Journal of Pharmaceutical Research Vol. 12, No. 4, October - December 2013 : 128-133.

FORMULATION AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES

Shanmukhi P [*1], Nagabhushanam M V [2], Ashok K [3], Beena Devi M [1]
Assistant professor, D.C.R.M Pharmacy college, Inkollu - 523 167, Prakasam Dt., Andhra Pradesh
Principal, D.C.R.M. Pharmacy College, Inkollu - 523 167, Prakasam Dt., Andhra Pradesh
Associate professor, QIS College of Pharmacy, Ongole - 523 001, Prakasam Dt., Andhra Pradesh

Received on : 19.08.2013 Revised : 27.12.2013 Accepted : 28.12.2013

ABSTRACT
In view of increasing bioavailability and percentage drug release by lymphatic drug delivery, Fosinopril loaded solid lipid nanoparticles were prepared by solvent emulsification and evaporation method. *In vitro* drug release studies revealed that 80% of the drug was being released from the optimized Fosinopril loaded solid lipid nanoparticles (SLNs) in 24hours. Optimized formulation and process parameters resulted in the production of Fosinopril loaded solid lipid nanoparticles with average particle size of 178.8 nm, zeta potential of -21mV and entrapment efficiency of 91.64% of 10 mg loading. *In vitro* characterization was carried out to evaluate the stability and release characteristics and kinetics. To analyze the release kinetics of drug from SLNs, drug release data was fitted into zero order, Korsmeyer-Peppas equation. Possible mechanisms for drug release might be anomalous diffusion or non-fickian diffusion. FTIR spectra, DSC thermograms revealed no significant interaction between drug and excipients. TEM photographs exhibited nanosized particles of Fosinopril. The stability studies performed for optimized SLN formulation at 4°C, 25°C, showed no significant change in % entrapment efficiency for one month. So, it was concluded that the optimized SLN formulation offers an efficient mode of delivery to the lipophilic antihypertensive drug, Fosinopril.

Keywords: *Fosinopril; Solid lipid nanoparticles; solvent emulsification and evaporation; anti hypertensive drug.*

INTRODUCTION

Solid lipid nanoparticles (SLN) have been reported as an alternative drug delivery system to traditional polymeric nanoparticles, and were introduced in early nineties. SLN are submicron (50-1000nm) colloidal carriers composed of the drug entrapped in physiological lipid which is dispersed in aqueous surfactant solution. This is the one of the most popular approaches to improve the oral bioavailability of poorly water soluble drugs[1, 2]. SLNs combine the advantages of different colloidal carriers like emulsions, liposomes, polymeric nanoparticles, etc. Additional advantages include, lack of coalescence after reaching to room temperature, better physical stability and lack of appreciable drug leakage from the particles. Also, they offer highest flexibility in controlling the release profile, cost effectiveness, excellent reproducibility, feasibility of large scale production, feasibility of incorporation of both hydrophilic and hydrophobic drugs.In recent years much work has been focused to develop SLNs as delivery systems for anti cancer drugs[3], peptides[4], anti viral drugs [5], non steroidal anti inflammatory drugs [6, 7], genetic material, antihypertensive drugs[2], cosmetics etc. SLNs find applications in site specific drug delivery, local action and enhancement of bioavailability [8]. SLNs are prepared from lipid, emulsifier and water or solvent by using different methods such as high pressure homogenization[9,10], hot[11] and cold; ultrasonication, solvent evaporation, emulsification solvent diffusion, microemulsion[2] etc. Mechanisms for enhancing the oral bioavailability of drug molecules by SLNs include enhancing dissolution or solubilisation, stimulation of lymphatic transport, increasing gastric residence time, enhancing intestinal permeability, reducing metabolism and efflux activity, preventing first pass metabolism etc.

In the present study, solid lipid nanoparticles are employed to incorporate the angiotensine converting enzyme inhibitor, Fosinopril, using glyceryl monostearate as lipid. Fosinopril belongs to the class IV in the bio-pharmaceutical classification system (BCS). The purpose of the study is to improve the antihypertensive activity of poorly water soluble drug Fosinopril by incorporating it into SLNs , and hence reduce its dose frequency and improve the patient compliance. This is achieved by incorporating the drug in a lipid vehicle using nano technology that delivers Fosinopril by lymphatic delivery.

