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Research Article

DEVELOPMENT AND CHARACTERIZATION OF SOLID LIPID NANOPARTICLES OF ACECLOFENAC BY MICROEMULSION TECHNIQUE USING LECITHIN, PLEURONIC F-68, AND SODIUM GLYCOCHOLATE

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ABSTRACT

The present study aimed to enhance the solubility of poorly soluble drug aceclofenac using nanonization technique. Solid-lipid nanoparticles of aceclofenac were prepared by the microemulsion technique. The organic phase contained the drug, lecithin, lipid, and the aqueous phase contained pleuronic F-68 and sodium glycocholate in varying concentrations. The formulations were optimized based on stirring rates, stirring time and ultrasound amplitude, and the number of cycles. Five different formulations were prepared. The formulations were characterized for particle size analysis, zeta potential, *in-vitro* release studies, and stability analysis. The formulations F1 and F2 were found to be acceptable in the size range and zeta potentials -20 mV indicated their physical stability. The formulation F2 exhibited enhanced dissolution rate and the release profile showed an initial burst release of drug followed by sustained release.

Keywords: Solid lipid nanoparticles, Aceclofenac, Microemulsion, Zeta potential, Lecithin, Sodium glycocholate, Pleuronic F-68.

1. INTRODUCTION

The drug delivery through the oral route for is one of the oldest, easiest, and widespread modes of drug delivery with numerous advantages like ease of administration and economic production possibilities, ease of handling and storage, self-administration, compliable, and can be easily made available to the mass of peoples. Nanoparticles are one of the several types of systems known collectively as colloidal drug-delivery systems which also include microcapsules, nanocapsules, macromolecular complexes, polymeric beads, microspheres, and liposomes. A nanoparticle is a particle-containing dispersed drug, with a diameter of 200 to 500 nm. The size of nanoparticles makes them unique at several steps of drug delivery and their performance within the body.

Today the concept of nanoparticles is widely used to deliver many drugs safely and effectively. Nanoparticles reduce toxicity by controlled disposition and improve the therapeutic efficacy of drugs. Drug-loaded nanoparticles can alter the drug's distribution and can thus reduce the drug's random and unwanted disposition. Formulations based on lipid carriers for oral delivery have not only proven their utility in mitigating poor and variable gastrointestinal absorption of poorly soluble lipophilic drugs but have also shown in many cases the ability to reduce or eliminate the influence of food on drug absorption. Lipid-based formulations currently occupy a small but successful role for dealing with poorly soluble drug delivery by mouth. By definition, formulations of solid-lipid nanoparticles are composed of solid lipids (*i.e.* solid lipids at room temperature), surfactants, and water. The inherent biocompatibility of lipids, self-assembly capabilities, the versatility of particle size, greater bioavailability, and low cost make lipid-based drug delivery systems attractive, and the subject of intensive research [1].

Solid lipid nanoparticles have a core matrix of solid lipids that can solubilize lipophilic molecules. Surfactants (emulsifiers) stabilize the lipid core. All the formulation excipients must have generally recognized as safe (GRAS) status for pharmaceutical applications [2]. Solid

lipid nanoparticles (SLN) offers an attractive means of delivering drugs, particularly for poorly water-soluble drugs. SLN is a product embedded at the outer shell in a biocompatible lipid core and surfactant, providing a strong alternative to polymeric systems in terms of lower toxicity. Besides, the manufacturing process can be modulated for the desired release of drugs, and the safety against drug degradation and the avoidance against organic solvents are other added benefits. This large-scale versatility can be of vital importance for the commercialization of new goods. The aforementioned features make SLN a fascinating carrier device for optimizing drug delivery.

As a novel colloidal drug carrier for intravenous applications, nanoparticles made from solid lipids are gaining great interest since they were introduced as an alternative particulate carrier system [3-5]. Compared with conventional carriers, the SLN blends the advantages of polymeric nanoparticles and o/w fat emulsions for drug delivery and thus provides an alternative method to current traditional carriers, in particular for the delivery of poorly water-soluble compounds for both pharmaceutical and cosmetic ingredients and good tolerability compared to polyester nanoparticles [6-8].

