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Development and Characterization of Solid lipid Nanoparticle of Diclofenac sodium in the treatment of ocular pain after photorefractive keratectomy

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Abstract: - The aim of this study was to prepare and evaluate incorporating solid lipid nanoparticles (SLNs) of diclofenac sodium for systemic delivery of the active after ocular application. Diclofenac sodium loaded solid lipid nanoparticles (SLNs) have been successfully developed using a microemulsion technique. Three different formulations were prepared It was found that variation in the amount of ingredients had profound effects on the diclofenac sodium loading capacity, the mean particle size, and size distribution of charge, morphology, and drug-lipid compatibility. At optimized process conditions, diclofenac sodium loaded SLNs showed spherical particles with a mean particle size of 450 nm and 60% diclofenac sodium incorporation efficacy was achieved. The SLNs were evaluated for in vitro drug release, *ex-vivo* permeation studies. The SLN sustained the drug release for 6 h in vitro. The results suggest enhancement in ocular delivery of diclofenac sodium with incorporating SLNs.

Key words: - solid lipid nanoparticles, diclofenac sodium, Ocular delivery, Analgesic activity.

Introduction- Delivery of drugs to the tear film is routinely done with eye drops, which are well accepted and for most patients easy to use. However, attainment of an optimal drug concentration at the site of action is a major problem. Poor bioavailability of drugs from ocular dosage form is mainly due to the pre-corneal loss factors which include tear dynamics, nonproductive absorption, transient residence time in the cul-de-sac, and relative impermeability of the corneal epithelial membrane In this study, SLN formulations as sustained ocular drug delivery systems are described. SLN formulations are adhesive, and could prolong the residence time of the dosage form in the eye and increase bioavailability.

Material and Method- Diclofenac Sodium (DS) was obtained from Alpha pharmaceuticals (Hyderabad, India), Gleceryl Behnate was purchased from Gattefosse France. Chloroform and methanol were purchased from Fine chemicals (Ahmadabad, India).

To prepare SLNs dispersion, DS, Gleceryl Behnate, of different compositions (Table 1) were dissolved in 10 ml mixture of Methelene Chloride. Organic solvents were completely removed using a rotoevaporator (Laborota 4000, Heidolph, Germany) to form a drug-embeded lipid layer. This layer was melted by heating at 65°C above the melting point of the lipid. Aqueous phase was prepared by dissolving polysorbate 80 (2% w/v) in double distilled water (10 ml) and heated to same temperature of oil phase. The hot aqueous phase was added to the oil phase and homogenization was performed (at 2500 rpm and 70°C) using a mechanical stirrer for 30 minutes. The coarse oil in water microemulsion so obtained was sonicated using probe soincator for 25 minutes. DS loaded SLN was finally obtained by allowing the hot nanoemulsion to cool at room temperature.

Table-1 Optimization of formulation

Formulation Code	Diclofenac Sodium	Gleceryl Behnate
B1	0.5%W/W	5%W/W
B2	0.75%W/W	5%W/W
B3	1%W/W	5%W/W

Particle size and zeta potential measurements

The particle size of the SLN was determined by phase angle light scattering (PALS) using Zetasizer Nano-Series (Nano- ZS, Malvern Instruments, England). Samples were diluted with double-distilled filtered water before measurement. The zeta potentials of the formulated SLN were also determined using the same instrument. For the zeta potential measurements, each sample was diluted with bidistilledwater and the electrophoretic mobility determined at 25 ◦C and dispersant dielectric constant of 78.5. The obtained electrophoretic mobility values were used to calculate the zeta potentials using the software.