Fosinopril may be used to treat mild to moderate hypertension, as an adjunct in the treatment of congestive heart failure, and to slow the rate of progression of renal disease in hypertensive individuals

***Correspondence :** priya_narendra@rediffmail.com

with diabetes mellitus and microalbuminuria or nephropathy. Lymphatic delivery is an alternative choice to avoid first pass metabolism in per oral drug delivery. Enhanced lymphatic transport of drugs reduces the hepatic first pass metabolism and improves bio-availability, because intestinal lymph vessels drain directly into thoracic duct, further into the venous blood, thus bypassing the portal circulation. Fosinopril is insoluble in water, with 36 % bioavailability. Glyceryl monostearate was used as lipid because it is biocompatible, and is compatible with the drug and is widely used in the preparation of SLNs. Sodium taurocholate was used as surfactant for organic phase and Tween80 was used as surfactant for aqueous phase. A combination of these two was chosen as they are easily available and compatible with each other.

MATERIALS AND METHODS

Fosinopril was a gift sample from Aurobindo Pharma (Hyderabad, India). Glyceryl monosterate (GMS) was obtained from Loba chemie (Mumbai, India). All other chemicals and reagents used were of analytical grade.

Fourier Transformed Infrared (FT-IR) spectroscopic analysis [12]

FT-IR spectra of the samples were obtained in the range of 1000 to 3500 cm^{-1}, to determine the compatibility of drug with lipid. FT-IR spectra were recorded by KBr pellet method. FT-IR analysis of pure drug, pure lipid, and the physical mixture of drug and lipid in the ratio of 1:1, were carried out. The peaks and spectra produced by the pure drug and lipid were compared with physical mixture. The spectral data are shown in Fig.1.

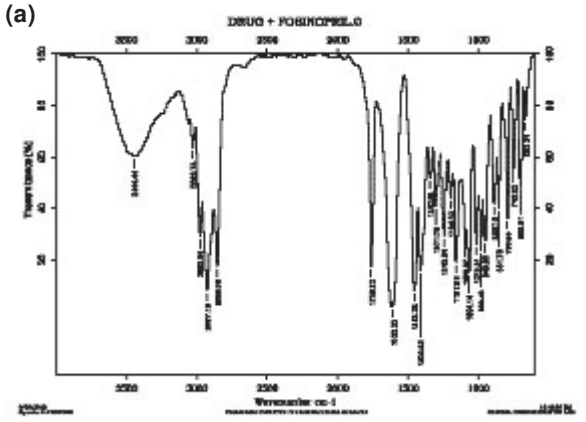


Fig.1: *FTIR spectra of (a) Fosinopril (b) Glyceryl monostearate (c) Physical mixture*

Formulation of Fosinopril loaded SLNs [2]

Solvent emulsification and evaporation method was selected to prepare solid lipid nanoparticles. In this method, accurately weighed amount of Glyceryl monostearate, 30 mg of Sodium taurocholate and 10 mg of Fosinopril were dissolved in 2ml of chloroform in a beaker. Aqueous phase having Tween80 is added to the organic phase and homogenized at 6000rpm for optimized time, in order to get coarse o/w dispersion. This coarse dispersion was subjected to ultrasonication at 120mV for optimized time, using probe sonicator. The resulted dispersion was kept for evaporation for removing the organic solvent under constant stirring at 100rpm, for optimized time. Solid lipid nanoparticles were formed at the bottom on evaporation of organic solvent.

Optimization of formulation and process variables

Three formulation variables, that is, the lipid content, concentration of Tween80 solution and volume of Tween80 solution; and three process variables, that is, homogenization time at 6000rpm, ultrasonication time at 120mV, and magnetic stirring time at 100rpm were studied and finally the formulation was optimized for maximum % entrapment efficiency (% EE), and percentage drug release (Table1).

