with a high melting point as the solid core, which is coated by nontoxic surfactants as the outer shell. The nanoparticles are in the submicron size range (50–1000 nm) and in the solid state at both body and room temperatures. Studies have shown that the physiochemical characteristics and stability of drug-loaded SLNs depend on the properties of drug and additives. Appropriate choice of lipids, surfactants, and their composition affect the particle size, long-term stability during storage, drug loading, and release behavior. It means that there is an optimal SLN formulation for each drug that can be obtained by investigating the effect of process variables on the characteristics of desired carriers. (4) SLNs come together the advantages of both polymeric nanoparticles and liposome's such as chance of controlled drug release and drug targeting, increased drug stability, incorporation of lipophilic and hydrophilic drugs etc. SLN production techniques include high shear homogenization and ultrasonication, high pressure homogenization, hot homogenization, cold homogenization, solvent emulsification and evaporation, etc. Lipid carriers used to prepare SLNs can be highly purified lipids such as tristearin or tripalmitine, for the lipophilic drugs SLNs serves as potential drug delivery but aqueous solubility of the drug serves as a limiting factor for its absorption. Although both hydrophilic and lipophilic drugs can be incorporated in to SLNs, loading of hydrophilic drugs is a great challenge as the drug has maximum tendency to partition in the water during the preparation process (5). The aim of this work was to assess the suitability of SLN for

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the encapsulation of Naratriptan is an acute treatment of migraine attacks with or without aura atypical drug for Administer orally with fluids. The maximum concentration of drug that could be used in those systems was estimated as well. Oral Administration Available as Naratriptan hydrochloride; dosage is expressed in terms of Naratriptan. Vascular Headaches Migraine by Oral is 1 or 2.5 mg as a single dose; individualize dosage selection, weighing the possible benefit (greater effectiveness) and risks (increased adverse effects) of the 2.5-mg dose. If headache recurs or only a partial response is achieved, may repeat dose once after 4 hours. In this work, Naratriptan-loaded SLNs were prepared by a mixture of Tripalmitine as the lipid core and combination of egg lecithin as emulsifier. The effects of lipid proportion in the lipid mixture, surfactant concentration, and surfactant composition on the particle size, drug loading, thermal characteristics, and drug release behavior of the resulting Solid lipid Nanoparticles drug delivery systems were investigated (6,7).

MATERIALS AND METHODS

Naratriptan was obtained as gift sample from GlaxoSmithKline India. Tripalmitine was purchased from the Ronak chemicals Gujarat India, Egg lecithin was obtained as gift sample from lipoid pharmaceuticals Germany, and Poloxamer 188 was obtained from Sigma-Aldrich Chemicals Private Ltd. Bangalore, India. All other chemicals were of analytical grade.

Preparation of Naratriptan SLN (8): SLNs were prepared via the hot high pressure homogenization technique proposed by (Ugazio et al.) The specified amounts of tripalmitine, and drug were melted in a water bath at 80 ^{0}C. Poloxamer 188 was mixed with deionized water containing 0.01% thiomersal as a preservative at 80 ^{0}C and 2000 rpm for 2 minutes and was added to the molten lipid mixture. The resulting emulsion was dispersed at 24,000 rpm within 5 minutes using a rotor-stator (Ultra-Turrax, IKA T18 basic; Staufen, India). Finally, the resulting nanoemulsion was gradually dispersed in 2^{0}C cooled water at a volume ratio of 1:10 under stirring at 3000 rpm. The SLNs dispersions consisted of 5% (w/v) lipid and 0.5% (w/v) drug stabilized by 0.5 to 1.5 % (w/v) of surfactant. An overview of the SLN formulations is given in Table 1.

Characterization of prepared Naratriptan SLNs

Measurement of particle size, Polydispersity index and zeta potential: The average Particle size of Naratriptan loaded SLNs was determined by laser scanning technique using (Malvern zeta sizer, Malvern instrument, Ltd UK.) after appropriate dilution with distilled water. The mean particle size, Polydispersity index and zeta potential were calculated for each formulation maintained at 25^{0}C and Polydispersity index are measure the size distribution of Nanoparticles population (9,10).

