Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

PREPARATION AND CHARACTERIZATION OF LORNOXICAM LOADED SOLID LIPID NANOPARTICLES MADE FROM DIFFERENT LIPIDS

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Received: 13 Sep 2013, Revised and Accepted: 18 Oct 2013

ABSTRACT

Objective: The objective of this study was to prepare solid lipid nanoparticles (SLNs) from different lipids and analyze their physicochemical characteristics.

Methodology: Lornoxicam loaded SLNs were prepared using different lipids (Glyceryl monostearate, Compritol 888, Cetyl palmitate, Precirol ATO5) by emulsification solvent evaporation method. The effect of different lipids and stabilizers on physicochemical characteristics of lornoxicam loaded SLNs were investigated.

Results :The entrapment efficiency of different lornoxicam loaded SLNs formulations was found to be ranged from 60.45±2.7 to 94.16±2.1 %.The results showed that the entrapment efficiency of SLNs dependent on lipophilicity of lipid .SLNs containing poloxamer 188 had smaller particle size with optimum zeta potential compared with other stabilizers (tween 80 and polyvinyl alcohol) formulated SLNs. The *in vitro* release of all four SLNs formulations were shown in similar patterns, within 8 h but the releasing rate of the four formulations were significantly different.

Conclusion: It can be concluded that the choice of lipid and stabilizer played important role on the physicochemical characteristics and *in vitro* release of SLNs.

Keywords: Lornoxicam, SLNs, *In vitro* release, Stabilizers.

INTRODUCTION

Solid lipid nanoparticles (SLNs) have recently attracted increasing attention as potential colloidal drug carriers system for controlled drug delivery. SLNs are composed of physiological and compatible lipids as the solid core, which is coated by nontoxic amphiphilic surfactants as the outer shell [1]. Studies have shown that the physiochemical characteristics and stability of drug-loaded SLNs depend on the properties of drug and ingredients [2]. Suitable choice of lipids, surfactants, and their composition affect the particle size, entrapment efficiency, zeta potential, stability during storage, and release behavior [3]. Lornoxicam is a potent nonsteroidal anti-inflammatory drug that is commonly used for the treatment of anti inflammatory, acute and chronic rheumatoid arthritis. The active drug substance is 6-chloro-4-hydroxy-2-methyl-N-2-pyridyl-2H-thieno-[2,3-e]-1,2-thiazine-3-carboxamide-1,1-dioxide.It is a yellow crystalline solid with a pKa of 4.7 [4].Lornoxicam produces gastrointestinal adverse effect. To avoid these side effects and to get better efficacy, the drug can be administered in the form of solid lipid nanoparticles as a delivery system [5]. The short half-life and poor solubility in water make lornoxicam a suitable candidate for controlled drug delivery [6]. In the present study, lornoxicam loaded SLNs were prepared using Glyceryl monostearate (GMS), Compritol 888(COM), Cetyl palmitate (CP) and Precirol ATO5(PRE) as lipid matrix by emulsification solvent evaporation method. The emulsification solvent evaporation method for preparation of solid lipid nanoparticles has been reported by various researchers [7-8]. The effects of lipids, surfactants and ratio between sodium cholate and lecithin composition on the particle size, entrapment efficiency, zeta potential and drug release behavior of the resulting lipid nanoparticles drug delivery systems were investigated for the purpose of choosing the right lipid matrix for a drug.

MATERIALS AND METHODS

Materials

Precirol ATO5 and Compritol 888 were gift from Gattefosse (Saint Priest, France). Glyceryl monostearate and Cetyl palmitate, Polyvinyl alcohol and Polysorbate 80 were procured from Himedia (Mumbai, India), Lecithin was procured from Acros Organics (New York), Sodium cholate was procured from Himedia (Mumbai, India), Poloxamer 188 was a gift from BASF (Germany),. Dialysis tube (Diameter 17.5 mm, flatwidth of 29.31 mm and pore size 2.4 nm) was obtained from Himedia (Mumbai, India), All other chemicals used in this research were of analytical grade.