Determination of drug content [13]

Total content of the Fosinopril was determined by dissolving 50µl formulation in 1ml of chloroform. An aliquot of 100µl of the above sample was diluted to

Table 1: *Composition of SLNs Formulations*

Formulation	Fosinopril (mg)	STC (mg)	GMS (mg)	Tween 80 conc. (%)	Tween 80 vol. (ml)	Homogenization time (min)	Sonication time (min)	Stirring time (h)
SLN 1	10	30	100	0.5	15	2	10	2
SLN 2	10	30	100	1	20	3	15	3
SLN 3	10	30	100	1.5	25	4	20	4
SLN 4	10	30	150	0.5	15	2	10	2
SLN 5	10	30	150	1	20	3	15	3
SLN 6	10	30	150	1.5	25	4	20	4
SLN 7	10	30	200	0.5	15	2	10	2
SLN 8	10	30	200	1	20	3	15	3
SLN 9	10	30	200	1.5	25	4	20	4

1ml with phosphate buffer pH 7.4 and Fosinopril content was determined by UV-Vis spectrophotometer (PG instruments limited, England). Total drug present in formulations were calculated using standard graph (Table2).

Table 2: *Drug Content, % EE, Cumulative % Drug Release at 24h*

S.No.	Formulation	Total drug content (mg)	% EE	Cumulative % drug release at 24h
1	SLN 1	6.12	42.32	58.11
2	SLN 2	6.53	46.55	60.18
3	SLN 3	5.97	40.53	54.62
4	SLN 4	9.01	80.02	75.29
5	SLN 5	9.57	91.64	80.33
6	SLN 6	9.30	86.23	78.54
7	SLN 7	7.1	54.78	64.76
8	SLN 8	8.5	75.41	73.24
9	SLN 9	7.9	63.29	69.62

Percentage Entrapment Efficiency (% EE) [14]

The percentage entrapment efficiency was determined by centrifugation method using cooling centrifuge (Remi electrotechnik limited, Mumbai, India). The undiluted sample was placed in centrifuge tubes and centrifuged at 14,000 rpm for 90 min at 4°C. The SLNs along with the encapsulated drug remained at the bottom of centrifuge tube and the unentrapped drug remained in the upper supernatant layer. The supernatant liquid was made up to desired volume with phosphate buffer pH 7.4 to measure the amount of drug using UV-Vis spectrophotometer at 208 nm (Table 2).The entrapment efficiency was calculated using following formula

% EE = (Total drug content - Unentrapped drug content) / Total drug content × 100

In-vitro drug release studies [3, 12, 15]

In-vitro drug release studies were performed using modified Franz diffusion cell (Delta Scientifics, Vijayawada, India) as shown in Fig.2. A dialysis membrane (Himedia, Mumbai) having pore size 2.4 nm, and molecular weight cut off 12,000–14,000, was soaked in double distilled water for 12 hours before mounting it on Franz diffusion cell. A volume of 2ml of Fosinopril loaded SLN formulation was placed in the donor compartment and the receptor compartment was filled with 10ml of phosphate buffer pH 7.4. The contents of the cell were stirred with the help of magnetic stirrer at 50rpm, at 37°C. 2 ml of samples were withdrawn from receiver compartment through side tube and replaced with the same volume of fresh phosphate buffer pH 7.4 at fixed intervals of 1, 2, 3, 4, 5, 6, 7, 8 and 24hours. The samples were analyzed for the drug content by using UV-Vis spectrophotometer at 208 nm (Table 2). Based on drug content, percentage entrapment efficiency, and *in vitro* drug release data, an optimized formulation was selected and following tests were performed for further evaluation of optimized SLNs formulation.