Fourier transform infrared (FTIR) spectroscopic analysis: The Fourier transform infrared spectroscopy (FT-IR) spectra were obtained using FT-IR (Shimadzu affinity 01 India.) The samples of Naratriptan, tripalmitin, Poloxamer 188, Naratriptan loaded SLNs and physical mixture of lipids and drugs were grounded and mixed thoroughly with potassium bromide, at 1:5 (sample: potassium bromide) weight ratio. FTIR spectrophotometer in the range of 400-4000cm^{-1} (11)

Scanning Electron Microscopy (SEM): The SEM analysis of prepared SLN was performed for morphological studies. The formulations are poured in to circular aluminum stubs using double adhesive tape, and coated with gold in HUS -5GB vacuum evaporator , and observed in (Hitachi S-3000N SEM) at an acceleration voltage of 10 Kv and a magnification of 5000 X (12,13).

Differential Scanning Calorimetry (DSC): DSC analysis was performed in order to investigate the melting and recrystallization behavior of crystalline materials like SLNs. DSC was performed by a Mettler DSC 821e (Mettler Toledo, USA). The samples were sealed in aluminium pans and measurements were recorded using DSC instrument. The samples were heated from 25 to 250^{0}C at a heating rate of 10^0 C /min under nitrogen atmosphere. (14,15)

Total Drug Content: From the prepared SLN formulation 1ml of SLN suspension is dissolved in the 10 ml of pH 7.4 Phosphate buffer. The amount of Naratriptan was determined using UV spectrophotometer at λ max = 223 nm. The placebo formulation prepared similarly to drug loaded SLN is used as blank. The total drug content was calculated. (16)

TDC= concentration ×dilution factor ×volume of formulation

% Entrapment efficiency : Entrapment efficiency was determined by measuring concentration of free drug in the aqueous phase using the ultra-violet spectroscopy (UV) method. Entrapment efficiency (%EE) were estimated using the total added drug, drug in precipitate (total drug added – free drug) and added excipients (lipid + surfactant mixtures), according to the following equations. (17)

%EE= {[total drug – free drug]/ total drug} ×100

In vitro diffusion studies: This is performed by using a modified Franz diffusion cell at 37^0 C which is fitted with a dialysis membrane having a molecular weight cut off 3500 Da. The membrane was soaked in boiling distilled water for 12 hours before mounting in a Franz diffusion cell. SLNs 2 ml is placed in to the donor compartment containing 20ml of PBS is used to fill receptor compartment. With one hour interval 1ml of sample is withdrawn and analysis using UV Visible spectrophotometer a 223nm.

In vivo studies: White albino rats of either sex (weighing between 250 and 300 g) were selected for the In vivo study. All animal experiments were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi, India. SLNs formulation and drug solution equivalent to 400 µg of Naratriptan were administered intranasally with the help of a micropipette (200µl) attached to low density polyethylene tubing with internal diameter 0.1 mm at the delivery site. Nanoparticulate formulation was prepared by dispersing NP in PBS (pH 7.4). Drug solution (0.4 ml) was injected intravenously into the tail vein of the rats. Subsequently, animals were sacrificed at different time intervals (n = 4 for each time point), and their brains were removed and homogenized in PBS (pH 7.4). The homogenate was centrifuged at 4000 rpm for 15 min. at 4 ^{0}C. The supernatant (100µl) was mixed with 100 µl methanol and kept in a refrigerator for 1 h. The mixture was then centrifuged at 4000 rpm for 15 min, and 20 µl supernatant was taken for analysis by UV Spectrophotometric method. (18)

Stability studies: A study was also carried out to assess the stability of SLNs containing drug Naratriptan. This was carried out according to the procedure described by Zhang et al. For this purpose the samples were taken in borosilicate glass vials and sealed, and the vials were Stored in room temperature (15^0 to 20^0C), refrigerator (3^0 to 5^0C), and 37 ^{0}C (relative humidity = 75%) over a period of 3 months. Samples were evaluated at 0, 1, 2, and 3 months for their drug content as well as any changes in their physical appearance. Chemical stability during the storage was checked by Fourier transform–infrared (FT-IR) studies after 3 months of storage (19).