Methods

Partition coefficient of drug in different lipids and phosphate buffer pH 7.4

10 mg of lornoxicam was added in a mixture of melted lipid (1 g) and 1 mL of hot pH 7.4 phosphate buffer (PB) and shaken for 30 minutes in a mechanical shaker (Remi,Mumbai) using a hot water bath maintained 10^{o}C above the melting point of the lipid [9]. The aqueous phase of the above mixture was separated from the lipid by centrifugation at 25000 rpm for 20 minutes in a high-speed cooling centrifuge. The clear supernatant was suitably diluted with pH 7.4 PB and the lornoxicam content was quantified using UV-visible spectrophotometer (Spectrascan UV2600, Thermoscientific) at 378 nm against a solvent blank. The partition coefficient of lornoxicam in lipid/ pH 7.4 PB was calculated using Equation:

Partition coefficient = C_L/C_A(1)

C_L is the amount of lornoxicam in lipid and C_A is the amount of lornoxicam in pH7.4 PB.

Preparation of SLNs

SLNs were prepared by emulsification solvent evaporation technique with slight modification [7]. The amount of different ingredients for preparation of SLNs as shown in Table 1. Lornoxicam (7.5mg) was dissolved in 3 ml of dichloromethane in which lipid and lecithin were previously dissolved. The inner oil phase was emulsified at 15000rpm (Ultra Turrax T-25 IKA Labortechnik) for 7 minutes with 15 ml of outer aqueous phase containing stabilizers and sodium cholate to prepare O/W emulsion. The emulsion was stirred at 700rpm for 3.5 hours using mechanical stirrer for complete evaporation of organic solvent. The lipid was precipitated out in the aqueous medium and formed solid lipid nanoparticles dispersion.

Purification of SLNs suspensions

Purification of lornoxicam loaded SLNs were carried out by dialysis bag method. The SLNs dispersion was taken in the dialysis bag and tied at both ends. The dialysis bag was kept into 50 ml of double distilled water containing 0.2% w/v sodium lauryl sulphate and stirred at 100 rpm for

20 minutes. 5 ml of sample was withdrawn at 5 minutes time intervals for 20 minutes. The samples were diluted appropriately and quantified the amount of drug by UV-VIS spectrophotometer (Spectrscan UV 2600, Thermo Fisher Scientific Inc, USA).

Table 1: Preparation of lornoxicam loaded SLNs.

Formulations	GMS (%)	COM (%)	CP (%)	PRE (%)	LEC (%)	SC (%)	POL (%)	POS (%)	PVA (%)
SLN-1	1				1.33	0.67	0.5		
SLN-2		1			1.33	0.67	0.5		
SLN-3			1		1.33	0.67	0.5		
SLN-4				1	1.33	0.67	0.5		
SLN-5				1	1.33	0.67		0.5	
SLN-6				1	1.33	0.67			0.5
SLN-7				1	1.5	0.50	0.5		
SLN-8				1	1.6	0.40	0.5		
SLN-9				1	1	1	0.5		

GMS: Glyceryl monostearate; COM: Compritol888; CP: Cetyl palmitate; PRE: Precirol ATO5; LEC: Lecithin; SC: Sodium cholate; POL: Poloxamer 188; POS: Polysorbate 80; PVA:Polyvinyl alcohol

Characterization of SLNs

The mean particle size and polydispersity index of the SLNs dispersions were determined by dynamic laser light scattering technique (90Plus Particle size analyzer, Brookhaven, New York, USA). The measurements of particle size were made in triplicate. Zeta potential of the formulations were determined by laser light scattering technique using Malvern zetasizer ver 6.00 (Malvern Instruments, Malvern, UK) after appropriate dilution with double distilled water. For the determination of Entrapment Efficiency (EE) and Drug Loading (DL) the lornoxicam loaded SLNs suspension (1 ml) was aggregated by acidifying to pH 1.2 with 0.1M HCl [10] and the admixture solution was immediately separated by using ultracentrifugation (Remi, Mumbai) at 10°C and 11000 rpm for 60 min. The clear supernatant was collected and the lornoxicam content in lornoxicam loaded SLNs was measured after dilution with methanol. The percentage EE was calculated by the following equation [11]:

$$\%EE = [Wa - (Ws + Wp)/Wa] \text{ X}100 \quad (2)$$

Where Wa is the quantity of drug presented in system, Ws is the quantity of drug presented in supernatant after the centrifugation, and Wp is the quantity of drug presented in the purification medium.