2. MATERIAL AND METHODS

2.1. Material

Aceclofenac had been collected from Cipla Ltd., Sikkim as a gift sample. Phospholipid 90 G was kindly gifted by Nattermann Phospholipid (Koln, Germany). Compritol-888 ATO (glycerol behenate) was obtained as a gift sample from Gattefosse (Weil A. R., Germany). Sodium Glycocholate was generously gifted by New Zealand Pharmaceuticals Ltd (New Zealand). Pleuronic F-68 was procured from Sigma Aldrich, USA. Dialysis membrane 12000-14000 dalton was procured from Himedia, India. Solvents of the reagent grade and deionized double-distilled water were used wherever required in experiments.

2.2. Methods

2.2.1. Drug excipients compatibility studies

The compatibility study of the drug with various excipients was performed by Fourier transform infrared spectroscopy (FTIR, Perkin Elmer, USA) technique. The Drug and various excipients (Compritol-888 ATO, Sodium Glycocholate, Pluronic F-68) were mixed thoroughly in a ratio of 1:1. Drug samples, as well as the mixtures of the drug and excipients, were stored at room temperature in closed vials. After 24 hours, the samples were scanned with an FTIR spectrophotometer for any type of incompatibility.

2.2.2. Preparation of solid lipid nanoparticles [9,10]

Beginning with a very fine powder is desirable to create solid lipid nanoparticles of a poorly soluble compound. For each formulation to be prepared every time, two different phases, organic phase (in chloroform & acetone, 1:1) and aqueous phase were prepared separately. The organic phase consisted of the drug, lecithin, and lipid while the aqueous phase consisted of Pleuronic F-68 and sodium glycocholate in different concentrations. All the components were mixed well to achieve uniformity.

The organic mixture was subjected to probe sonication at different cycles and amplitudes for a certain period (Sartorius, ultrasonic-homogenizer-Labsonic-M). The microemulsion resulting from sonication was warmed to get a clear organic phase. The resultant organic phase was added to the aqueous phase which consists of dissolved surfactant and co-emulsifier at 70°C with homogenization at 4000-6000 rpm (Remi, RQT-124A) for one hour. After homogenization, the resulting emulsion was cooled at 0°C and again homogenization was done at the same speed for further one hour and stored at 2-8°C.

2.2.3. Characterization of solid lipid nanoparticles

2.2.3.1. Particle size analysis

The polydispersity index (PI) and mean particle size had been determined by the Photon Correlation Spectroscopy (PCS) with a Malvern Mastersizer (Malvern Instruments UK). The samples were diluted with double distilled water to suitable scattering intensity and redispersed by handshaking before the measurement.

2.2.3.2. Zeta Potential Analysis [11-13]

A Malvern Mastersizer Nano-ZS (Malvern Instruments UK) had calculated the zeta potential. It is a measurement of electrical charge on the particle surface which indicates the physical stability of colloidal systems. The zeta potential values above 30mV indicate long term electrostatic stability of aqueous dispersions. The Helmholtz-Smoluchowsky equation is used to determine the zeta potential values. The study was conducted using a transparent disposable zeta cell, at a field strength of 20 V/cm in distilled water. Measures were conducted in triplicate.

2.2.3.3. Microscopy

Microscopy was performed using a photomicroscope (radical instrument) at a magnification of 4x1000. The measurements were done in triplicate.

2.2.3.4. Scanning electron microscopy (SEM)

The shape and size of the nanoparticles were determined by the SEM. Before the analysis, all the samples were diluted with ultra-purified water to obtain a suitable concentration. The samples were then placed onto a sample holder (stub) and dried under a vacuum. Subsequently, they were subjected to gold-palladium coating (JFC 1200 Fine Coater, JEOL, Japan) and analyzed by electron microscope scanning (JSM 6301F, JEOL, Japan).

2.2.3.5. In-vitro dissolution study

The dissolution properties of the solid-lipid nanoparticles were estimated *in-vitro*. 10 ml test tube open at both ends was taken and 5 ml of solid lipid nanoparticle dispersion was transferred into the test tube. The one open end of the test tube was tied with a 200 nm dialysis membrane (MW-12400 Daltons, Sigma Aldrich). The membrane end of the tube was dipped in a beaker containing 100 ml of dissolution media (pH 6.8 phosphate buffer). The temperature of the medium and stirring rate was maintained at 37±2 °C and 100 rpm respectively. One ml of dissolution medium was taken out at a fixed interval and instantly replaced with a fresh medium to sustain the sink condition. The amount of drug released was assessed by U.V. spectroscopy (U.V. Spectrophotometer Shimadzu-1700) at 275 nm. All the measurements were done in triplicate and the same procedure was repeated for the rest of the formulations.