RESULTS

Characterization of SLN: It can be seen that the particle size of tripalmitin-SLN dispersion was smaller, the particle size of SLN that used Poloxamer 188 as emulsifier was larger than that of egg lecithin. For example, the particle size of formulation F_6 consisting of Naratriptan and egg lecithin was 133 ± 2.4 nm, while formulation F_3 which consisted of Naratriptan and Poloxamer 188, was 175 ± 2.2 nm and mixture of surfactant (1:1) (F_8) was 110± 1.4nm An identical dependency of the release on the size was obtained for different size Naratriptan loaded SLNs. The highest cumulative amounts of drug were obtained from the smaller particle size of TP-SLN dispersion (formulation F_8) the lowest cumulative amounts of drug were obtained from the larger particle size of TP-SLN dispersion (formulation F_3).

FTIR spectroscopy: FTIR spectroscopy was used to examine the interactions between lipid, drug and other excipients (Shimadzu affinity 01) From the FTIR graphs of pure drug Naratriptan, optimized formulation and physical mixture it is inveterate that there are no particular interactions between the lipids and drug. FTIR Spectra of pure drug, lipoidic Excipients, physical mixture of lipid and drug and drug loaded SLN are shown in the fig 1.The spectrum of physical mixture was equivalent to the spectrum of crystalline drug with sharp vibration bands indicating crystalline. This showed that there are no interactions with simple physical mixing of drug, lipid carrier and other excipients. The FTIR spectrum of Naratriptan showed a strong C=O stretch band (Amide I) around 1730 cm^{-1} and an Amide II due to N-H bend at 1420 cm^{-1}. These peaks were, however, completely masked in the FTIR spectrum of SLN.

Scanning Electron Microscopy (SEM): The SEM photograph of optimized formulation reveals that particles are roughly spherical and somewhat uniformity is observed. Fig 2 shows the SEM photographs of Naratriptan loaded solid lipid nanoparticles.

Differential Scanning Calorimetry (DSC): In the development of SLNs the confirmation of desired physical state melting and recrystalision behavior of matrix lipid is of crucial importance which can be determined by the DSC. When the DSC thermo grams of the bulk lipids and corresponding SLNs are compared the difference in the position and shape of the signals are usually observed. The DSC curve of the pure drug Naratriptan shows that it is in crystalline anhydrous state, exhibiting a sharp endothermic peak at 243^0C, corresponding to its melting point 239 ^{0} C and for the optimized formulation peak is at 192^0C. See fig. 3.

%Entrapment Efficiency: Similar entrapment efficiency means that the amount of drug in the precipitate did not change via different formulations. But, for constant amount of

entrapped drug, upon increasing surfactant concentration the amount of excipients increases, which results in reduced drug loading. The percentage concentration of lipid and surfactant is increased to decrease the entrapment efficiency of drug in colloidal carrier shown in table 2. This may be due to increased solubility of Naratriptan in the aqueous phase as the percentage of Poloxamer 188 increased.

***In vitro* diffusion studies:** The Modified Franz diffusion cell with dialysis membrane was used in our study. This dialysis membrane allowed the transfer of drug immediately to the receiver compartment. The % drug release of Naratriptan from 9 different formulations of SLNs is shown in the Figure 4. The Naratriptan released from SLNs up to 25 hours.

***In vivo* studies:** The results of in vivo studies showing the time profile of Naratriptan concentration in brain after IN administration of SLNs and drug solution and intravenous (IV) administration of drug solution. After the initial 30 min, the drug concentration in the brain was found lower for IN-delivered NP (0.0337 ± 0.001%) than the IV administered Naratriptan solution (0.05 ± 0.004%). As time progressed, the concentration increased and then remained at a constant level from IN-delivered SLNs, but reduced in the case of drug solution by the same route. Thus, after 3 h, IN SLNs showed 10.86 times higher accumulation (0.0489 ± 0.003%) of drug (almost constant) in the brain compared with IN drug solution (0.0045 ± 0.005%). At the end of 1, 2 and 3 h, IN NP showed 1.82, 2.34 and 6.35 times higher accumulation of drug in brain than the drug solution administered IV The corresponding values were also higher (1.27, 1.73 and 10.86 times, respectively) for the SLNs than those of the nasal Naratriptan solution. The results of two-way ANOVA followed by Bonferroni post-tests showed statistically significant differences ($p < 0.05$) between the IN drug solution and IN SLNs at each time point except at 30 min.