Drug loading was calculated by using following equation:

$$\%DL = \frac{\text{Amount of entrapped drug}}{\text{Total amount of lipid}} \text{X}100 \quad (3)$$

In vitro release study

In vitro drug release study of lornoxicam suspension, Lornoxicam loaded SLNs was studied by dialysis bag diffusion method [12]. SLN suspension equivalent to 2.5 mg Lornoxicam was taken in dialysis bag and tied at both ends. The dialysis bag was immersed in a receptor compartment containing 50 ml of pH 7.4 PB stirred at 100 rpm and temperature of 37± 0.5°C. The receptor compartment was covered with aluminum foil to prevent evaporation of the medium. 5 ml of aliquots were withdrawn at various time intervals(0.5,1,2,3,4,5,6,7,8h) for 8h and replaced with fresh volume of dissolution medium, diluted appropriately, and concentration of the drug was measured by UV-Visible spectrophotometer at 378 nm. The experiments were performed in triplicate.

In vitro release kinetics

In vitro release kinetics of different SLNs formulations were determined and analyzed with a view to find the drug release patterns. The first order and higuchi equation were determined by using the following equations [9].

First order release model= ln (Q_0-Q) vs. t (4)

Higuchi model = Q vs. $t^{1/2}$ (5)

Where Q_0 = amount of drug (mg) released at zero hour.

Q= amount of drug (mg) released at time t hour.

Stability study

Different formulations of lornoxicam loaded SLNs were stored at 4±2°C and 25±2°C for 90 days. The mean particle size and zeta potential of Lornoxicam loaded SLNs were determined.

Statistical analysis

All results were expressed as the mean value ±S.D. Statistical data were analyzed, one-way analysis of variance, followed by Dennett's t-test was performed and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION:

Partition coefficient

Partition coefficient of lornoxicam is shown in Table 3 and the Partition coefficient was in the order of PRE (2.80) >CP (2.27) > COM (1.70) > GMS (1.45). PRE is the most lipophilic among the 4 lipids and had a higher affinity for lornoxicam. PRE SLNs (SLN-4) exhibited the highest entrapment of lornoxicam (94.16%), while GMS SLNs (SLN-1) showed the lowest entrapment efficiency (60.45%). Hence, an initial study of the partitioning nature of the drug between the melted lipid and aqueous media can provide some idea about the entrapment in the SLNs formulation.

Table 2: Characterizations of lornoxicam loaded SLNs.

Formulations	MPS (nm)	PDI	EE (%)	ZP (mV)
SLN-1	318.5±4.1	0.286±0.004	60.45±2.7	-32.7±3.1
SLN-2	289.6±4.7	0.274±0.006	67.21±3.6	-35.2±2.7
SLN-3	246.1±4.9	0.234±0.005	73.34±2.5	-37.6±1.9
SLN-4	228.6±3.4	0.294±0.008	80.26±3.2	-31.4±2.1
SLN-5	352.2±5.1	0.329±0.009	66.51±2.4	-24.1±2.4
SLN-6	274.5±4.9	0.316±0.007	71.45±3.9	-16.3±3.2
SLN-7	206.9±4.1	0.261±0.005	94.16±2.1	-36.3±2.5
SLN-8	240.7±5.1	0.266±0.007	86.25±4.1	-36.8±2.9
SLN-9	252.6±4.3	0.272±0.005	85.11±3.2	-37.0±3.1

MPS: Mean particle size; PDI: Polydispersity index; EE: Entrapment efficiency; ZP: Zeta potential Data presented as mean ± S.D (n = 3).

