International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 295-304 www.ijprajournal.com ISSN: 2249-7781

Development And Characterization of NSAIDs Loaded SLNsfor Topical Drug Delivery System.

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Submitted: 09-03-2023 Accepted: 18-03-2023

ABSTRACT
Solid lipid nanoparticles (SLNs) have shown potential as a novel lipid-based drug delivery system for the topical applications of innumerable therapeutic compounds. However, the mechanisms governing the absorption and cellular uptake of SLNs through topical route, along with the mechanism of drug release from SLNs. Aceclofenac is a non-steroidal anti-inflammatory drug, has been used in the treatment of rheumatoid arthritis and osteoarthritis. The overall goal of the present study was to formulate and evaluate aceclofenacemulgel. Which will increase skin penetration of drug in comparison with present marketed preparations of the drug. This study was conducted to develop a gel formulation of aceclofenac using various types of gelling agents Carbopol 934, Stearic acid, Beeswax, Tween 20, Bees wax, Carnauba wax, Cetyl alcohol, Lecithin soya. The drug release from all gelling agents through a dialysis membrane was evaluated. All gels showed acceptable physical properties concerning color, homogeneity, consistency, and pH value. Among all the gel formulations, carbopol formulation showed superior drug release.

Keywords - Solid lipid nanoparticles, topical delivery, drug release mechanism, formulation, Aceclofenac, Carbopol 934, Stearic acid, Beeswax, Tween 20, Bees wax, Carnauba wax, Cetyl alcohol, Lecithin soya

I. INTRODUCTION

Solid lipid nanoparticles (SLN) have been established as an alternative particulate carrier system by various research groups. Recently, increasing attention has been focused on these SLN as colloidal drug carriers combining advantages of polymeric nanoparticles, fat emulsions, and liposomes, but simultaneously avoiding some of their disadvantages During the past several years, SLN began to act as a topical carrier not only for pharmaceutical molecules, but also for cosmetic products(Chawla & Saraf, 2012; Khurana et al., 2013; Torchilin, 2006). Compared with conventional carriers such as cream, tincture, and emulsion, SLN combines their advantages such as controlled release, in vivo good toleration, and protection of active compounds Especially, SLN can favour drug penetration into the skins, maintain a sustained release to avoid systemic absorption, act as a UV sunscreen system, and reduce irritation (Shetty et al., 2022).

Lipid nanoparticles as drugs delivery system were considered from the beginning of the 19th century by professor R.H. muller from Germany and professor M.gascom from Italy these nanoparticles are manufactured from solid or mixture of solid and lipids and stabilized by emulsifiers triglycerides, fatty acids, steroids, and waxes(Chawla & Saraf, 2011). Lipid nanoparticles have many advantages in comparison to other particulate system such as the ease of large scale production, biocompatible and biodegradable nature of the materials, low toxicity potential, possibility of controlled and modified drug release, drug solubility enhancement and the possibility of both lipid used in these nanoparticles are biocompatible and completely tolerated by the body like hydrophilic and lipophilic drug incorporation (P. Sharma et al., 2005).

Lipid nanoparticles are different from micro emulsion, which are the clear thermodynamically stable dispersion of oil and water that are the stabilized by surfactants and cosurfactants, the most importantparameters in lipid nanoparticlescharacterization are particles size and size distribution, zeta potential, polymorphism, degree of crystallinity, drug loading entrapment efficiency, and drug release (Ana et al., 2022). particles with size ranging from 200–400 nm show an occlusive effect due to a film formation on the skin, which can enhance drug penetration into specific skin layers (Imran et al., 2022)(Krysiak & Stachewicz, 2022).

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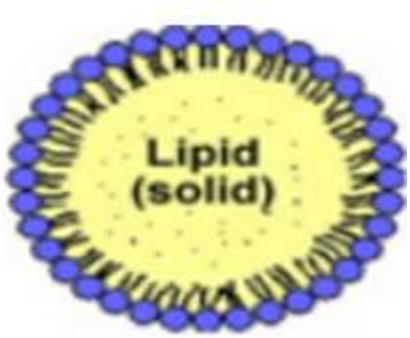
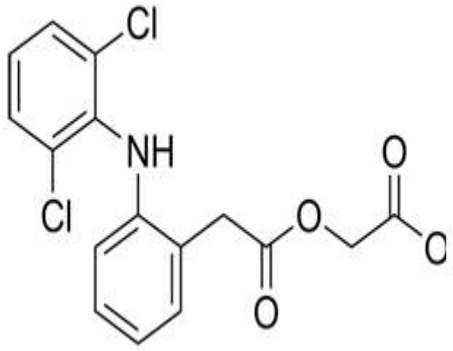