Table – 02 Particle size and zeta potential of formulation

Batch of SLN	Zeta Pottential mV ±SD., n=4	Particle size in nm
B1	-12.2+0.5	254
B2	-29.1+0.7	300
B3	-49..5+2.3	323

Transmission electron microscope- Morphological examination of the NPs was performed using a transmission electron microscope (TEM). A drop of NP suspension was placed on a copper grid with a Formvar film. Copper grids coated with Formvar/carbon were glow-discharged for 15 sec to impart hydrophilicity to the surface and facilitate spreading of the SLNs samples onto the grid. Diluted SLN samples (10 µl of a 1 % w/w dispersion) were placed on the grid. After 2 min, excess sample was blotted off with filter paper and 6 µl of aqueous 2 % (w/w) PTA were added. After 30 sec, excess solution was removed and the grid was dried at room temperature. Samples were viewed in a TECNAI 200 Kv TEM

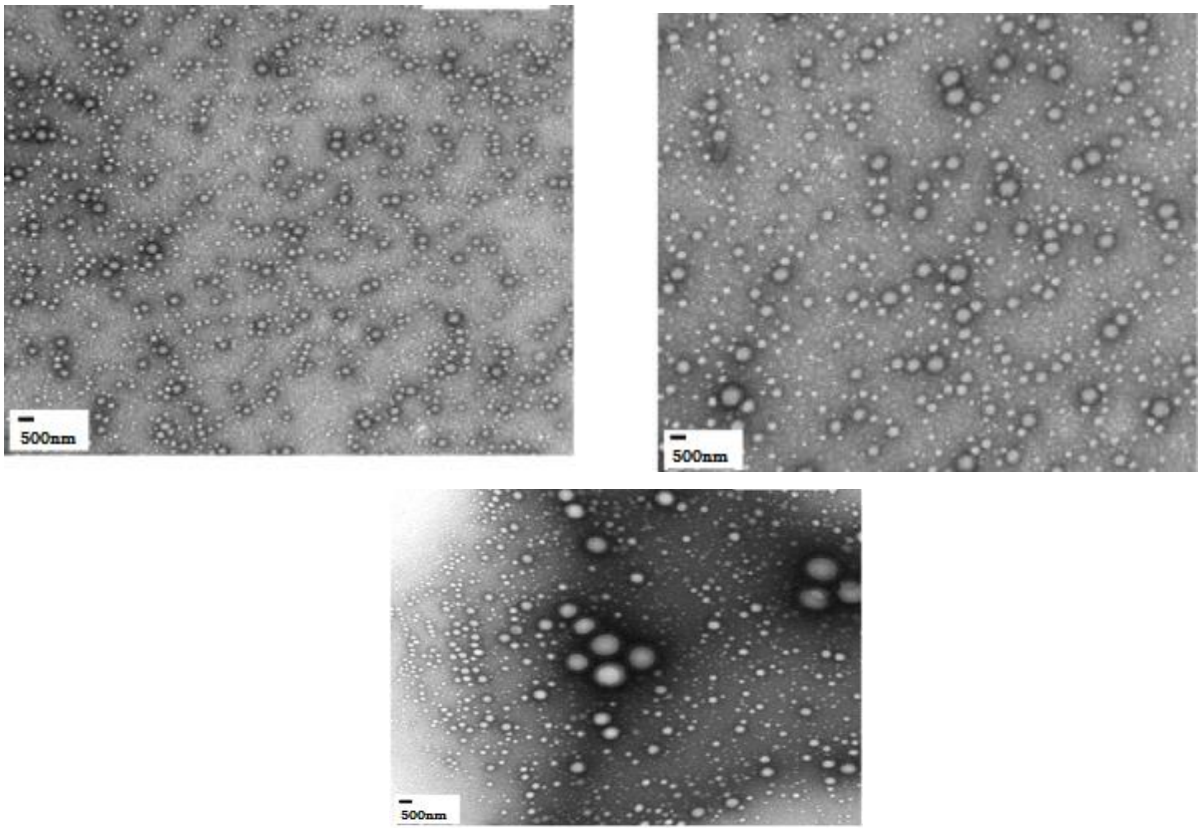

Fig- 01 TEM Micrograph of Solid Lipid Nanoparticle B1,B2,B3 formulation

Drug release-

In vitro drug release studies were performed in vertical Franz diffusion cell. Phosphate buffer pH 7.4 (24 ml) was placed in the receiver compartment. A 0.5 gm of SLN of diclofenac was placed in the donor compartment. A dialysis membrane was used to separate the donor and receiver compartments. The diffusion cells were maintained at (37±0.5ºC) with stirring at 600rpm throughout the experiment. At fixed time intervals, 5 ml of the sample was withdrawn from receiver compartment though side tube and analyzed by UV-Visible spectrophotometer at 276nm. Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of diclofenac release from SLN.

