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Original Article

Formulation and Evaluation of Topical Solid Lipid Nanoparticulate System of Aceclofenac

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ABSTRACT

The objective of the present study is to evaluate the potential use of solid lipid nano particles (SLN) in dermatology. The main aim of present project is to formulation and evaluation of topical solid lipid nanoparticulate system of Aceclofenac. Aceclofenac is a more potent anti-inflammatory agent belongs to non-steroidal anti-inflammatory drugs commonly used in the treatment of rheumatoid arthritis. Solid lipid nanoparticles (SLN) can improve permeability of drug and further decrease irritation potential due to entrapment. The Preparation contains six formulations by using lipid extrusion method. The prepared batches of topical solid lipid nanoparticulate system of Aceclofenac can be evaluated for preliminary evaluations like visual appearance, drug content, particle size analysis, and zeta-potential, and SEM, DSC and *in-vitro* release profile. The formulations F6 gave the highest drug content and better encapsulation. But the particles size is slightly increased because lipid quantity is increased. So F6 formulation is taken for incorporation into gel. The SLN incorporated gel was prepared and evaluation studies were performed by albino rat abdomen skin. The *ex-vivo* skin permeation studies show better release than plain SLN.

Keywords: Active Pharmaceutical Ingredient (API), Particle Size Distribution (PSD), Solid lipid nano particles (SLN), Biopharmaceutical Classification System (BCS).

1. INTRODUCTION

Topical drug delivery system

Most drugs are either absorbed into the body through the digestive system or injected into subcutaneous tissue or muscle. An alternative route, Transdermal (transcutaneous) drug administration, enables drug to pass across the epidermis and into blood vessels of the dermis. The drug is released continuously at a controlled rate over a period of one to several days. This method of administration is especially useful for drugs that are quickly eliminated from the body[1, 2]

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Advantages of Topical Drug Delivery Systems: [3, 4]

- Avoidance of first pass metabolism.
- Convenient and easy to apply.
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input.
- Improved patient compliance and reduced inter-and intra-patient variability.
- Easy termination of the medication when needed.
- A relatively large area for application in comparison with buccal or nasal cavity.
- Avoidance of gastro-intestinal incompatibilities.
- Ability to deliver drug more selectively to specific site
- Improving physiological and pharmacological response.
- Self-administration is possible with these systems.

Disadvantages of topical drug delivery system[5]

- Skin irritation or contact dermatitis due to the drug, excipients and enhancers of the drug used to increase percutaneous absorption.
- Poor permeability of some drugs through skin.
- Possibility of allergic reactions.
- Enzyme in epidermis may denature the drug.
- Drugs of large particle size are not easy to absorb through skin.

Solid lipid nanoparticles[6]

SLN's are colloidal carriers developed in the last decade (1990s) as an alternative system to the existing traditional carriers (emulsions, liposome's, and polymeric nano particles) [6]. Nano particles made from solid lipids are attracting major attention as novel colloidal drug carrier for various applications as they have been proposed as an alternative particulate carrier system. The SLN's are sub micron colloidal carriers (50-1000nm) which are composed of physiological lipid, dispersed in water or in an aqueous surfactant solution.[7]

The advantages of solid lipid nano particles containing aceclofenac for topical use over other aceclofenac conventional dosage forms (ointment, In-situ gels, creams, lotions, etc) are;

- Convenient and easy to apply.
- Ability to easily terminate medication when needed.
- A relatively large area of application.
- Ability to deliver drug mores electively to specific skin site.
- Provide suitability for self-medication.

2. MATERIALS AND METHODS

Aceclofenac was obtained as a gift sample from Dr Reddy's labs, Hyderabad, Carbopol 934, Stearic acid, Beeswax, Span 20, Tween 20 from Loba Chemie Pvt.Ltd, Mumbai. All other chemicals used were of analytical grade.

Preparation of SLN by lipid extrusion technique[7, 8]

- Lipid extrusion is carried out at temperatures above the melting point of the lipid and is like the homogenization of blank formula.
- A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device, Polytron PT1600E homogenizer.

Table 1: Formulation design for lipid extrusion method

Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)
Aceclofenac	25	25	25	25	25	25
Bees wax	500	600	800	1000	1500	2000
Carnauba wax	250	300	600	1000	1500	2000
Cetyl alcohol	250	300	400	600	800	800
Lecithin soya	200	200	200	200	200	200
Tween 20	250	250	250	250	250	250
Distill water (ml)qs	50	50	50	50	50	50

