AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Aceclofenac Loaded Solid Lipid Microparticle: Preparation, Characterization & In-Vitro Study

Dilip Patel[1*], Roohi Kesharwani[1], Bhaskar Mazumder[2]

1 Chandra Shekhar Singh College of Pharmacy, Kausambi, Allahabad, U.P. India.

2 Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

ABSTRACT

The aim of the study is to prepare solid lipid Microparticle (SLM) dispersion of aceclofenac for the treatment of inflammation and allied condition. SLM prepared by melt emulsification & solvent evaporation methods were characterized by Malvern instrument for particle size and particle size distribution and zeta potential analysis. The particle size of dispersion was further confirmed by scanning electron microscopy (SEM) studies. IR study of pure drug, Stearic acid and drug loaded solid lipid Microparticle were performed. In-vitro release study was performed on modified franz diffusion assembly which showed that drug release maximum 79.86% in 24 hours.

Keywords; Aceclofenac, Solid lipid Microparticle (SLM), Microparticle, Lipid Microparticle

*Corresponding Author Email: dilippatel87mph@gmail.com

Received 02 October 2013, Accepted 09 October 2013

Please cite this article in press as: Patel D. *et al.*, Aceclofenac Loaded Solid Lipid Microparticle: Preparation, Characterization & In-Vitro Study. American Journal of PharmTech Research 2013.

INTRODUCTION

There is growing interest and investment in the use of lipid-based systems in drug discovery and product development to overcome the limitations associated with traditional formulations such as poor aqueous solubility and stability, membrane permeability, drug efflux and availability1. These systems offer large variety of options such as solutions, suspensions, emulsions, microemulsions, self-emulsifying drug delivery systems (SEDDS), liposomes, self-microemulsifying drug delivery systems (SMEDDS), self-nanoemulsifying drug delivery systems (SNEDDS), dry emulsions, solid lipid microparticles (SLMs), and solid lipid nanoparticles (SLNs).[1] Micro particles are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target to specific sites.[2] Solid lipid microparticles (SLMs) attract increasing attention as alternative delivery systems. SLMs combine the advantages of different traditional carriers; for example, they can be produced on a large industrial scale and they are toxicologically highly acceptable and allow the control of drug release. SLMs appear promising as drug carrier systems for topical application.[3,4] Aceclofenac is a potent analgesic, antipyretic and anti-inflammatory agent has been approved for the treatment of various kinds of pain, osteoarthritis and rheumatoid arthritis. An arthritic condition demands a controlled release drug delivery system for prolong period so that can satisfy the goals of the treatment like reduction of the pain and inflammation, slowing the disease progression and prevention of adverse reaction.[5,6]

In this study, we prepared solid lipid microparticles by emulsification and low-temperature solidification method.[7,8] The main aim of this investigation was to develop and evaluate solid lipid microparticles of aceclofenac for topical delivery. Furthermore, in the present investigation, we aimed at fabricating SLM of aceclofenac by using simple laboratory method and with easily available solid lipids such stearic acid and characterized it for assay and in vitro release.

MATERIALS AND METHODS

Materials

Aceclofenac was kindly gifted by Cipla Sikkim India, Stearic acid, Pluronic F68 and Lecithin were purchased from Himedia Mumbai India, The other chemicals were of analytical reagent grade.

Preparation of SLM dispersion:

Aceclofenac loaded SLM were prepared by an emulsification and low-temperature solidification method. Aceclofenac was dissolved in ethyl alcohol and mixed with acetone solution containing

Stearic acid and pure lecithin. The mixtures were then added to pluronic F68 solution, stirred at 1000 rpm for 0.5 h at 60^0C temperature. The mixed solution was transferred to icy water bath and stirring for four hour at 1000 rpm.

Table I: Composition of SLM dispersion.