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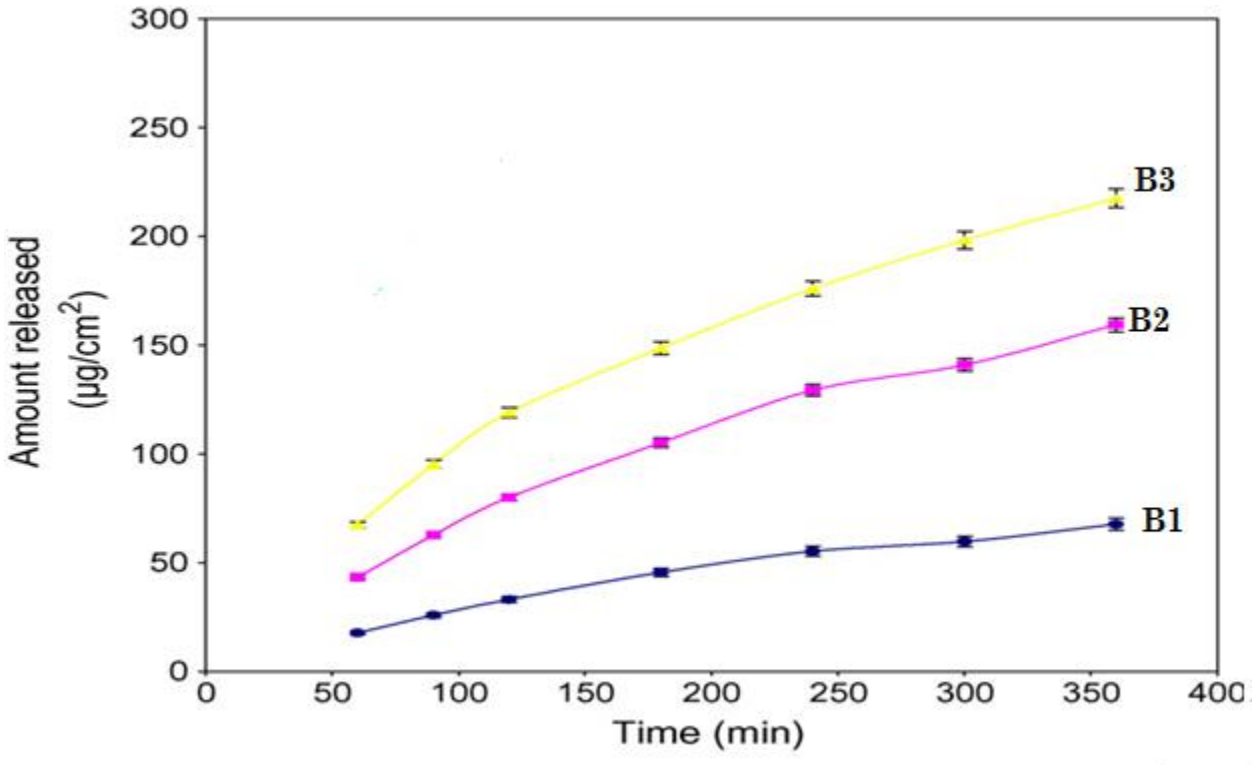

Fig 02 Drug release from formulation in PBS 7.4

Entrapment Efficiency (EE) was determined by measuring the concentration of free drug (unentrapped) in aqueous medium [20]. The diluted aqueous medium containing the DS SLN was subjected to ultra-filtration to separate the free drug from encapsulated drug. Centrisart tubes, which consists of filter membrane (M.wt. cut off 20,000 Da) at the base of the sample recovery chamber was used. About 1ml of the diluted formulation was placed in the outer chamber and sample recovery chamber was placed on top of the sample and centrifuged at 4000 rpm for 15 min. The formulation was diluted to 1000 times using the same buffer used in the preparation of the SLN. The SLN along with encapsulated drug remained in the outer chamber while the aqueous phase moved into the sample recovery chamber through filter membrane.

$$EE - \frac{\text{Wt of drug used in the formulation- Wt of drug aqueous phase}}{\text{Wt of drug used in formulation}} \text{ X 100}$$