Fig.1 structure of solid lipid nanoparticle (SLN)

DRUG PROFILE

Aceclofenac

aceclofenac is a non- steroidal agent with marked anti-inflammatory and analgesic properties. The mode of action of Aceclofenac is largely based on the inhibition of prostaglandin synthesis. Aceclofenac is a potent inhibitor of the enzyme cyclo-oxygenase, which is
involved in the production of prostaglandins aceclofenac is nonsteroidal anti-inflamatory drug (NSAIDs) anlog of diclofenac.it is used for the relief of pain and inflammation in rheumatoidarthritis, osteoarthritis and ankylosing spondylitis(Fischer & Ganellin, 2010).



Chemical structure - ($C_{16}H_{13}Cl_2NO_4$),
Molar mass - 354.18 $g \cdot mol^{-1}$
Chemically name - [(2-{2, 6-dichlorophenyl),amino}phenyl, acetooxyacetic acid],
Properties - is a crystalline powder with a molecular weight of 354.19. It is practically insoluble in water with good permeability.
Solubility - poorly soluble and highly permeable drug.

Machnism of action -Aceclofenac works by inhibiting the action of cyclooxygenase (COX) that is involved in the production of prostaglandins (PG) which is accountable for pain, swelling, inflammation and fever. The incidence of gastric ulcerogenicity of aceclofenac has been reported to be significantly lower than that of the other frequently prescribed NSAIDs, for instance, 2-folds lesser than naproxen, 4-folds lesser than diclofenac, and 7-folds lesser than indomethacin(Karmoker et al., 2016).
Route of administration – mouth, topical
Side effects - Aceclofenac should not be given to people with porphyria or breast-feeding mothers, and is not recommended for children. It should be avoided near term in a pregnant woman because of the risk of having a premature closure of ductus arteriosus leading to fetal hydrops in the neonate.
Adverse effects - Nausea, vomiting, diarrhoea, flatulence, constipation, dyspepsia, abdominal pain, melaena, haematemesis, ulcerative stomatitis, exacerbation of colitis and Crohn's disease
Precautions: Aceclofenac is not recommended if you have any bleeding disorder. It may cause severe swelling and bleed in the stomach, colon, and anus. Pregnancy- This medicine is not recommended in pregnant women. Breast-nutrition- This medicine is not recommended for breastfeeding women.

Formulation development

1. **Physical Examination**: The prepared formulations were inspected visually for their color, homogeneity, consistency, grittleness and phase separation (Khalil et al., 2013)(Suthar et al., 2013).
2. **Measurement of pH**: The pH of the formulations was determined by using digital pH meter. 1gm of prepared sample was dissolved in 100ml of distilled water and it was kept a side for 2 hours. The measurement of pH of each formulation was done in triplicate and average values were calculated (Khalil et al., 2013)(Suthar et al., 2013).
3. **Particle shape and surface morphology** - Transmission Electron Microscopy (TEM) (Philips CM12 Electron Microscope, Eindhoven, The Netherlands) was used for the determination of SLN morphology. The aqueous dispersion (one drop) was placed over a 400-mesh carbon-coated copper grid followed by negative staining with a phosphotungstic acid solution. For the scanning electron microscopy (SEM) examination, the SLN were dried for 24 h before the analysis. The prepared samples were characterized for shape and surface structure by SEM (Leo 435 VP, Cambridge, UK) (Hu et