2.2.3.6. Physical stability study [15-17]

The physical stability study of the Solid lipid nanoparticles was conducted at 25 °C. The changes in settling behavior, appearance, and Ostwald ripening, were recorded at predetermined time intervals of 24hrs, 1 week, and 2 weeks.

3. RESULTS AND DISCUSSION

3.1. Characterization of solid lipid nanoparticles

Five different formulations were prepared based on optimized parameters. Compositions of different formulations are discussed in table 1. These formulations were characterized for particle size, zeta potential, microscopy, SEM analysis, and *in-vitro* dissolution study.

Table 1: Composition of different solid lipid nanoparticle formulations

S.No.	Drug (mg)	Compritol 888 ATO (%W/V)	Soya lecithin (% w/v)	Pleuronic F-68 (% w/v)	Sodium. Glycocholate (mg)
F1	100	0.5	1.5	0.5	--
F2	100	1.0	1.0	1.0	10.0
F3	100	1.5	0.8	1.5	10.0
F4	100	2.0	0.4	2.0	15.0
F5	100	2.5	0.2	2.5	20.0

3.1.1. Particle size analysis

The average particle size and polydispersity index of the formulations were measured. The average particle sizes of formulations F1 to F5 were found to be 177.8, 139.4, 240.6, 492.9, and 713.5 nm respectively. The polydispersity index of these formulations was found to be 0.465, 0.528, 1.00, 0.448, and 0.509 respectively. Size distribution in F2 formulation is given in Fig.-1. Table 2 represents the mean PCS diameters and PI values obtained with different formulations and the mean particle size of F2 formulation was observed to be 139 nm.

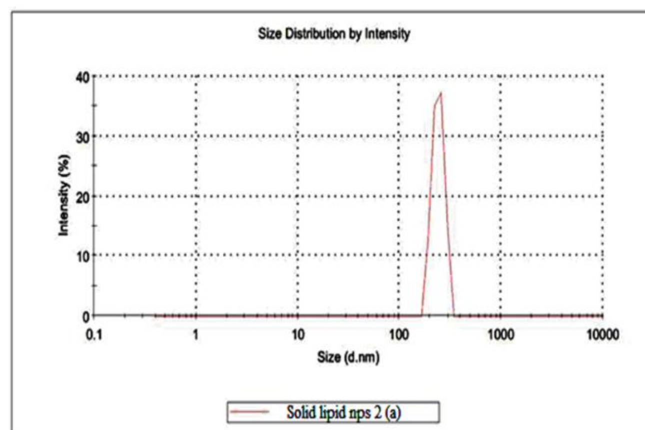

Fig. 1: Size distribution of SLN formulation-2A

3.1.2. Zeta Potential

The zeta potential value of -24.2 mV indicated that a higher amplitude pressure and cycles could provide more energy to comminute particles leading to the attainment of the smaller size.

To investigate the surface properties of the solid lipid nanoparticles, the zeta potential of all the five formulations was determined. The nanoparticles of formulation F1, F2, F3, F4 and F5 had a zeta potential of -23 mV, -24.2 mV, -31.7 mV, -24.32 mV and -22.18 mV respectively. It was found that the zeta potential of the formulations F1 and F2 is around ±20 mV. This showed both formulations were physically stable. The value of the zeta potential of each formulation is presented in Table 2. Fig. 2 shows the peak of zeta potential of formulation F2. The mean size of F4 and F5 significantly increased reaching values higher than the acceptable range. Thus the F4 and F5 formulations were rejected due to the higher distribution of particle size than the appropriate range. The formulation F3 was also rejected due to the high value of polydisperse particles (non-uniform particle size distribution). The formulations F1 and F2 were found in the acceptable nano range, thus these formulations were selected for the dissolution studies.