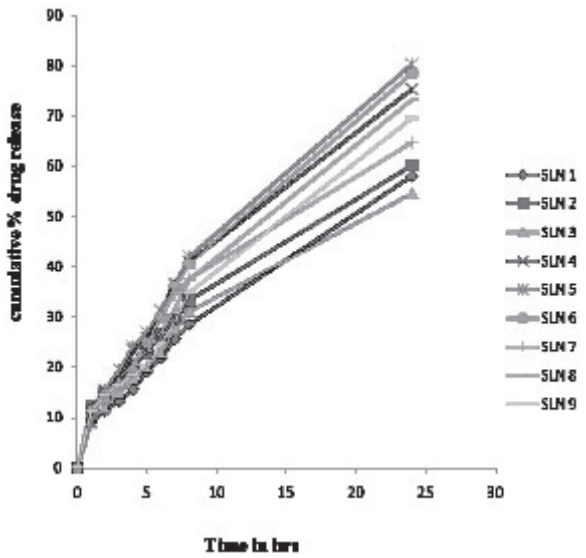


Fig. 2: *Percentage Drug Release Graph of Fosinopril SLN Formulations*

Characterization of optimized formulation (SLN-5)

Particle size analysis and Zeta potential [13]

The average particle size, polydispersity index and zeta potential (æ) of the optimized formulation were measured by photon correlation spectroscopy using Zetasizer Nano ZS (DTS Ver.5.10, Malvern Instruments, UK). The sample of dispersion was diluted with double distilled water to get optimum kilo counts per second (Fig. 3, 4).

Transmission Electron Microscopy (TEM) [16]

TEM (Hitachi, H-7500, Germany) is a method of probing the microstructure of delicate systems such as micelles, liquid crystalline phases, vesicles, emulsions and nanoparticles. The shape and size of optimized formulation was examined under TEM and photographs were taken (Fig. 5).

Differential Scanning Colorimetry (DSC) [5]

DSC (DSC200F3 Maia, Mumbai, India) was used to investigate the melting point and crystalline behavior of the crystalline materials. A heating rate of 10 K/ min was employed in the range of room temperature to 300°C. Analysis was performed under nitrogen atmosphere; about 100mg of sample was used for analysis. The samples were weighed into standard aluminum pans and an empty pan was used as reference. The samples subjected to analysis were of pure drug, pure lipid, physical mixture of pure drug and lipid and the optimized formulation (Fig. 6).