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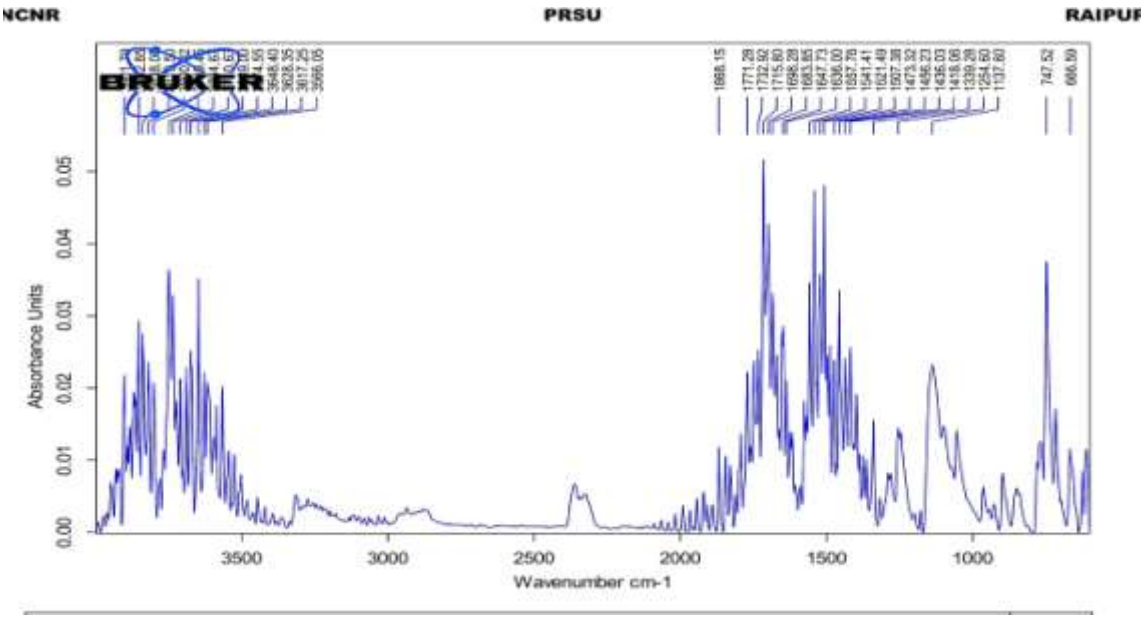
al., 2002).

4. **Zeta potential measurement** - Zeta potential of the SLN's was measured by malveren zeta sizer.
5. **Scanning electron microscopy (SEM)** - Surface morphology of the specimen will be determined by using a scanning electron microscope(V. K. Sharma et al., 2011).
6. **Drug content** - 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. (Souto et al., 2004)
7. **X-ray diffraction (XRD)** - The internal crystalline structure was investigated by small-angle XRD. Copper X-rays (1.542 A°) were produced by X-ray generation (FLCU 4KE, Bruker, Germany) and operated at 40 KV and 45 mA. Experiments were performed in triplicate. (Ezhilarasi et al., 2016)
8. **Differential Scanning Calorimetry (DSC)** - DSC was used to investigate the melting and recrystallization behaviour of crystalline materials like SLN (Hou et al. 2003). The transition temperature of lipid nanoparticles was measured in triplicate using modulated DSC (TA Instruments 2910) with a programmed heating rate of 10°C/min. (Chawla & Saraf, 2011)
9. **In-vitro release studies for SLN** - 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 at 240 nm (Parhi & Suresh, 2010).
10. **FTIR Analysis** - The IR spectra of pure drug aceclofenac, Drug loaded SLN, are shown in graph. This result indicates that there is no chemical interaction between the drugs. However, in the IR spectrum of aceclofenac loaded SLN peaks corresponding to aceclofenac disappear.