When IV-delivered drug solution was compared with IN-delivered SLNs, the results were found to be highly significant with $p < 0.001$, except at 45 min. This could be related to the longer residence time of the SLNs in the rat nasal cavity, which provides the opportunity for sustained drug delivery to the brain. Also, their small diameter potentially allows SLNs to be transported transcellularly through olfactory neurons to the brain via the various endocytic pathways of sustentacular or neuronal cells in the olfactory membrane. Thus, the results of the present investigation prove that Naratriptan could be transported directly to the CNS after nasal delivery of SLNs, thereby enhancing drug concentration in the brain and also providing sustained delivery of Naratriptan.

Stability studies: Stability of a drug in a dosage form at special environmental conditions is important, because it determines the expiry date of that particular formulation. Changes in the physical appearance, like color, odor, taste, or texture of the formulation indicate the drug gives instable. The chemical changes that may occur in the formulations are ascertained through chemical analysis only. Hence, the stability and chemical interaction of the drug in the Solid lipid nanoparticles were studied. Table 3 shows the stability studies results of Naratriptan solid lipid Nanoparticales. There were no changes in their physical appearance. The total drug content in the formulations was determined at time 0 and after 1, 2, and 3 months of storage at room temperature (15^0 to 20^0C), refrigerator (3^0 to 5^0C), and 37^0C (relative humidity = 75%). It was observed that the initial drug content and the drug contents of the samples analyzed after 1, 2, and 3 months of storage at various conditions were similar, indicating there were no significant changes in the physical as well as chemical characteristics of the formulations. Chemical interaction between the drug and polymer, if any, during the storage conditions was studied using FT-IR. No significant changes were observed in the IR spectra of the drug-loaded SLNs just after the formulation (zero time) and after 3 months of storage. These results indicated the physically and chemically stable and retain their pharmaceutical properties at various environmental conditions over a period of 3 months.

DISCUSSION

In this study the effect of surfactant composition and its concentration on the properties of Naratriptan loaded SLNs prepared from tripalmitin, egg lecithin and Poloxamer 188 was studied. It was found that particle size decreased by increasing surfactant concentration or by reduction of surface tension due to solid lipid Nanoparticales. The surfactant type can also affect the stability of particles during periods of storage,[20] egg lecithin as a lipoidic and zwitterionic surfactant and also provide the electrostatic stabilization by egg lecithin, triglycerides such as tripalmitin is used as lipoidal barrier it gives to cross blood brain barrier of hydrophilic drug the mixture of tripalmitin and egg lecithin is more suitable as compared to tripalmitin and Poloxamer 188 and more suitable for the production of SLNs. High entrapment efficiency (98.3%) indicated a good compatibility

Between Naratriptan and the tripalmitin core of SLNs. The chemical composition of tripalmitin and egg lecithin it shows good stability after measuring the three month did not show any effect on physical stability and effusion of drug molecules from SLNs. Drug release from Nanoparticales with more tripalmitin and mixture of surfactant content than the single type of surfactant it gives the 98.3% of drug release and higher degradation rate, hydrophilicity, and less crystallinity of the tripalmitin structure. Drug release was expatiating to be slower from more lipophilic matrices. The partical size is 110±1.4 is produced by mixture of surfactant for investigating the effect of surfactant composition on the size, PDI, and zeta potential of SLNs It is clearly shown that lecithin alone as emulsifier was not sufficient for stabilizing SLNs, and particle agglomeration occurred leading to an average particle size of 700 nm. These results can be attributed to the hydrophilic-lipophilic balance (HLB) value of surfactants that is required for stabilizing the lipid core whereas the HLB value of egg lecithin is 8. The HLB value of lecithin is not sufficient for stabilizing the average particle size with 100% Poloxamer 188 was found to be 250 nm, but combination of surfactants reduced the particle size and PDI. Poloxamer 188 and egg lecithin had a significant effect on Zeta potential of particles prepared in the presence of 100% Poloxamer 188 as surfactant was minimum –14.35 ± 4.04 due to the nonionic structure, whereas SLNs that contained 100% egg lecithin as a zwitterionic surfactant possessed the highest zeta potential of –30 mV. Combination of these surfactants changed the surface properties of the SLNs. Slight variation of the zeta potential from –15 to –16 mV for SLNs prepared.