Table 3: Effect of partition coefficient of different lipids on entrapment efficiency of lornoxicam.

Formulations	Lipids	Partition coefficient	Entrapment efficiency
SLN-1	GMS	1.45	57.11
SLN-2	COMPRITOL 888	1.70	63.28
SLN-3	CETYL PALMITATE	2.27	67.89
SLN-4	PRECIROL ATO 5	2.80	71.26

Characterization of SLNs

Lornoxicam loaded SLNs were prepared by using emulsification solvent evaporation method. The mean particle size and entrapment efficiency of different lornoxicam loaded SLNs formulation is shown in Table 2. The mean particle size of lipid nanoparticles was found to be ranged from 206.9±4.1 to 318.5±4.1nm and polydispersity index was found in between 0.234 and 0.329. The choice of stabilizers is an important parameter to be considered in optimizing any nanoparticles formulation, not only to control the particle size but also to stabilize the SLNs dispersions. SLNs containing poloxamer 188 showed smaller in particle size as compared with tween 80 and polyvinyl alcohols. The entrapment efficiency of different lornoxicam loaded SLNs formulations was found to be ranged from 60.45±2.7 to 94.16±2.1 %.The entrapment efficiency of the four SLNs(SLNs-1to SLNs-4) were significantly different and they were in the order of SLN-4 > SLN-3 > SLN-2 > SLN-1. The entrapment efficiency of SLNs was enhanced with increasing the lipophilicity of lipid, since the higher lipophilicity of the lipid resulted in increased accommodation of lipophilic drugs [13-14]. The Sodium cholate/lecithin ratio influenced on the entrapment efficiency and average size of lornoxicam loaded SLNs.There highest entrapment efficiency and narrowest size distribution of lornoxicam loaded SLNs showed when the sodium cholate/lecithin ratio was maintained at 0.33. However further Increase of sodium cholate fraction which resulted in the low entrapment efficiency and increase in average particle size of lornoxicam loaded SLNs. Zeta potential is a key factor to evaluate the stability of colloidal dispersion. The zeta potential values of different SLNs formulations are shown in Figure 1. The zeta potential values of different SLNs formulations were found to be ranged from −16.3 mV to −37.6 mV. The nanoparticles are thermodynamically unstable systems and for the stability of nanoparticles, a zeta potential value should be above +30 mV or below -30 mV [15]. The range of zeta potential obtained for SLNs with Polyvinyl alcohol was not high enough to provide a strong electrical field around the particles. This could explain the aggregates of SLNs that contained polyvinyl alcohol as stabilizer during the storage of a long period of time.