Table 2: Particle size determination and zeta potential measurement

S.No.	Code	Particle size (d.nm)	Polydisper-sity Index (PdI)	Zeta Potential (mV)
1.	F1	177.8	0.465	-23
2.	F2	139.4	0.528	-24.2
3.	F3	240.6	1	-31.7
4.	F4	492.9	0.448	-24.32
5.	F5	713.5	0.509	-22.18

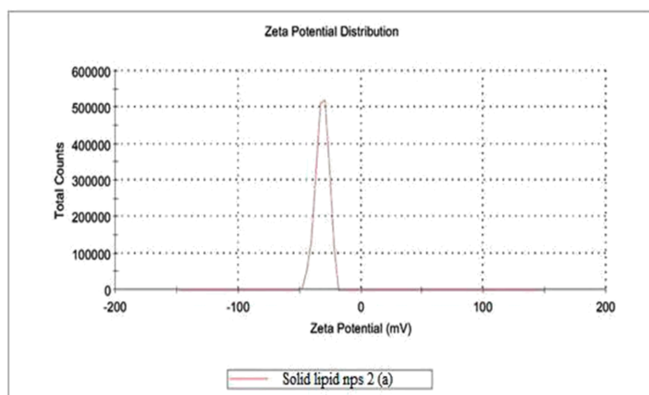

Fig. 2: Zeta Potential distribution of formulation-2A

3.1.3. Microscopy

A Photomicroscope study showed that the particles obtained were in the range of nanoparticles. A Photomicrograph of formulation F2 is given in Fig.-3.

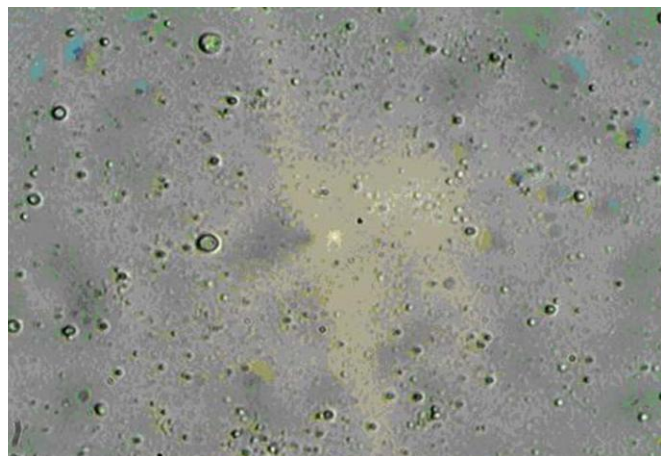

Fig. 3: Photomicrograph of formulation 2A

3.1.4. Scanning Electron Microscopy (SEM)

Morphological characterization was done by SEM. The micrograph indicated that production parameters affected the crystal morphology. On low amplitude pressure and cycles of ultrasonication, larger crystals with heterogeneous size distribution were obtained. While at optimized amplitude pressure and cycles of ultrasonication, smaller, uniform, and particles with narrow size distribution were obtained. SEM photographs of formulation F1 and F2 are given in Fig.-4 and 5 respectively.

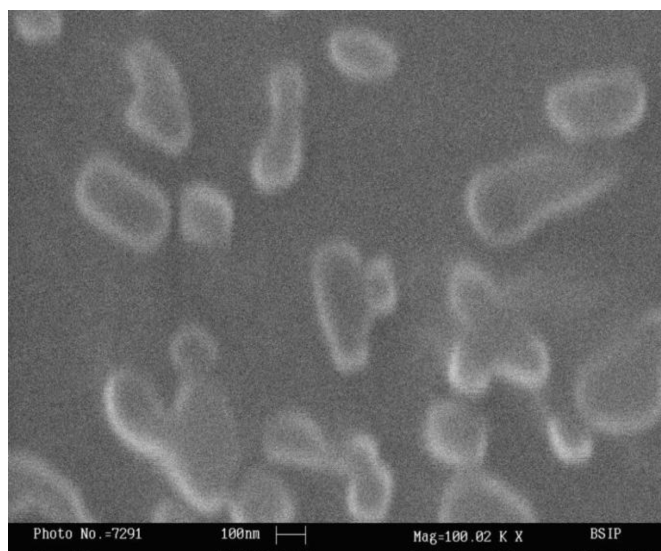

Fig. 4: SEM image of formulation-2A

Table 3: Release profile of solid lipid nanoparticles of aceclofenac

Time (min)	Formulations				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
15	16.72	24.12	17.75	8.92	10.28
30	22.32	28.62	21.11	13.43	12.07
60	24.56	36.22	26.82	16.35	15.52
120	26.96	40.03	28.9	21.03	19.62
180	35.01	45.37	34.26	24.8	22.37
240	41.32	47.31	39.49	31.16	25.93
300	46.10	52.06	43.96	34.09	28.69
360	52.77	56.00	48.29	39.08	31.46
420	58.23	60.54	51.21	46.19	34.82
480	61.47	65.12	55.56	48.79	39.13
540	64.67	70.12	59.79	53.37	43.79
600	67.51	76.43	63.11	54.08	45.43

3.1.5. *In-vitro* release study

The release study of solid lipid nanoparticles was done for all the formulations in phosphate buffer pH 6.8. The results indicated (Table 3) that the cumulative % drug release increased with decreasing particle radius. The dissolution rate of lipid nanoparticle F2 was 55.5 % dissolved within 60 minutes and 94.5 % dissolved in 120 minutes.