Formulation code	Amount of drug(mg)	Amount of lipid Stearic acid(mg)	Amount of Lecithin(mg)	Amount of Pluronic F68(%w/v)
SLM-1	100	900	--	1.5
SLM-2	100	850	50	1.5
SLM-3	100	800	100	1.5
SLM-4	100	750	150	1.5
SLM-5	100	700	200	1.5

Characterization of Aceclofenac Loaded Solid Lipid Microparticle:

Particle size and Particle size Distribution:

Particle size and polydispersity index (PI) which is a measure of the distribution of microparticle population were determined by using Malvern Mastersizer 2000MU (Malvern instrument UK detection limit 0.01–1,000 μm). All samples were analysed in triplicate with an obscuration value above 10%. The obtained data were evaluated using the volume distribution ($d_{10\%}$, $d_{50\%}$, $d_{90\%}$) which means that if the diameter 90% ($d_{90\%}$) is registered as 100 μm, this indicates that 90% of particles have a diameter of 100 μm or lower.

Zeta Potential (ζ)

The zeta potential was measured by using the Zetasizer2000 (Malvern Instruments Ltd., Malvern, UK).

Scanning Electron Microscopy

The morphological observation of NLC was studied by scanning electron microscope (JEOL-JSM-6360 Japan). One drop of sample was placed on a slide and excess water was left to dry at room temperature. then the slide was attached to the specimen holder using a double coated adhesive tape and gold coated under vacuum using a sputter coater (Model JFC-1100, JEOL, Japan)for 10 minute and investigated at 20kV.[8,9]

Drug entrapment efficiency determination

A volume of 2.0 ml of each drug-loaded sample was centrifuged at 6000 rpm for one hours to separate the lipid and aqueous phase. The supernatant was then diluted with methanol and analyzed by UV-VIS spectrophotometer at 273 nm using a UV-1800 Shimadzu spectrophotometer. The entrapment efficacy of microparticle was calculated as follows:

$$EE = \left(\frac{Wa - Ws}{Wa}\right) \times 100$$

Where EE is entrapment efficiency, Wa stands for the mass of Aceclofenac added to the formulation and Ws is the analyzed weight of drug in supernatant.[8,10]

Infra-Red Spectroscopy

An IR spectrum reveals the characteristic peaks of all functional groups present in a sample. In order to ascertain successful entrapment, the drug, lipid, their physical mixture and NLC were subjected to FTIR studies. IR spectra of aceclofenac (ACLO), stearic acid (SA), and NLC formulation were recorded using Shimadzu model 8400, IR Spectrophotometer between the ranges of 500 cm-1 to 4000 cm-1.

In-Vitro Drug Release Studies

The in vitro release studies were performed using modified Franz diffusion cell to evaluate the amount of aceclofenac released from each formulation. Dialysis membrane 70 (Hi-Media, Mumbai, India) having pore size 2.4 nm, molecular weight cut-off between 12,000-14,000 was used and mounted on the Franz diffusion cells. The surface area of the release membrane was 3.14 cm^2. The receptor medium was approximately 45 ml and composed of phosphate buffer saline (PBS), pH 7.4, and stirred by magnetic bar at 700 rpm to avoid different concentrations within the acceptor medium and to minimize stagnant layers. SLM dispersion (equivalent to 1mg of drug) was placed in the donor compartment. During the experiments, the solution in receptor side was maintained at $37^0C \pm 0.5^0C$. After certain time interval, 3-ml of the sample medium were withdrawn from receiver compartment through side tube and same volumes of freshly prepared receptor medium were added. The samples were analyzed by UV-VISIBLE spectrophotometer at 273nm. For each formulation, the release study was performed in triplicate. Concentration of aceclofenac was corrected for sampling effects according to the following equation:

$$Cn = Mn [Vt/ (Vt-Vs)] \times [(Cn-1)/ (Mn-1)]$$

Where Cn is the corrected concentration of the nth sample, Mn is measured concentration of the nth sample, Vt is the volume of the dissolution medium, Vs is the volume of the sample withdrawn, Cn-1 is the corrected concentration of previous sample[11,12.]

RESULTS AND DISCUSSION

Preparation and characterization of SLM dispersion

The d_{90} for microparticulate dispersion determined using Malvern Mastersizer showed size ranging from 36 to 50 micrometer. Particle size distribution of SLM dispersions were given in table 4.The samples have different zeta potential values because the surface charge depends on lipid matrix used. The higher magnitude of zeta potential indicates the stability of formulation.