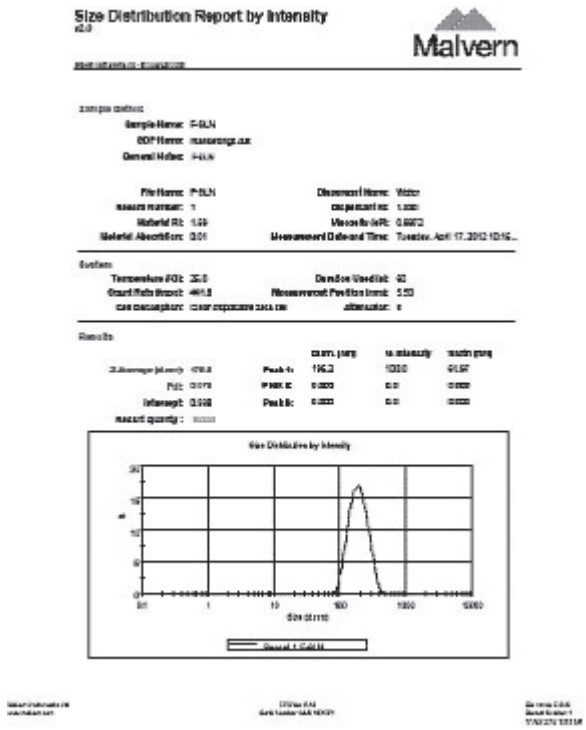


Fig.3: *Particle size analysis*

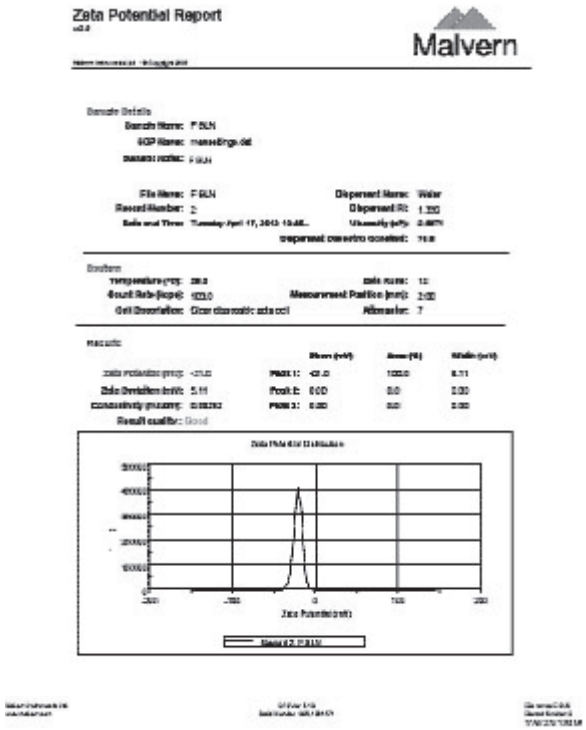


Fig.4: *Zeta Potential Report*

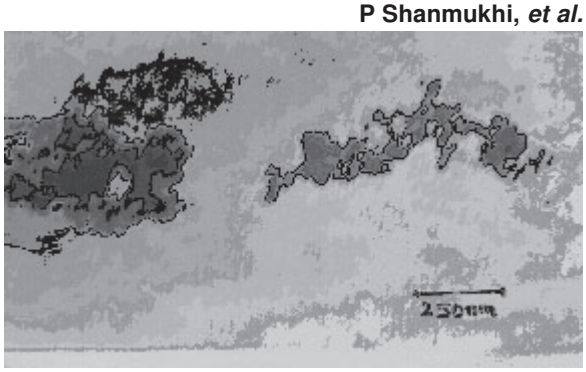
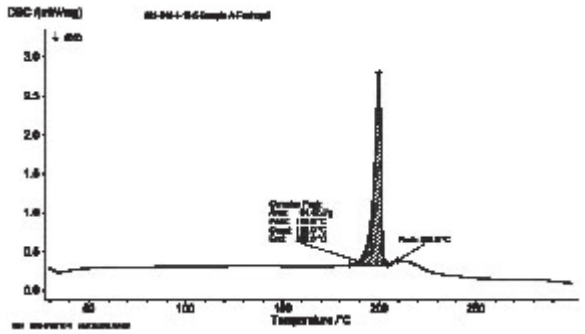
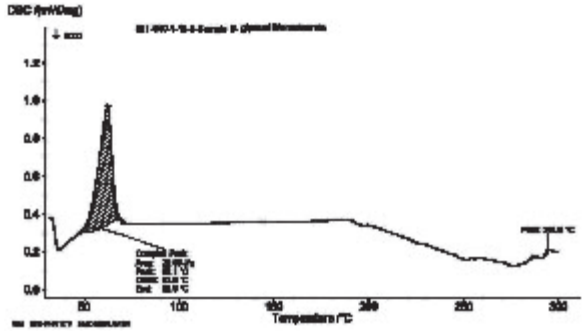


Fig.5: *TEM image*

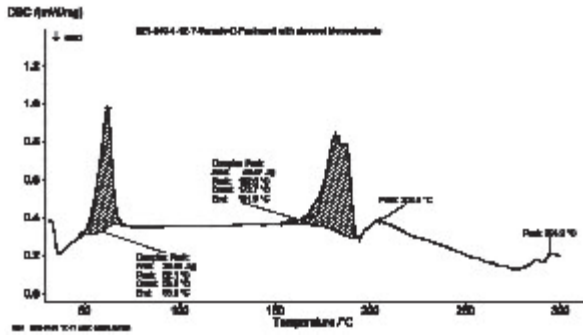
(a)



(b)



(c)



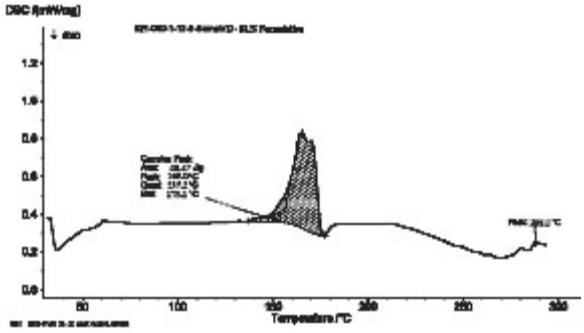


Fig.6: *DSC Thermograms of (a) Fosinopril (b) Glyceryl monostearate (c) Physical mixture (d) SLP optimized formulation*

Stability studies [14]

The stability study was performed as per modified ICH guidelines. The formulation was stored at 25°C ± 2°C and 60 ± 5% RH using stability chamber (Technico, Chennai, India) and at 4°C ± 2°C in a refrigerator. The % EE was estimated on 15th and 30th day (Table 3).