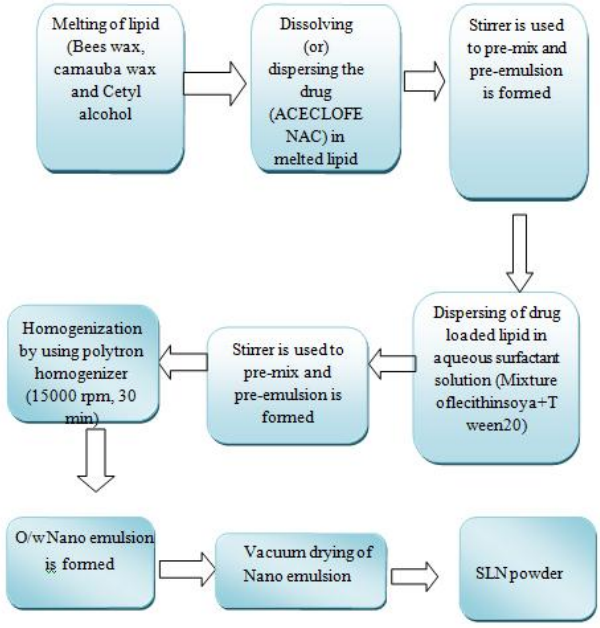

Fig 1: Schematic representation of SLN preparation by lipid extrusion

Evaluation of SLN

Particle size analysis [9]

The particle size should be less than 1000 nm in nano particles. It can be analyzed by using Malvern particle size analyzer. Particles in the size range of colloids display constant random thermal motion which is known as Brownian motion. This motion causes the intensity of light scattered by the particles to vary with time. The larger the particles lower their motion and hence the smaller the variation in intensity of light scattered. Photon correlation spectroscopy uses the rate of change in the intensity to determine the size distribution of particles. The zeta sizer has a correlate with 64 channels. Each of this channel measures changes in light fluctuation over a defined time span.

Zeta potential measurement [10]

Zeta potential of the SLN's was measured by malveren zeta sizer.

Procedure

The zeta size mainly consists of laser which issued to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light split provide an incident and reference beam. The incident laser beam passes through the centre of the sample cell, and the scattered light at an angle of about 130 is detected. When an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed and this information is passed to the digital signal possessor and then to a computer. Zeta sizer software produces a frequency spectrum from which the electro phoretic mobility hence the zeta potentials calculated.

Scanning electron microscopy (SEM) [11]

Surface morphology of the specimen will be determined by using a scanning electron microscope.

Procedure

The samples are dried thoroughly in vacuum desiccator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the sample sputter coating unit (ModelE5100 Polaron U.K) in Argon at ambient of 8-10 with plasma voltage about 20mA. The sputtering was done form early 5minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current about 80 mA. The condenser lens position was maintained in range of 4.4 to 5.1. The objective lens aperture has a diameter of 240 microns and working distance WD=39mm.

Drug content [12]

The drug equivalent to10 mg of formulation was taken and dissolved in small quantity of methanol. Then the solution was filtered through whatman filter paper in 25 ml volumetric flask and volume was made up to the mark by methanol to give concentration of 1000 µg/ml. of Aceclofenac. Then 1 ml was pipette out in100 ml. volumetric flask to give concentration of 10µg/ml and then absorbance was measured at 240 nm.

***In-vitro* release studies for SLN**[13]

In-vitro release studies were carried out by modified Franz diffusion cell, 10 mg equivalent weight of SLN was placed on a cellophane membrane which was placed between donor and receptor compartment of diffusion cell assembly. The donor compartment is wetted by 0.5 ml of phosphate buffer. The donor compartment is filled by 50ml phosphate buffer pH 7.4. The receptor compartment was continuously stirred using the magnetic stirrer. The temperature was maintained 35°C. The study was carried out for 24hrs, and the sample was withdrawn every 30 minutes time interval and same volume was replaced with free phosphate buffer. The content of Aceclofenac from withdrawn sample was measured after suitable dilution at240 nm.

Gel preparation

Procedure

Required quantity of Carbopol 934 was taken and hydrated insufficient quantity of water for24 h. Further, the hydrated gel was stirred for 4 hrs. The gel was neutralized by tri-ethanolamine (added drop by drop) until a clear transparent gel was obtained.[13]

Physical Evaluations of gel:

Rheological studies[14]

Rheological properties (study of deformation and flow of matter) are required in various pharmaceutical areas. It helps to monitor the effect of vehicles consistency on release of drug from the preparations and subsequent percutaneous absorption. Also, it is important from the manufacturing point of view. Viscosity measurements were carried out using a Brookfield viscometer (T–bar spindle). The formulation of SLN based gel was kept in 100 ml beaker and dial readings was noted at 3, 5, 6, 10, 12, 20, 30, 50 and 60 rpm. The speed was then successively lowered, and the corresponding dial readings were noted.

Preparation of SLN incorporated gels

About 50 mg drug equivalent weight of SLN was incorporated in to gel by mechanical mixing.