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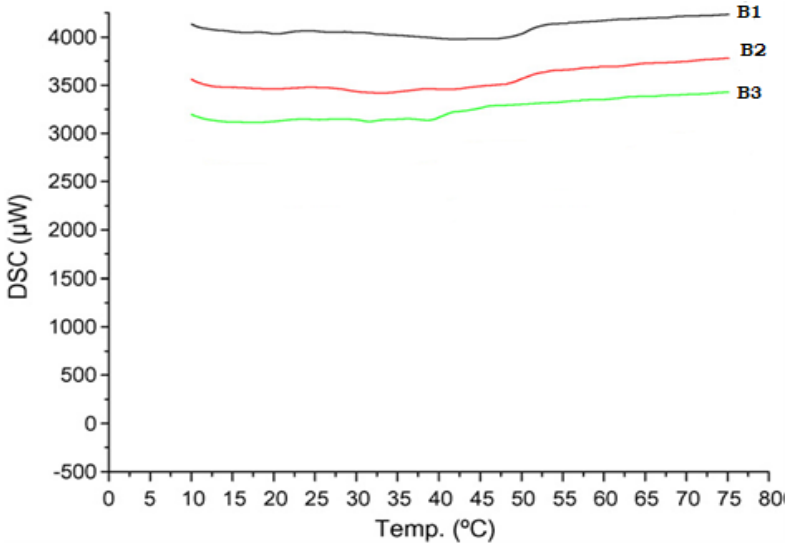
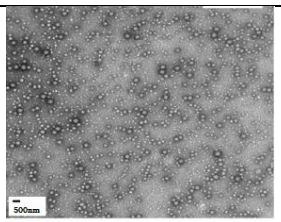
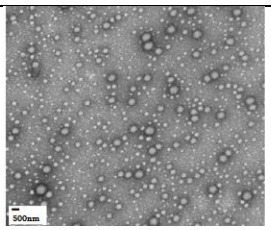
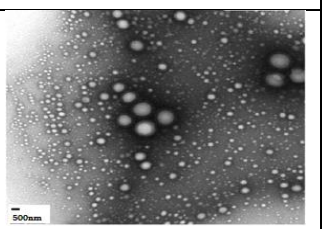
Table 03 Entrapment efficiency of Formulation

Formulation	% EE
B1	94.2 ± 1.5
B2	94.0 ± 0.4
B3	93.8 ± 1.2

Differential study calorimetry - DSC was used to underline the high drug encapsulation potency of the SLN. The higher the enthalpy of the transitions, the more crystalline the SLN and consequently, the more difficult it will be for any drug to be encapsulated. The DSC result is presented in Fig. 3



Characteristics	**B1**	**B2**	**B3**
Marphology			
Zeta Pottential mV ±SD., n=4	-12.2+0.5	-29.1+0.7	-49..5+2.3

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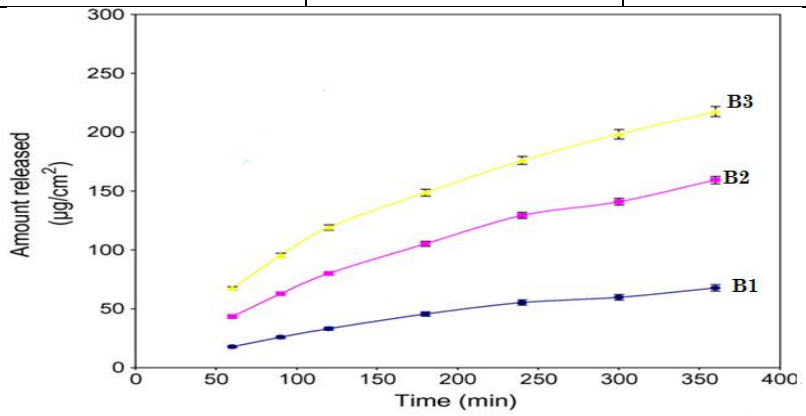
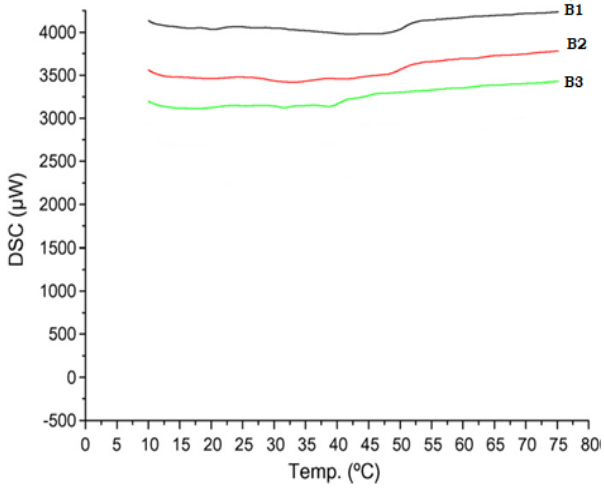
Particle size	254	300	323
Drug release			
Entrapment efficiency	94.2 ± 1.5	94.0 ± 0.4	93.8 ± 1.2
DSC			