The surface charge values are negative for empty and drug-loaded samples. Moreover, these values are lower in drug-loaded samples than empty e.g. zeta potential of blank SLM was -8.9 while for SLM-4 dispersion it was -7.6. The presence of drug causes a diminution of surface charge of all the investigated samples because probably a share of drug is situated on the lipid microparticles surface.

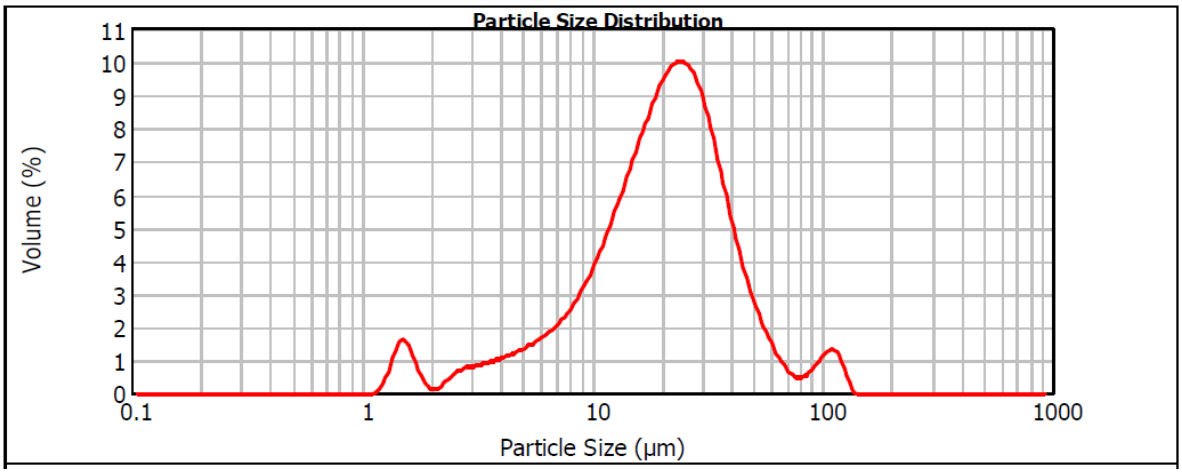

Figure 1: Particle size distribution of Blank SLM dispersion

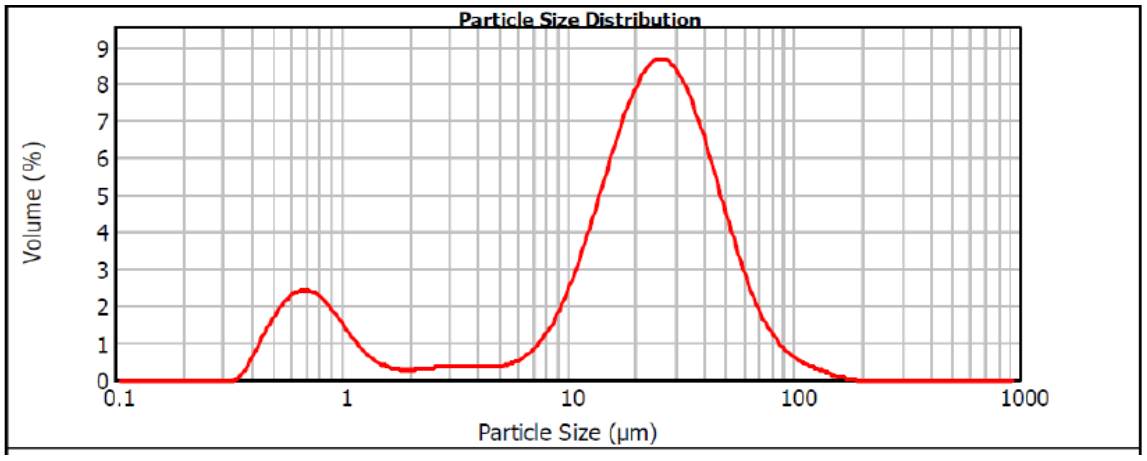

Figure 2: Particle size distribution of Blank SLM-1 dispersion

Table II: Particle size distribution zeta potential and entrapment efficiency of different formulations of SLM and blank formulation