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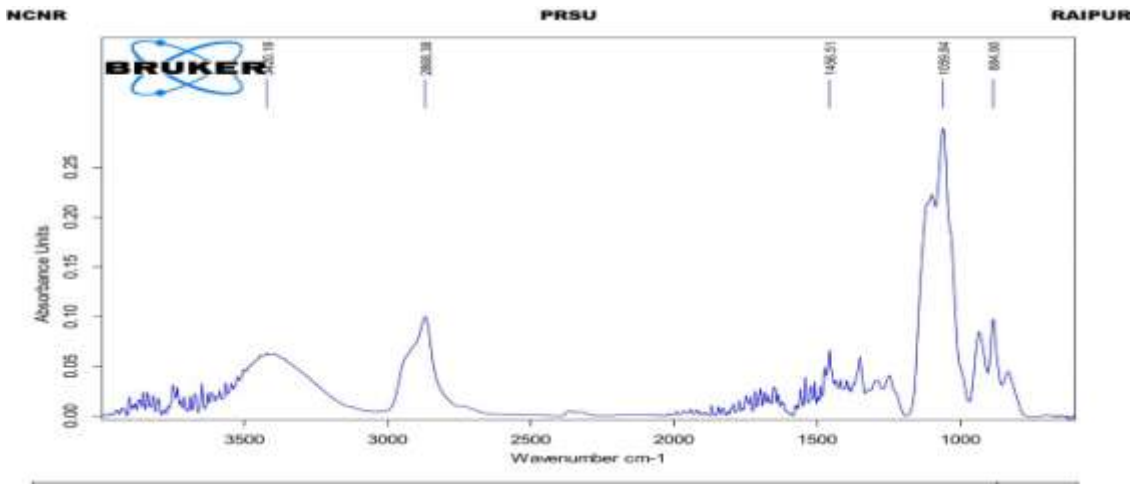
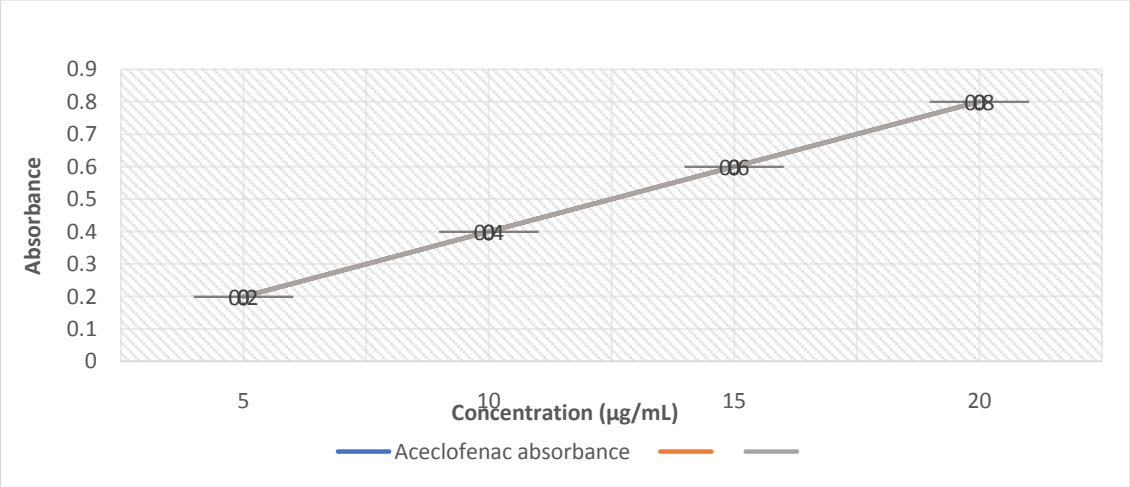


11. Standard Graph of AceclofenacIn Phosphate Buffer pH 7.4 / Determination of wavelength of maximum absorbance (λ max)

S.N.	Concentration (µg/mL)	Aceclofenac absorbance
1	5	0.2
2	10	0.4
3	15	0.6
4	20	0.8
5	25	1.0
6	30	1.2

Table 1: Calibration curve of Aceclofenac



METHOD OF PREPARATION

SLN were prepared by ultrasonic emulsification technique, with slight modifications. Briefly, ACF were dissolved in a minimum quantity of Cetyl alcohol at 50°C. After evaporation of Cetyl alcohol, the melt was dispersed in preheated water (50°C) containing a

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mixture of emulsifier (soya lecithin and Tween 20, at a ratio of 1:1) under mechanical stirring (Remi, India) at 4000 rpm for 10 min. The resulting emulsion was subjected to ultrasonication using a probe sonicator (Soniweld, India) for 6 min at 40 watts. Further, the dispersion was immediately dispersed in cold distilled water (4°C), followed by continued mechanical stirring for 10 min

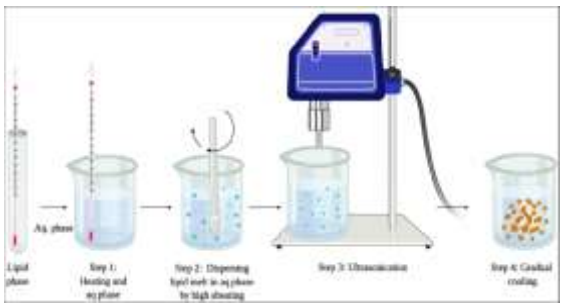

Fig 2 ultasonication technique

The suspension was then filtered through a 0.45 mm filter to remove impurities from the material. (Castelli et al., 2005; Luo et al., 2006)

Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)
Aceclofenac	500	500	500	500	500	500
Bees wax	500	600	800	1000	1500	2000
Carnauba wax	750	900	1200	1500	1700	1800
Cetyl alcohol	350	500	700	800	900	900
Lecithin soya	210	210	210	210	210	210
Tween 20	240	240	240	240	240	240
Distill water (ml)qs	60	60	60	60	60	60

Table 2. Formulation design for ultrasonic emulsification technique

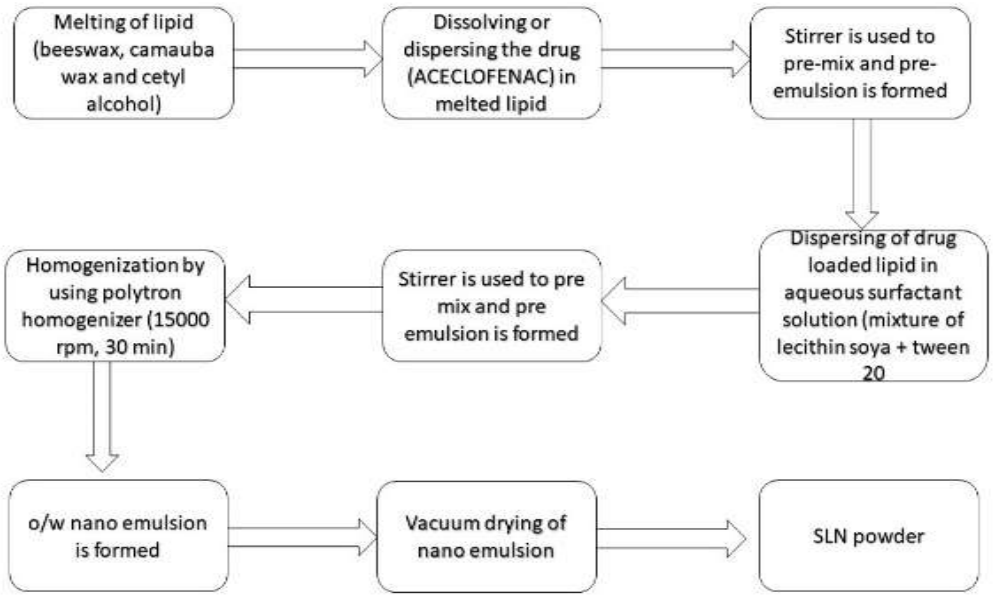

Fig 3: Schematic representation of SLN preparation by lipid extrusion (Castelli et al., 2005)

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II. RESULTS AND DISCUSSION

1. **Physical Examination** - The prepared aceclofenac formulations when subjected for colour appearance were white viscous preparation with a smooth homogenous texture and glossy appearance, non-grittiness with good consistency.

S.N.	CHARACTERISTICS	RESULTS
1	Colour	white viscous
2	Texture	smooth homogenous texture
3	Appearance	Glossy
4	Grittiness	non-grittiness
5	Consistency	Good consistency

Table 3 physical examination of drug

2. **Measurement Of pH** - The pH value of all the prepared formulations was ranging from 5 to 6.8, which is considered acceptable to avoid the risk of irritation upon application to the skin.
3. **Particle size and zeta potential** - The average particle size and size distribution of the SLN were determined by photon correlation spectroscopy using a Zetasizer DTS version 4.10 (Malvern Instruments, UK). Measurements were performed in distilled water adjusted with a solution of sodium chloride at a concentration of 0.1 mmol/L, to a conductivity of 50 mS/cm at 25°C. The surface charge of SLN was determined by measuring zeta potential of the lipid nanoparticles.