***EX- VIVO* release studies for SLN incorporated gel** [15]

Ex-vivo release studies were carried out by modified Franz diffusion cell. 10 mg equivalent weight of SLN incorporated gel was placed on an albino rat abdomen skin which was placed between donor and receptor compartment of diffusion cell assembly. The donor compartment is filled by 50ml phosphate buffer pH 7.4. The receptor compartment was continuously stirred using the magnetic stirrer. The temperature was maintained 35°C. The study was carried out for 24 hrs and the sample was withdrawn at 30 minutes time interval and same volume was replaced with free phosphate buffer. The content of Aceclofenac from withdrawn sample was measured after suitable dilution at 240 nm.

3. RESULTS AND DISCUSSION

Melting point determination

The melting point of prepared SLN powder was evaluated and mean melting point in range of 195 to 197oC.

Calibration of Aceclofenac in phosphate buffer pH 7.4

The prepared test concentration like 5, 10, 15, 20, 25 (mcg/ml) are measured absorbance in UV spectrophotometer at wavelength of 239 nm and shown in figure 2.

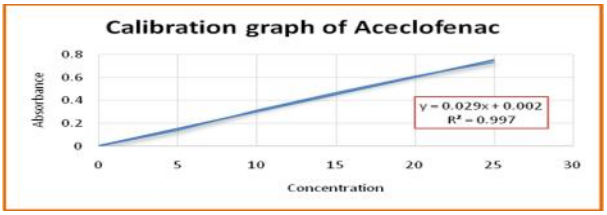

Fig 2: Standard plot of Aceclofenac in Phosphate Buffer pH 7.4

Compatibility studies

The drug and excipients compatibility studies are measured in FTIR. Based on interpretation values of drug with excipients shows ideally compatible and shown in figure 3.

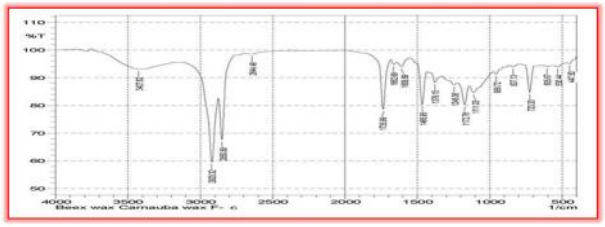

Fig 3: FTIR Spectrum of optimized formulation F6

Band position(cm-1)		Assignment
1666	C=C	stretching of the aliphatic non-conjugated alkene
1612	C=O	stretching of the ketone
1724	C-Cl	stretching of chlorine
1063	COO	stretching of the ether
1010	O-H	bending of the alcohol

Percentage drug content and entrapment efficiency

The prepared formulations evaluated for drug content and entrapment efficiency of all formulations F6 SLN formulation shows high drug content 94.23 and better entrapment efficiency 92.23 ± 0.87.

Evaluations of gel formulation

The prepared gel shows off white appearance and pH in the range of 7 to 8. Viscosity was determined by Brookfield's viscometer at different rpm shown in table 2.

Table2: Viscosity of optimized SLN gel formulation

RPM	Viscosity of gel (Cp)
3	58975.3
5	38763.8
6	37367.5
10	22022.5
12	13137.3
20	7680.25
30	6706
50	4415.75
60	3146.75

Particle size distribution

The particle size analysed by using Malvern particle size analyser the mean particle size of the all formulations was found to be 46.33 nm, 63.65 nm, 93.85 nm, 116.6 nm, 287.9 nm, 302.2 nm respectively, indicating, that the particles fell in an acceptable nano metre range.

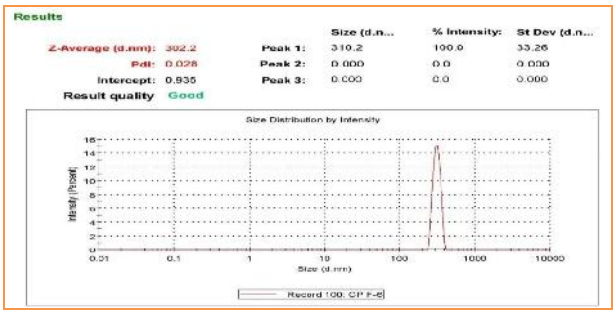

Fig 4: Particle size distribution of optimized formulations F6

Zeta potential

The Zeta potential studies were carried out and the results were found to be -18.5, -15.6, -33,-23.1, -19.7, -29.9 for all formulations with respectively, which indicated that formulations were stable.

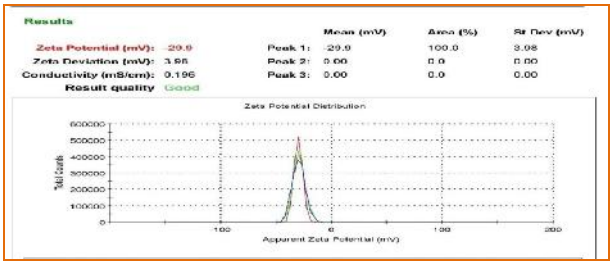

Fig 5: Zeta potential report for the optimized formulation F6

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) studies were carried out for the optimized formulations F6 the photographs revealed that the Aceclofenac-SLNs were smooth and near to spherical. The particles were found to be in clusters.