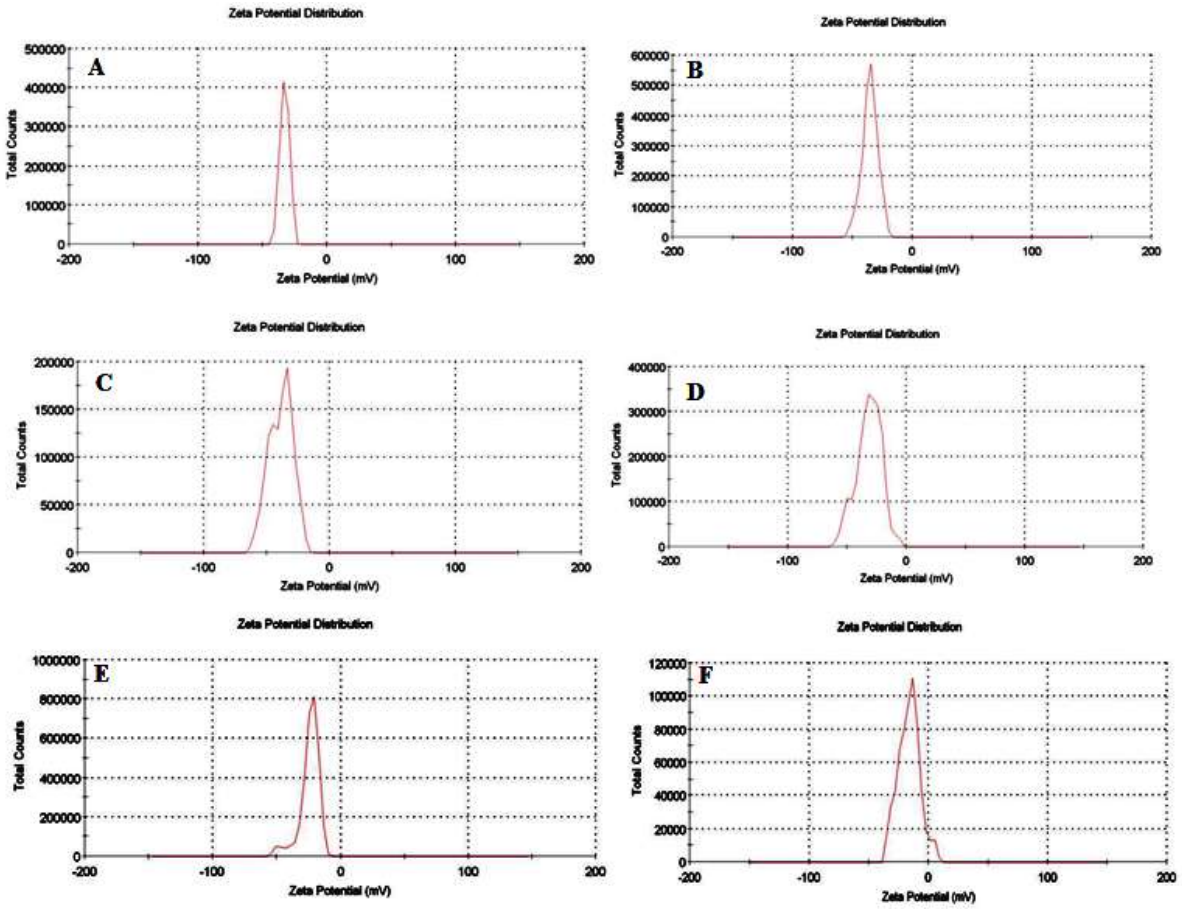

Fig. 1: Zeta potential of (A) SLN-1,(B)SLN-2,(C)SLN-3,(D)SLN-4,(E)SLN-5,and (F)SLN-6.

***In vitro* release study and release kinetics**

Solubility of lornoxicam was more in alkaline medium so pH 7.4 PB was used as the diffusion medium to study the release patterns between the different SLNs formulations. The release profiles indicated that SLNs dispersions showed a sustained release of the lornoxicam from the lipid matrix when compared with lornoxicam suspensions. *In vitro* releases of lornoxicam from the four SLNs formulations in pH 7.4 PB are shown in Figure 2. The four different SLNs showed a similar biphasic drug release pattern with a faster

release within the initial 3h and a sustained release afterwards. The lipid affected the release significantly and the higher lipophilic lipid, the slower the release rate [16]. Comparative t_{25}, t_{50} (time taken for 25%, and 50% of lornoxicam to be released) of lornoxicam suspension and the lornoxicam loaded SLNs in pH 7.4 PB is shown in Table 4. The sustained release is probably due to diffusion of drug from the lipid matrix. PRE is the most lipophilic lipid in this study, had the highest sustained release effect [16]. All the lornoxicam loaded SLNs formulations (SLN-1 to SLN-4) were found to be linear with first order release rate with R^2 values in the range of 0.96-0.98. The amount of drug released vs. square root of time were plotted for different SLNs formulations (SLN-1 to SLN-4) and R^2 value of each SLNs formulation was found to be 0.99.Thus the rate of drug release from SLNs were by diffusion controlled process.