Formulation code	Mean volume distribution (µM)			% Obscuration	Zeta Potential (mV)	%EE
	$d_{10\%}$	$d_{50\%}$	$d_{90\%}$			
SLM-1	0.876	22.216	50.993	12.16	-6.2	79.867
SLM-2	0.865	21.060	47.608	12.19	-6.8	80.887
SLM-3	0.991	20.825	43.625	12.16	-6.9	81.876
SLM-4	0.855	17.080	36.234	12.30	-7.6	81.890
SLM-5	0.864	17.088	37.067	12.34	-8.2	82.874
B-SLM(Blank)	6.273	20.833	43.928	14.00	-8.9	

Scanning Electron Microscopy

SEM allowed us to obtain more information about particle size and shape, spherical and disc like particle with a size in the micrometer range were observed for the SLM formulation (Figure 3), which was in agreement with the size data determine by Malvern mastersizer.

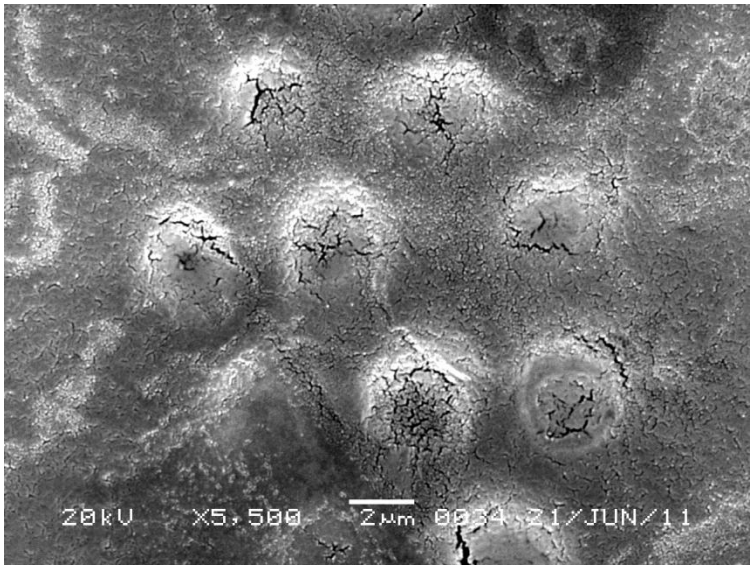

Figure 3: SEM image of SLM

Drug entrapment efficiency determination

It has been observed that by increasing the lecithin content lead to better entrapment efficiency by providing more space to incorporate the drug. Increments of the lecithin content reduce the possibility of drug to enter the external phase, which accounted for the enhancement of entrapment efficiency. Higher percentage entrapment 82.87% was found when the amount of cosurfactant (Lecithin) was increased from 50 mg to 200 mg.

Infra-red spectroscopy

For FTIR study about 1-2 mg of sample were mixed with anhydrous potassium bromide. The IR spectra of pure drug aceclofenac, SA, SLM, are shown in Figure 4,5, and 6 respectively. The IR spectrum of pure drug, aceclofenac, which is 2-[2-[2-(2,6-dichloroanilino) phenyl]acetyl]oxyacetic acid shows a hydroxyl broad band at 3,280 cm^{-1}, a carbonyl peak at 1,718 cm^{-1}, an aryl chloride peak at 1,055 cm^{-1}, C–H bending at 750 cm^{-1} and NH stretching at 3,317 cm^{-1}(19, 20). However, in the IR spectrum of aceclofenac loaded SLM (Figure 6) peaks of NH group, carbonyl group, hydroxyl group and aryl chloride group are absent. It is evident that the IR spectrum of SLM resembles that of the lipid (Figure 6) thus proving that the lipid forms the outer core and the drug has been successfully incorporated inside.[13,14]