Optimized parameters of ACF-loaded SLN

Parameters	SLN
Particle size	189±9.2 nm
Zeta Potential	-33.5±0.12 mV

Table. 3 Particle size and zeta potential

4. **Surface morphology** - TEM and SEM images of the optimized SLN have been presented in fig 7, respectively. Both techniques confirmed that the SLN were spherical in shape and well dispersed. The size of the SLN, determined by the Zetasizer, was found to be smaller than that shown by TEM results. TEM images of SLN show spherical and homogeneous particles ranging approximately from 160 to 270 nm in size, and the SEM photograph suggest that the SLN possessed a slightly rough surface.

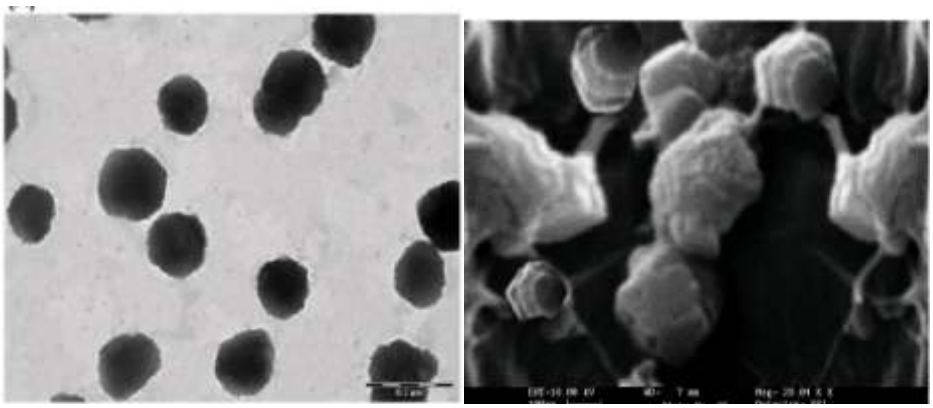

Fig. 4 SEM photograph of ACF-loaded SLN (bar 100 nm). (B) TEM photograph of ACF-loaded SLN (X1,00,000).

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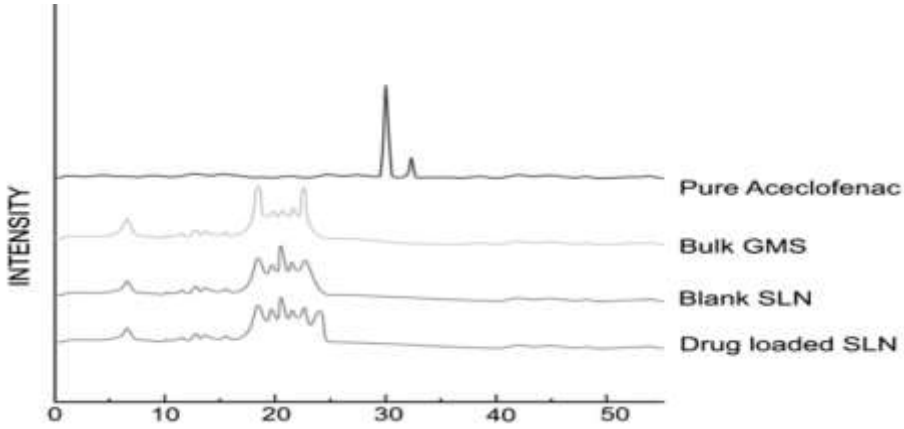
5. **Percentage drug content and entrapment efficiency** - The prepared formulations evaluated for drug content and entrapment efficiency of all SLN formulation shows high drug content 93.19 and better entrapment efficiency 92.19 ± 0.65.
6. **X-ray diffraction (XRD)** - The XRD patterns of ACF, bulk lipid, and drug-loaded SLN are shown in graph, revealing significant difference between diffraction of ACF and drug-loaded SLN. It could be inferred via XRD that the ACF existed in the amorphous form, because of the absence of a sharp peak of ACF in the diffraction pattern of drug-loaded SLN. The XRD of SLN was broader and much weaker than that of bulk lipid, confirming that the GMS in SLN was partially recrystallized.



XRD of ACF-loaded SLN

7. **Differential scanning calorimetry (DSC)** - The XRD patterns of ACF, bulk lipid, and drug-loaded SLN are shown in graph, revealing significant difference between diffraction of ACF and drug-loaded SLN. It could be inferred via XRD that the ACF existed in the amorphous form, because of the absence of a sharp peak of ACF in the diffraction pattern of drug-loaded SLN. The XRD of SLN was broader and much weaker than that of bulk lipid, confirming that the GMS in SLN was partially recrystallized.