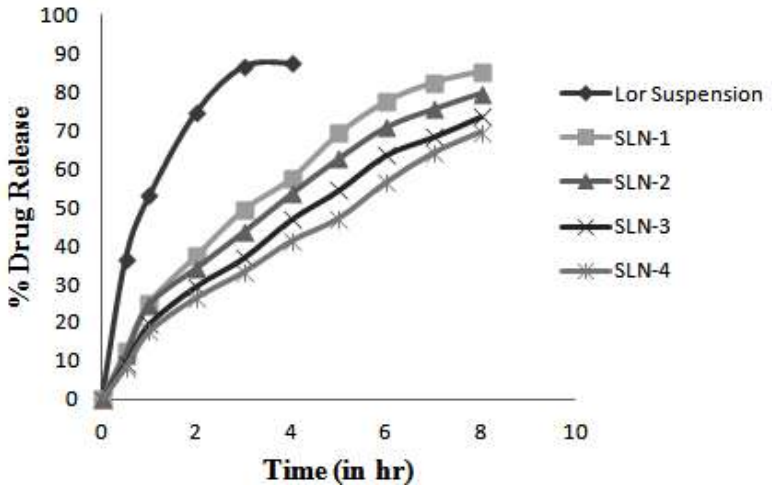

Fig. 2: In vitro release profiles of lornoxicam suspension and different SLNs formulations.

Table 4: Comparative t_{25} and t_{50} of lornoxicam loaded SLNs.

Parameters	Time (in minutes)				
	Lornoxicam suspension	SLN-1	SLN-2	SLN-3	SLN-4
t_{25}	21	58	69	90	112
t_{50}	56	187	236	270	320

Stability study

The mean particle sizes of SLNs formulations stored at 4±2°C and 25±2°C are shown in Figure 3. The Mean particle size of the SLN-4 stored at 4±2°C increased from 228.6 nm to 319.3 nm in 90 days, whereas the SLN-4 stored at 25±2°C increased from 228.6 nm to 347.6 nm. But the CP SLNs (SLN-3) showed good stability when compared with other SLNs formulations. The zeta potential of different SLNs formulations measured after 90 days of storage at 4±2°C and 25±2°C are shown in Table 5. The changes in the zeta potential were more prominent for all the lornoxicam loaded SLNs stored at 25±2°C when compared with those stored at 4±2°C.

Table 5: zeta potential values of the lornoxicam loaded SLN dispersions stored at 4°C and 25°C after day1 and day90.

FC	Zeta potential (mV)			
	4±2°C		25±2°C	
	Day 1	Day 90	Day 1	Day 90
SLN-1	-32.7	-29.5	-32.7	-26.1
SLN-2	-35.2	-32.1	-35.2	-28.1
SLN-3	-37.6	-34.6	-37.6	-32.7
SLN-4	-31.4	-30.7	-31.4	-28.2