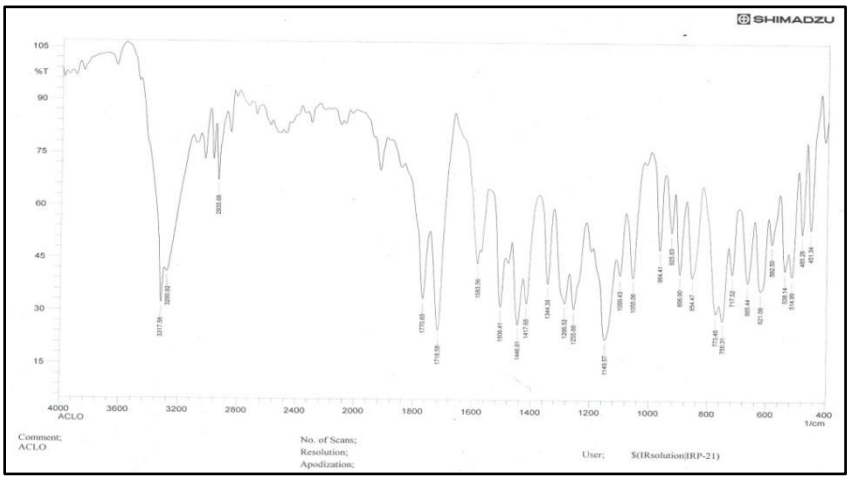

Figure 4: IR spectrum of aceclofenac

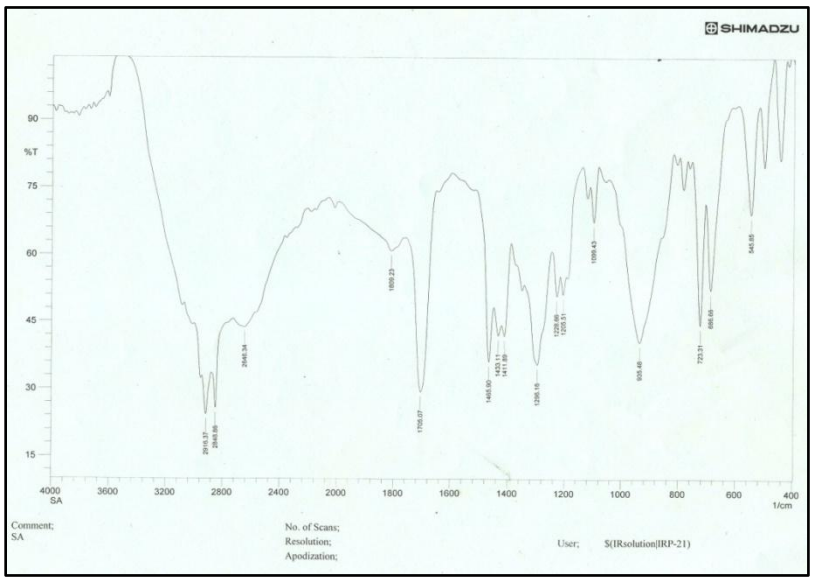

Figure 5: IR spectrum of Stearic acid

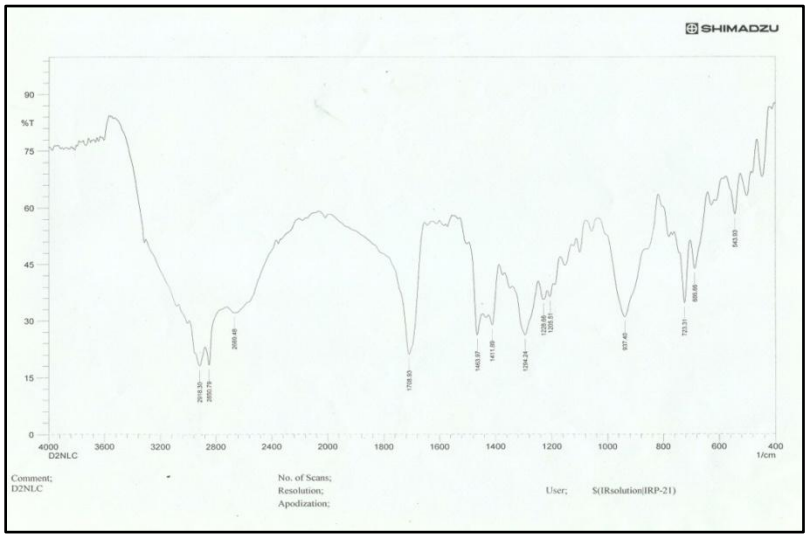

Figure 6: IR spectrum of aceclofenac loaded SLM

In-vitro Drug Release Studies:

The *in-vitro* release of the aceclofenac from SLM is found to be biphasic with the initial burst effect is slight, which was followed by much slower gradual release of the drugs which portrayed in Figure 3. The initial burst release might be due to the presence of unentrapped drug in the SLM dispersion. Another reason might be due to most of the liquid lipid being located in the outer shell of the nanoparticles, which lead to a drug-enriched shell that is related to burst release at the initial stage. The amount of drugs released by the initial burst effect varies with batches and upto 3 – 12 % of the drugs can be released during the initial release phase and this phase may extent up to 2 hours. The initial burst release is seen in almost all the batches. It is found to be of advantageous since it will provide for the achievement of initial therapeutic concentration. The decrease in release rate is due to the exhaustion of the drugs on the surface and the increase in diffusional path-length as the drug continues to dissolve and diffuse from the matrix.

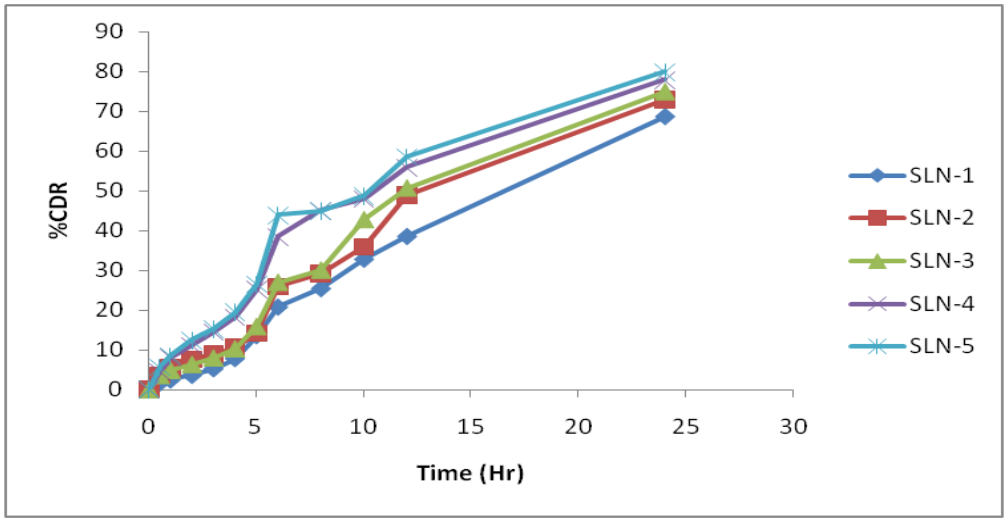

Figure 7: Comparative drug release profile of aceclofenac loaded SLM.

Table III: Percentage release of aceclofenac from different dispersion

Sampling Time (Hr)	SLN-1	SLN-2	SLN-3	SLN-4	SLN-5
0.5	1.71±0.28	3.58±0.68	3.58±0.45	4.51±0.86	5.60±0.56
1	2.46±0.52	5.41±0.45	4.92±0.66	8.03±1.67	8.52±1.56
2	3.62±1.56	7.60±0.57	6.39±0.87	11.05±1.43	12.43±2.06
3	5.27±1.65	8.91±2.56	8.00±1.34	14.54±0.45	15.27±1.87
4	7.85±0.45	10.53±1.87	10.34±1.89	18.18±0.35	19.52±0.45
5	13.58±1.67	14.22±0.34	15.92±0.56	25.25±1.45	26.53±0.56
6	20.78±1.87	25.92±0.69	27.04±1.56	38.44±2.08	43.80±0.34
8	25.41±1.78	29.18±0.45	30.12±1.45	44.95±1.88	44.85±0.56
10	32.79±0.67	35.90±2.12	42.79±0.26	47.98±2.10	48.67±1.95
12	38.54±0.63	48.76±1.45	50.74±0.88	55.86±2.08	58.46±1.56
24	68.66±2.67	72.85±0.88	74.98±0.46	77.89±0.68	79.86±1.54

The release rates became faster when the lecithin concentration was increased in the SLM dispersion. The SLM dispersion containing no lecithin (SLM-1) showed a 68.66% release, whereas the SLM dispersion containing 50 mg of lecithin (SLM-2) released up to 72.85% (table III) of aceclofenac within 24 hrs. by further increasing the concentration of lecithin from 50 mg to 200 mg drug release was increases respectively (i.e. from 72.85% to 79.86%).This revealed that the lecithin played an important role in the release of aceclofenac from the SLM dispersion.