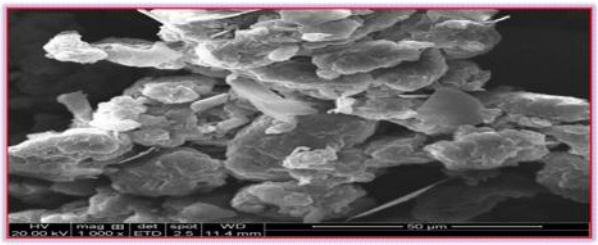

Fig 6: SEM for optimized formulation F6

In vitro release study

Formulations F1, F2, F3, F4, F5, and F6 were subjected to *in-vitro* release studies. The release studies were performed in a phosphate buffer of pH 7.4, suspending the formulation with 10 mg equivalent of the drug results shown in table. The results revealed that, about 89.43% of drug was released from F6, formulation respectively in a span of 24 hrs of study. So F6 formulation is taken for incorporation into gel

Table3: *In vitro* release study of formulations in phosphate buffer pH 7.4

Time (hrs.)	%Cumulative drug release					
	F1	F2	F3	F4	F5	F6
0.5	5.04	6.12	8.71	10.14	12.6	13.02
1	7.91	9.24	11.03	12.66	15.79	17.06
2	9.02	11.16	13.18	14.09	17.22	21.11
3	11.09	12.98	15.66	17.43	21.14	25.7

4	14.12	17.04	19.21	22.32	26.51	30.49
5	18.79	21.08	24.54	27.43	32.68	35.24
6	23.42	27.67	29.23	32.54	36.4	39.55
7	29.31	32.22	35.16	38.31	40.15	43.16
8	33.62	36.41	40.39	42.93	44.95	48.49
9	38.25	40.43	44.85	46.41	48.83	52.86
10	43.19	44.03	48.42	50.94	52.35	57.36
12	49.69	50.21	54.43	56.49	59.23	62.12
24	62.11	64.69	70.68	75.64	82.07	89.43

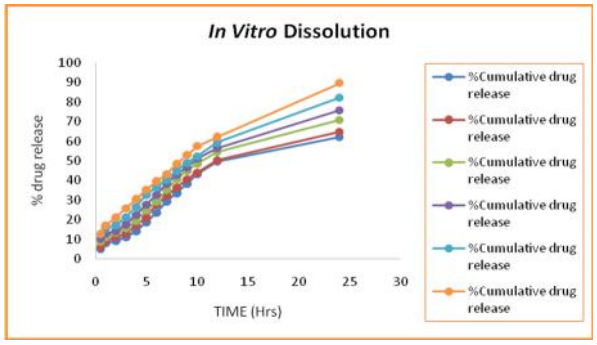

Fig 7: % cumulative drug release of all SLN formulations

***Ex- vivo* Release study**

The SLN incorporated gel was prepared and evaluation studies were performed by albino Rat abdomen skin. The *ex-vivo* skin permeation studies show better release than plain SLN was shown in figure.

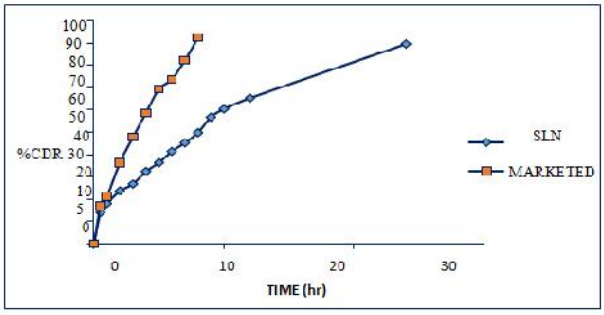

Fig 8: Comparative *EX- VIVO* drug release profiles of marketed and SLN gel

4. CONCLUSION

The drug loaded SLNs were prepared by lipid extrusion method. The SLNs were characterized on basis of preliminary evaluations like visual appearance, drug content, particle size analysis, zeta-potential, SEM, DSC and *in-vitro* release profile. The mean particle size of the all formulations was found to be 46.33 nm, 63.65 nm, 93.85 nm, 116.6 nm, 287.9 nm, 302.2 nm respectively, indicating, that the particles fell in an acceptable nano meter range. The Zeta potential studies were carried out and the results were found to be -18.5, -15.6, -33, -23.1, -19.7, -29.9 for all formulations with respectively, which indicated that formulations were stable, and stability was also confirmed by stability studies.

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