TABLE 3: *Stability Studies (% Entrapment Efficiency of SLN5 Stored at 4°and 25°)*

Formulation	% EE on 1st day	% EE at 4°C		% EE at 25°C	
		on 15th day	on 30th day	on 15th day	on 30th day
SLN-5	91.64	90.53	90.12	89.79	89.62

Drug release kinetics [12, 17, 18]

Different kinetic models such as zero order (cumulative amount of drug released vs time), first order (log cumulative percentage of drug remaining vs time), Higuchi model (cumulative percentage of drug released vs square root of time), Korsmeyer-Peppas model and Hixson-Crowell model were applied to interpret the drug release kinetics. Based on the highest regression values (R^2) for correlation coefficient for formulations, the best-fit model was decided (Table 4).

Table 4: *Drug Release Kinetics Data of Optimized Formulation SLN5*

Batch code SLN-5	Zero order Regression (R^2)	First order Regression (R^2)	Higuchi's model Regression (R^2)	Hixson-Crowell Regression (R^2)	Korsmeyer-Peppas model		Best fit model
					Regression (R^2)	Slope (n)	
(Optimized formulation)	0.9749	0.9689	0.9869	0.9529	0.9934	0.7	Zero order and Korsmeyer-Peppas's model

RESULTS AND DISCUSSION

FT-IR spectroscopic studies revealed that characteristic peaks of drug and lipid were present in the physical mixture and no major shifting and appearance of new peaks was observed. This indicates that there is no significant evidence of chemical interaction or incompatibility between the drug and the lipid.

The percentage entrapment efficiency was found to be 91.64% of 10mg loading. The *in-vitro* drug release studies indicate the cumulative amount of drug released with respect to time. This data indicates that about 80% of the drug was released from the optimized Fosinopril loaded solid lipid nanoparticles. The average particle size was 178.8 nm with polydispersity index of 0.078 and the particle size distribution was found to be normal and uniform. The zeta potential was found to be -21mV. Zeta potential is an important parameter that influences stability. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy redispersion. In case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desirable. The TEM morphology is confirming the spherical shaped particles in nanometric range.

The DSC curve of pure drug Fosinopril exhibits a sharp endothermic peak at 199.8°C. The thermogram of glyceryl monostearate displayed endothermic peak at 62.1°C. The thermogram of physical mixture displayed endothermic peaks at 62.1°C, 182.0°C and the SLN formulation thermogram displayed complete disappearance of characteristic peak of Fosinopril, indicating that the drug was molecularly dispersed within the lipid matrix, which was accompanied by the formation of a new endothermic peak at 163°C. DSC measurements showed that the optimized SLN-5 formulation was less ordered arrangement of crystals, and this was favorable for increasing the drug loading capacity. For the less ordered crystal or amorphous state, the melt of the substance did not require or just required less energy than the perfect crystalline substance which needed to overcome lattice force. As a result, the higher melting enthalpy values should suggest higher ordered lattice arrangement and vice versa. Therefore, it is concluded that the lipid within nanoparticles should be in a less ordered arrangement compared to the bulk materials corresponding to the DSC analysis.

The percentage entrapment efficiency was used to predict the stability of the preparation. The mean values of these parameters were compared with that obtained on 1st day. There was no significant change in % entrapment efficiency at storage temperatures after one month of SLNs production which indicates the stability of preparation.