Result and discussion

Particle size and zeta potential- For ocular administration, irritation and tear wash out may occur on administration of large-sized particles, since smaller particles are better tolerated Very small particles as nanoparticles possess adhesive properties , which could prolong the residence time of the drug in the cul-de-sac, prevent tear wash out (due to tear dynamics), and increase ocular bioavailability. The zeta potentials of the drug-loaded SLN presented in Table 1 indicate very stable particles. 1.0%DS obsessed the highest absolute zeta potential, it possessed higher

crystallinity, lower drug load and higher particle size than other drug-containing SLN. These factors coupled with the tendency to agglomerate eliminate it from being the best SLN in this study.

Encapsulation efficiencies above 90% were recorded for the SLN batches the high drug loading was also contributed by the behaviour of DS in the presence of a as diclofenac shows thermotropic liquid crystalline behaviour and may participate in the microstructure of the system. The encapsulation efficiencies (mean±S.D., $n = 3$) obtained for the drug-loaded SLN are presented in Table 1. There was no significant difference ($p < 0.05$) between the loading efficiencies of the SLN, but there was a significant difference ($p < 0.05$) in the loading efficiencies of SLN with. This was due to complex formation between lipid and the drug, and the participation of drug in the structures formed at the particle surface.

DSC was used to underline the high drug encapsulation potency of the SLN. The higher the enthalpy of the transitions, the more crystalline the SLN and consequently, the more difficult it will be for any drug to be encapsulated. The DSC result is presented in Fig. 2. which showed a slight endothermic signal at about 50 ◦C, confirming their higher crystallinity.

Drug release shows the release profile of DS in phosphate buffer (pH 7.4) using dialysis membrane as the release barrier. There was burst release of DS from SLN formulated within the first 125 min. This was due to the more crystalline nature of this SLN, which resulted in more drugs in the periphery and bulk aqueous medium than in the core of the lipid nanoparticles. The profiles of the lipid containing SLN revealed sustained release of the active with regards to ocular delivery. Higher quantity of DS was released in the batch loaded with higher amount of DS.

Poor bioavailability of drugs from ocular dosage form is mainly due to the tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium. After topical ocular application, drugs may be absorbed into the eye through the corneal or conjunctival and scleral route. In fact, conjunctiva is a conduit for drug clearance into the systemic circulation The route through conjunctiva and sclera is important mostly for very hydrophilic and large molecules that are not able to penetrate through the corneal barrier . Most clinically used ocular drugs have adequate lipophilicity for corneal absorption, and such properties are sought in the development of new ocular drug. This SLN formulation of DS, which showed high *in vitro* ocular transport would offer two advantages in terms of ocular drug

delivery. First, encapsulation of the DS into the lipophilic particle would facilitate transport through the corneal route. Lipid formulations are also known to enhance the absorption of certain drugs and together with polysorbate 80, may also have inhibitory effects on some drug efflux transporters

Conclusion- The high encapsulation efficiency and high permeation achieved with this novel SLN formulation indicate this SLN could be an effective drug delivery system for ocular active drugs. DS-containing SLN evaluated in this work showed that the performance of this analgesic drug for ocular application could be improved by formulation as SLN.

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