CONCLUSION

The objective of study was to assess the formulation and characterized to enhance the entrapment of water soluble drug in to SLN prepared by high pressure homogenization method. The hydrophilic drug had been successfully incorporated in to SLNs and the Purpose of controlled release has been achieved. Results show that on combination of two emulsifying agents such as Poloxamer 188 and egg lecithin in ratio of (1%) was found to have significant effect on particle size and entrapment efficiency (EE) ($p>0.005$). However effect of these various Poloxamer 188 and egg lecithin concentration on drug release was found to be not significant but the release profiles of Naratriptan loaded SLNs are amenable to slow delivery of the drug. The major outcome of this work was the successful entrapment of a hydrophilic drug with in a lipid core. Despite of the low zeta potential the prepared SLN were stable. It can be concluded that using mixture of Poloxamer 188 and egg lecithin (F_8) concentrations in optimum formulation better narrow size is achieved and by this SLN approach and preparation high pressure homogenizer method the drug release can be sustained and may lead to the avoidance of frequent drug administration.

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Table 1. The compositions of the tested SLN dispersion formulations

Formulation	TP%	P188%	EL%	Surfactant Mixture(1:1)
F_1	5	0.5	-	-
F_2	5	1	-	-
F_3	5	1.5	-	-
F_4	5	-	0.5	-
F_5	5	-	1	-
F_6	5	-	1.5	-
F_7	5	-	-	0.5
F_8	5	-	-	1
F_9	5	-	-	1.5

TP - Tripalmitin; EL- egg lecithin; P188- Poloxamer 188;

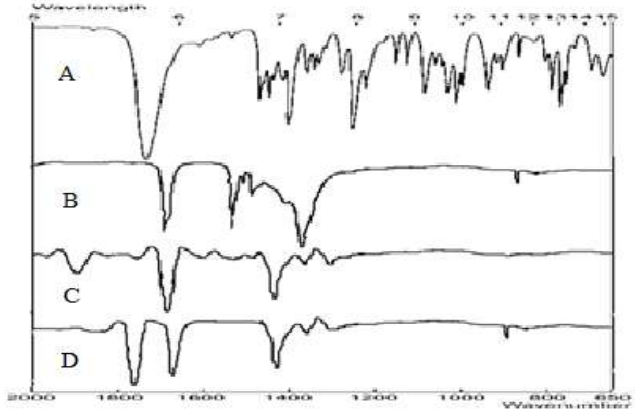

Figure1. FTIR of Naratriptan pure drug (A), Tripalmitin (B), Egg lecithin(C) and SLNs (D)

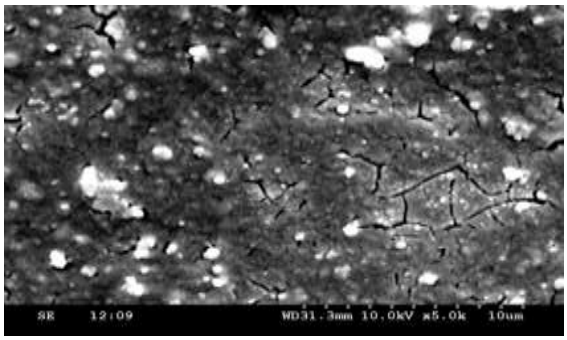

Figure2. SEM photographs of naratriptan SLNs.

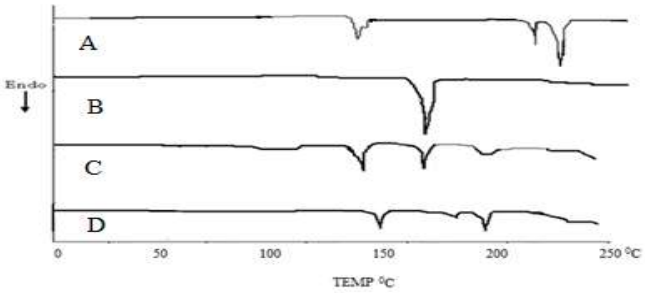

Figure3. DSC thermogram of the ingredients and lipid Nanoparticales (Naratriptan, tripalmitine, egg lecithin and naratriptan loaded SLNs).

Table 2. Size, zeta potential and entrapment efficiency and (mean of S.D. n =3) of Naratriptan SLNs of different lipids with different emulsifying agent.