Conclusion

On the basis of best fit with highest correlation (R^2), it was concluded that, the optimized formulation SLN-5 follows Zero order, Korsmeyer-Peppas models and the 'n' value was 0.7. According to Korsmeyer-Peppas kinetic model, if n'' is between 0.45 and 0.89, it indicates anomalous diffusion or non-fickian diffusion and it refers to combination of both diffusion and lipid erosion controlled rate release of Fosinopril from solid lipid nano particles.

ACKNOWLEDGEMENT

The authors wish to thank Management of D.C.R.M. Pharmacy College, Inkollu, Prakasam Dt., Andhra Pradesh for providing necessary facilities. Also, thanks to Aurobindo Pharma (Hyderabad, India) for providing the authentic sample of drug.

REFERENCES

1. Gasco MR. Method for producing solid lipid microspheres having a narrow size distribution. United states patent US 188837; 1993.
2. Ekambaram P, Abdul hasan sathali A, Priyanka K. Solid lipid nanoparticles: a review. Sci Reviews and Chem Commun 2012; 2(1): 80-102.
3. Kusum Subedi R, Kanga K, Choia H. Preparation and characterization of solid lipid nanoparticles loaded with doxorubicin. Eur J Pharm Sci 2009; 37: 508-513.
4. Hu FQ, Hong Y, Yuan H. Preparation and characterization of solid lipid nanoparticles containing peptide. Int J Pharm 004; 273: 29-35.
5. Huabing C, Xueling C, Danrong D, Wei L, *et al*. Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. J Controlled Release 2006; 110: 296-306.
6. Sedef E M, Sevgi G, Yildiz O, Ahmet A. Preparation and *in vitro* evaluation of indomethacin loaded solid lipid microparticles. Acta Pharm Sci 2009; 51: 203- 210.
7. Chauhan1 MK, Pathak AM. Evaluation of Solid Lipid Nanoparticles for the Delivery of Flurbiprofen. J Pharm Cosmetology 2011; 2: 7.
8. Manjunath, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as Drug Delivery Systems. Methods Find Exp Clin Pharmacol 2005; 27(2): 127-144.
9. Cavalli, Caputo R, Gasco O, MR. Solid lipospheres of doxorubicin and Idarubicin. Int J Pharm 1993; 89: 9-12.
10. Mehnert W, Mader K. Solid lipid nanoparticles Production, characterization and applications. Adv Drug Delivery Revs 2001; 47: 165–196.
11. Santos, Mehnert, Schaller W. Drug targeting by solid lipid nanoparticles for dermal use. J Drug Target 2002; 10: 489-95.
12. Dhanalakshi P, Rahul N, Chakrapani M, Venkatkrishnakiran P. solid lipid nanoparticle systems for delivery of drugs to the brain. Int J Biopharma 2012; 3(2): 70-77.
13. Vinay Kumara V, Chandrshekar D, Ramakrishna S, Kishan V. et al. Development and evaluation of nitrendipine loaded solid lipid nanoparticles: Influence of wax and glyceride lipids on plasma pharmacokinetics. Int J Pharm 2007;335:167-175.
14. Priyanka K, Abdul Hasan Sathali A. Preparation and evaluation of montelukast sodium loaded solid lipid nanoparticles. Pharm 2012; 4(3): 129-137.
15. Gambhire MS, Bhalekar MR, Gambhire VM. Simvastatin loaded Solid lipid nanoparticles: Formulation optimization using Box Behnken design, characterization and in vitro evaluation. Current Pharma Res 2011; 1(2): 157-164.
16. Dong Zhi H, Changsheng X, Kaijin H, Changhong Z. The production and characteristics of solid lipid nanoparticles (SLNs). Biomaterials 2003; 24: 1781–1785.
17. Suvakanta D, Narasimha Murthy P, Prasanta Chowdhury L N,. Kinetic Modeling On Drug Release From Controlled Drug Delivery Systems. Acta Poloniae Pharm Drug Res 2010; 67(3): 217-223.
18. Raslamol K, Saraswathi R, Subash S Pillai, Dilip C, Sankar C, Krishnan PN. Studies on formulation and development of oxybutynin chloride matrix tablet for oral extended release therapy and *In-vitro* evaluation. Res J Pharm, Biol and Chem Sci 2010; 1(3): 490.