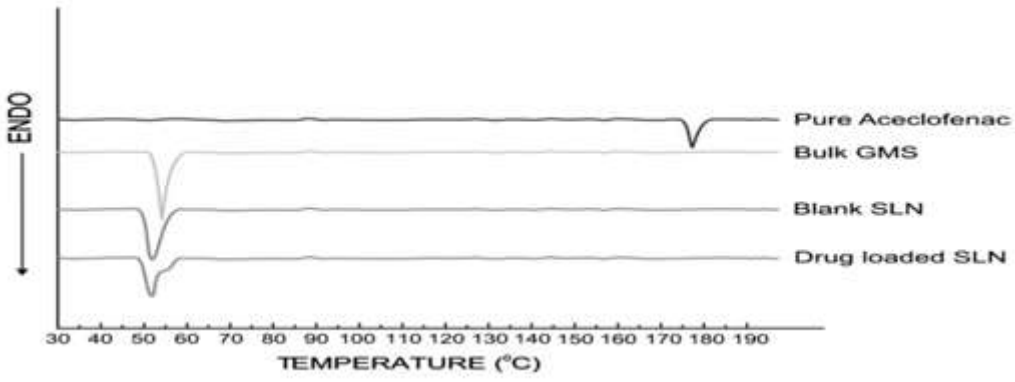


Fig. 5DSC curve of ACF-loaded SLN

8. **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 100 mg equivalent of the drug results shown in table. The results revealed that, about 90.55% of drug was released from F6, formulation respectively in a tween 20 of 24 hrs of study. So F6 formulation is taken for incorporation into gel.

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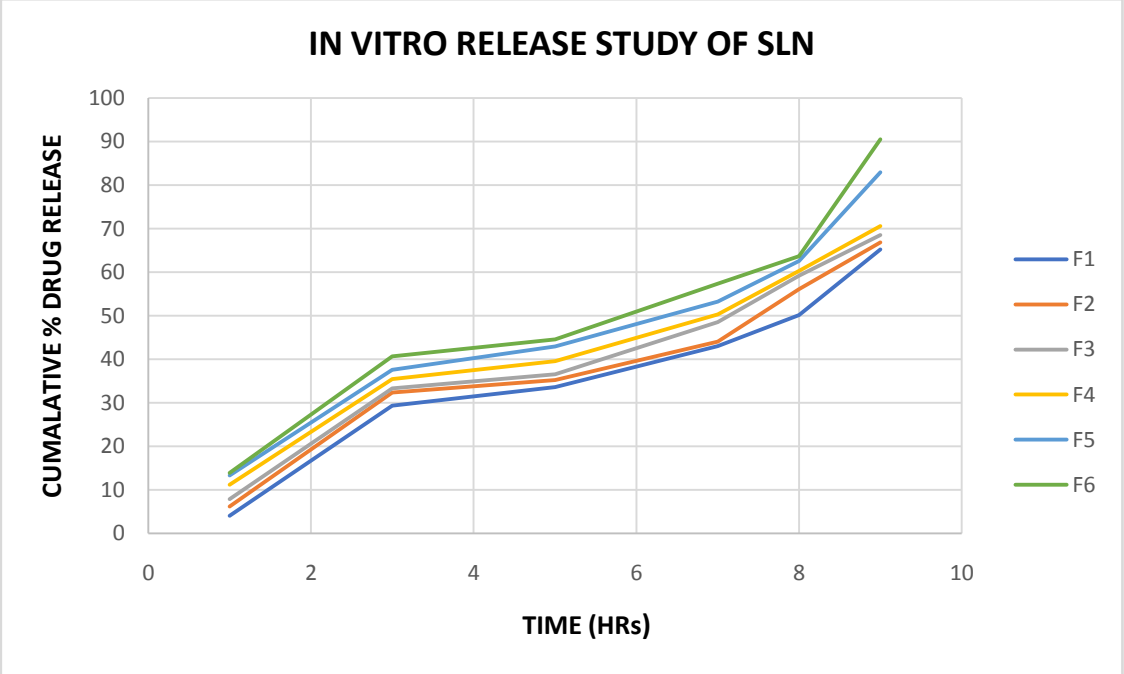