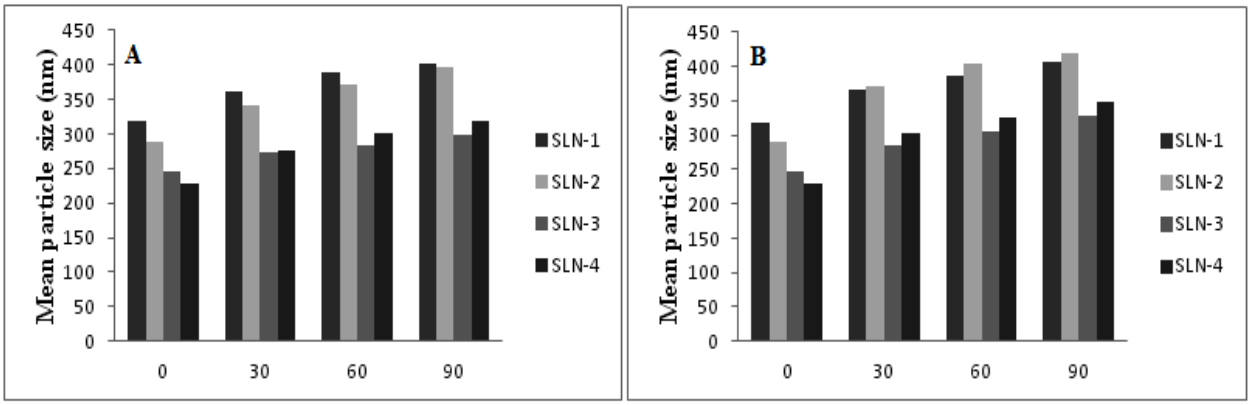

Fig. 3: Mean particle size of the lornoxicam loaded SLNs dispersions stored (A) at 4°C and (B) at 25°C after 0, 30, 60, and 90 days.

CONCLUSIONS

Lornoxicam loaded SLNs were prepared by using emulsification solvent evaporation method. The highly lipophilic lipid had significant influences on the physicochemical characteristics of lornoxicam-loaded SLNs. The highly lipophilic lipid resulted in higher drug incorporation and in vitro sustained release. SLNs containing poloxamer 188 had smaller particle size with optimum zeta potential when compared with other stabilizers. Thus the appropriate selection of lipid and stabilizer is important in the formulation of lornoxicam loaded SLNs.

REFERENCES

1. Mehnert W, Mader K. Solid lipid nanoparticles production, characterization and applica- tions. Adv Drug Deliv Rev 2001; 47:165-96.
2. Lim SJ, Kim CK. Formulation parameters determining the physiochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid. Int J Pharm 2002; 243:135-46.
3. Kim BD, Na K, Choi HK. Preparation and characterization of solid lipid nanoparticles (SLN) made of cacao butter and curdlan. Eur J Pharm Sci 2005; 24:199-205.
4. Balfour JA, Fitton A, Barradell LB. Lornoxicam, A review of its pharmacology and therapeutic potential in the management of painful and inflammatory conditions. Drugs 1996; 51(4):639-657.
5. Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Rel1999; 59: 299–307.
6. Meastrelli F, González-Rodríguez ML, Rabasco AM, Mura P. Effect of preparation technique on the properties of liposomes encapsulating ketoprofen-cyclodextrin complexes aimed for transdermal delivery. Int J Pharm 2006; 312:53-60.
7. Battaglia L, Trotta M, Gallarate M, Carlotti ME, Zara GP, Bargoni A. Solid lipid nanoparticles formed by solvent-in-water emulsion-diffusion technique: Development and influence on insulin stability. J Microencapsul 2007; 24:672–684.
8. Liu D, Jiang S, Shen H, Qin S, Liu J, Zhang Q, Li R, Xu Q. Diclofenac sodium loaded solid lipid nanoparticles prepares by emulsion/ solvent evaporation method. J Nanopart Res 2011; 13: 2375-2386.
9. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Rel 2004; 95:627-638.
10. Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ, Zeng S. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloid Surf B biointer 2005; 45: 167–173.
11. Shah M, Pathak K. Development and statistical optimization of solid lipid nanoparticles of simvastatin by using 2^3 full-factorial design. AAPS Pharm SciTech 2010;11:489–496.
12. Kyo YC, chung JF. Physicochemical properties of nevirapine loaded solid lipid nanoparticles and nano structured lipid carrier. Colloids sur B biointer 2011;83:299- 306.
13. Kumar VV, Chandrasekar D, Ramakrishna S, Kishan V, Rao YM, Diwan PV. Development and evaluation of nitrendipine loaded solid lipid nanoparticles :influence of wax and glycerides lipids on plasma pharmacokinetics. Int J Pharm 2007; 335:167–175.
14. Ozyazici M, Gokçe EH, Ertan G. Release and diffusional modeling of metronidazole lipid matrices. Eur J Pharm Biopharm 2006; 63:331-339.
15. Muller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. Adv Drug Deliv Rev 2001; 47: 3–19.
16. Reddy LH, Murthy RS. Etoposide loaded nanoparticles made from glyceride lipids: formulation, characterization, in vitro drug release, and stability evaluation. AAPS PharmSciTech 2005; 6: E158–E166.