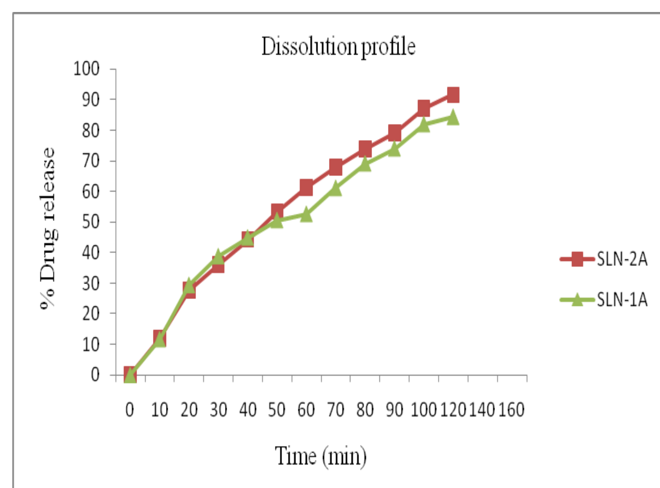

Fig. 6: Dissolution profile of formulations SLN-1A and SLN-2A

Whereas in formulation F1 (having slightly larger particles than F2 formulation) only 49.5 % released in 60 minutes and, 87.4 % released in 120 minutes. It could be described based on the Noyes-Whitney equation, which states that the rise in surface area (A) and saturation solubility (Cs) due to radius reduction may increase the velocity of the dissolution of poorly soluble compounds.

From the analysis of dissolution, it was obvious that F2 had a faster rate of dissolution compared to F1 formulation so that the F2 formulation was further selected for model fitting. For all the formulations the drug release was measured in 10 hours. Fig. 6 indicates the in-vitro drug release profile of the formulation F2.

3.1.6. *Release kinetics*

The data acquired from F2 formulation release studies were fitted into different kinetics models. The highest value of the regression coefficient was found to be 0.9945 with the Peppas model. The value of n (0.9945) obtained by the Korsmeyer-Peppas equation indicated the non-fickian release of drug from the formulations. Mathematical models for the drug release profile based on the regression coefficient (R) and (k) value is given in Table 4.

Table 4. Mathematical models for drug release profile based on regression Coefficient (R) and (k) value.

Formulation code	Zero-order		First-order		Peppas model		Hixon Crowell model	
	R	k	R	k	R	k	R	k
SLN-2A	0.7180	0.0098	0.7291	-0.0001	0.9945	0.3776	0.7245	0.0000

3.1.7. Physical stability study of drug nanoparticles

For two weeks, we researched the physical stability of aceclofenac nanoparticles at 25°C. Physical stability parameters were observed at fixed intervals, such as a change in appearance, Ostwald ripening, and settling behavior. Light microscopy was observed for Ostwald ripening. It is the mechanism by which smaller particles are consumed due to increased solubility resulting from the higher curvature of smaller particles as defined by the Ostwald Freundlich equation for the growth of larger particles. The observation confirmed that no crystal growth was there at the predetermined intervals. Visually we noticed changes in appearance and settling behavior. The result suggested no settling, and nearly all lipid nanoparticles were stable.

4. CONCLUSION

The solid lipid nanoparticles of aceclofenac were successfully developed. The lipid nanoparticles with suitable and desired characteristics may be prepared by the microemulsion technique. Owing to reduced particle size and huge surface area, the device exhibited an increased dissolution rate. The release profile of aceclofenac from the solid lipid nanoparticles was found to be amenable to the slow delivery of the drug.

5. ACKNOWLEDGEMENTS

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Abbreviations:

SLN-Solid lipid nanoparticles, GRAS- Generally Recognized as Safe, FTIR- Fourier Transform Infra-Red, PI- Polydispersity Index, MW- Molecular weight, SEM-Scanning Electron Microscopy.

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