Table 4: In vitro release study of SLN

TIME (HRS)	CUMULATIVE % DRUG RELEASE					
	F_1	F_2	F_3	F_4	F_5	F_6
1	4.02	6.12	7.81	11.14	13.26	13.89
3	29.31	32.33	33.25	35.41	37.56	40.64
5	33.62	35.23	36.56	39.54	42.89	44.56
7	43.02	44.06	48.56	50.26	53.23	57.36
8	50.14	56.12	59.23	60.25	62.56	63.71
9	65.23	66.85	68.55	70.56	82.96	90.55

III. SUMMARY AND CONCLUSION

SLNs have demonstrated significant improvement in drug penetration through the skin by forming an occlusive layer. It has been recommended that the use of appropriate formulation base/semi-solid dosage form, such as gel will further enhance the effective topical application of SLNs. The optimized protocol of the ultrasonic emulsification method was successfully applied for preparation of ACF-loaded SLN. Furthermore, the ACF-loaded SLN gel provided higher localization of drug in the epidermal layer as compared with the simple gel. The size of the SLN and close contact with the SC proved enhanced skin deposition of drug. The results suggest that ACF-loaded SLN could have promising potential as an alternative against conventional topical delivery of ACF.

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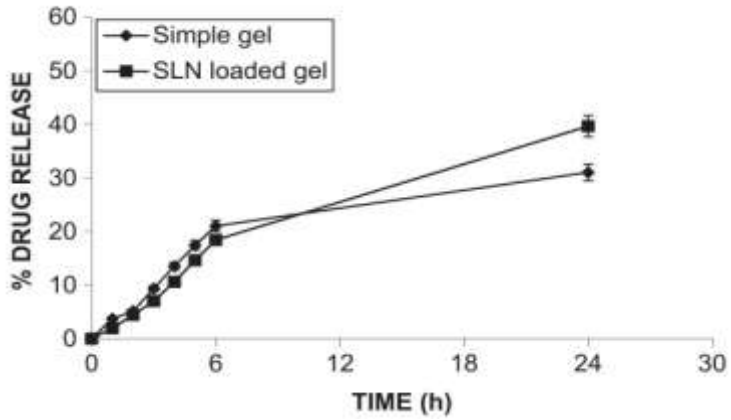

Fig 6 In vitro skin permeation profile of ACF from SLN. Values represent mean SD (n=6)

formulations of gel of ACF were successfully prepared using Aceclofenac, Carbopol 934, Stearic acid, Beeswax, Tween 20, Bees wax, Carnauba wax, Cetyl alcohol, Lecithin soya by ultrasonic emulsification technique. We have manufactured gel with quality and product consistent by a production ultrasonic emulsification technique which avoids costly technology, equipment and lengthy manufacturing process.The gel was evaluated for parameters like Physical Examination, Measurement of pH, Particle shape and surface morphology, Zeta potential measurement, Scanning electron microscopy (SEM), Drug content, X-ray diffraction (XRD), Differential Scanning Calorimetry (DSC), In-vitro release studies for SLN.

The results shows that colour is white viscous, texture is smooth homogenous texture, appearance is glossy, non-grittiness and consistency is good. The pH value of all the prepared formulations was ranging from 5 to 6.8, which is considered acceptable to avoid the risk of irritation upon application to the skin. The average particle size and size distribution of the SLN were determined 189±9.2 nm and zeta potential is -33.5±0.12 mV. TEM images of SLN show spherical and homogeneous particles ranging approximately from 160 to 270 nm in size, and the SEM photograph suggest that the SLN possessed a slightly rough surface. The results revealed that, about 90.55% of drug was released from F6, formulation respectively in a tween 20 of 24 hrs of study.

Gels of aceclofenac SLN have been successfully prepared and characterized. The viscosity of prepared gels was independent of time and shear. Gels were stable over high as well as low temperatures which can be helpful in their long-term stability and transportation.

The rational of the present study was to increase the penetration of drug into the skin. In this study, an attempt was made to prepare and characterize gel of Aceclofenac. This formulation was also found to be stable when compared with other formulations. The results indicated that SLNs which have least particle size can release properly from nanoparticles. FT-IR spectra studies indicated that there was no interaction of lipid and poloxamer with drug.

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