Formulation	Particle Size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment Efficiency(%)	Drug loading (%)
F_1	210 ±9.1	0.60 ± 0.04	–14.35 ± 4.04	85.70 ± 0.1	12.75±0.62
F_2	190 ±5.4	0.45 ± 0.03	–15.45 ± 2.06	86.40 ± 0.6	10.85± 0.35
F_3	175 ± 2.2	0.39 ± 0.07	–13.45 ± 3.08	90.10 ± 0.9	9.50 ± 0.10
F_4	148 ± 4.6	0.24 ± 0.04	–19.50 ± 2.07	91.20 ± 0.2	13.65 ±0.50
F_5	139 ± 6.7	0.25± 0.03	–22.40 ± 1.00	90.60 ± 0.5	12.42 ±0.45
F_6	133 ± 2.4	0.24 ± 0.02	–25.10 ± 0.63	93.90 ± 0.3	10.30 ±0.23
F_7	129± 1.9	0.23 ± 0.01	–14.25 ± 4.04	92.70 ± 0.2	12.65 ±0.50
F_8	110± 1.4	0.22 ± 0.03	–16.21 ± 4.02	95.20 ± 0.1	15.30 ±0.23
F_9	122± 2.0	0.23 ± 0.09	–15.33 ± 5.08	94.50 ± 0.8	14.42 ±0.45

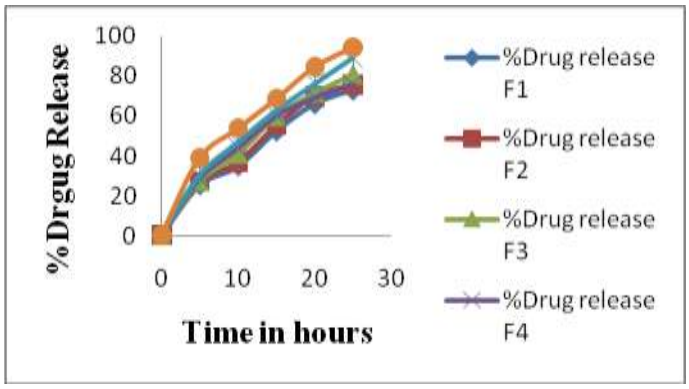

Figure4. Release profiles of naratriptan from slns prepared with different in the lipid and surfactant.

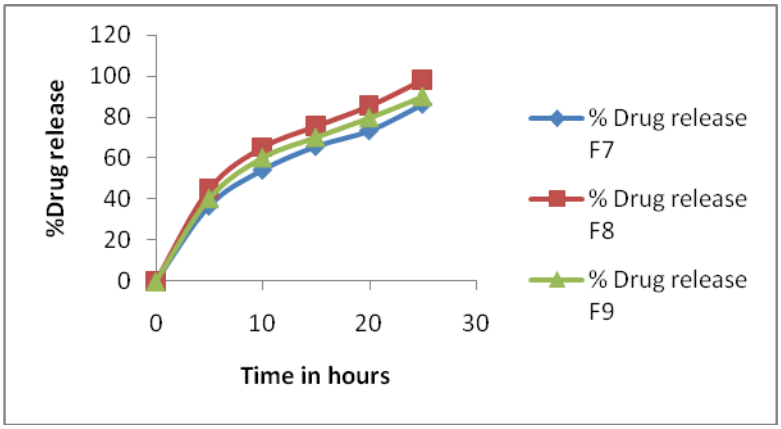

Figure.5 Release profiles of naratriptan and surfactant mixture.

Table 3. Shows the stability studies results of Naratriptan solid lipid Nanoparticales.

Temp.	**Evaluation parameter**	**Observation (month)**			
		0	1	2	3
15–20^{0}C	Physical appearance	Straw yellow	No change	No change	No change
	FT-IR	Performed	-	-	Nsc
	Drug.content(%wt/wt)	15.30 ± 0.23	15.29±0.20	15.26±0.16	15.24± 0.14
3–5^{0}C	Physical appearance	Straw yellow	No change	No change	No change
	FT-IR	Performed	-	-	Nsc
	Drug.content(%wt/wt)	15.30±0.23	15.30 ± .34	15.27±0.21	15.25 ±0.15
37^{0}C RH=75%	Physical appearance	Straw yellow	No change	No change	No change
	FT-IR	Performed	-	-	Nsc
	Drug.content(%wt/wt)	15.30 ±0.23	15.29±0.25	15.25±0.14	15.22 ±0.22

–, not performed; Nsc, no significant change. n = 3 ± SD.

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