CONCLUSION

In the present work SLMs containing aceclofenac produced by emulsification and low-temperature solidification method by varying the solid lipid and co-surfactant lecithin concentration. The study indicated that the amount of solid lipid and lecithin were significantly affecting the particle size as well as entrapment efficiency. d90% of SLM-1 was found to be 50.993 µm where dispersion containing higher amount of lecithin having d90% 37.067 µm. release profile of SLM dispersion also influenced by the content of lecithin as well as solid lipid. Dispersion containing higher amount of lecithin (SLM-5) release 79.86% drug in 24 hour and dispersion containing no lecithin release only 68.66% drug.

REFERENCES

1. Chukwuebuka E Umeyor, Franklin C Kenechukwu, Emmanuel. M. Uronnachi1, Uduma. E. Osonwa1, Calistus. D. Nwakile. Solid Lipid Microparticles (SLMS): An Effective Lipid Based Technology for Controlled Drug Delivery Am. J. PharmTech Res. 2012; 2(6).
2. Haznedar S, Portune B. Preparation and in vitro evaluation of eudragit microspheres containing acetazolamide, Int J Pharma 2004; 269: 131-140.
3. Amal H. El-kamel, Iman M. Al-fagih, Ibrahim A. Alsarra. testosterone solid lipid microparticles for transdermal drug delivery. Formulation and physicochemical characterization. J Microencapsulation, August 2007; 24(5): 457–475
4. Jaspart S, Piel G, Delattre L, Evrard B. Solid lipid microparticles: Formulation, preparation, characterization, drug release, and applications. Expert Opinion on Drug Delivery. 2005; 2:75–87.
5. Insel PA Analgesic-antipyretics and anti-inflammatory agent: drug employed in the treatment of rheumatoid arthritis and gout. In L. S. Goodman, A. Gilman, T. W. Rall, A.S. Nies, P. Taylor, The pharmacological basis of therapeutics, McGraw-Hill International edition, New York: 1922: 638-681.

6. Jayanthi B., Madhusudhan S., Mohanta GP. Manna PK. Preformulation Characterization, Designing and Formulation of Aceclofenac Loaded Microparticles" Int J Drug Develop Res2012 ; 4(3).

7. Mishra S, Suryawanshi R, Chawla V, Saraf S**.** Fabrication and characterization of solid lipid microparticles of ketoprofen. Ars Pharm. 2011; 52(1): 12-15.

8. Patel Dilip, Dasgupta Sandipan et al., Nanostructured Lipid Carriers (NLC)-Based Gel for the Topical Delivery of Aceclofenac: Preparation, Characterization, and In Vivo Evaluation. Sci Pharm. 2012; 80: 749–764

9. Nasr M, Mansour S, Mortada ND, Shamy AA. Lipospheres as Carriers for Topical Delivery of Aceclofenac: Preparation, Characterization and In Vivo Evaluation. AAPS PharmSciTech. 2008; 9-1:154-162.

10. Hu QF, 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. colloids and surface B: Biointerfaces. 2005; 45: 167-173

11. Mazumder, B., Dey, S., Bhattacharya, S., Sarkar, S., Mohanta, B., Studies on formulation and characterization of cellulose-based microspheres of chlorpheniramine maleate. Arch Pharm Sci Res. 2009;1:66-74

12. Sachan, N.K., Bhattacharya, A. Modeling and characterization of drug release from glutinous rice starch based hydrogel beads for control drug delivery. Int. J. Health Res 2009;2(1), 93-99.

13. Bhalekar M R, Pokharkar V, Madgulkar A, Patil, N, Patil N K. "Preparation and Evaluation of Miconazole Nitrate-Loaded Solid Lipid Nanoparticles for Topical Delivery." AAPS PharmSciTech, 2009;10(1).

14. Chawla V, Saraf S. Glyceryl Behenate and Its Suitability for Production of Aceclofenac Solid Lipid Nanoparticles. J Am Oil Chem Soc, 2011; 88:119–126.