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FORMULATION AND EVALUATION OF DIACERIN LOADED SOLID LIPID NANOPARTICLES

GUGULOTHU B AND CHINTHALA P*

Department of Pharmacy, Chaitanya Deemed to be University, Himayathnagar, Hyderabad, Telangana, India-500075

***Corresponding Author: Prof. Praveena Chinthala: E Mail: praveenamr18@gmail.com**

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ABSTRACT

Introduction: Osteoarthritis (OA) is one of the most common forms of arthritis. Diacerin, is used in the treatment of osteoarthritis. It is classified as BCS class II drug with low solubility and high permeability. In this work, Diacerin loaded solid lipid nanoparticles (SLNs) were developed to improve the oral bioavailability.

Methods: The Diacerin loaded nanoparticles were prepared by hot homogenization followed by ultrasonication method using varying concentrations of lipids such as Trimyristin, Tripalmitin and Tristearin. Total six formulations were developed (F1-F6). Further, various physicochemical properties, morphology of SLNs were characterized. The physical stability study was conducted on optimized formulation at room temperature and refrigerated conditions for 3 months.

Results: Among all the formulations, the F3 formulation prepared with Tripalmitin was selected as the best based on particle size, percentage entrapment efficiency, and *in-vitro* drug release. The optimized F3 formulation exhibited a particle size of 179 nm, an entrapment efficiency of 96.1%, and an *in-vitro* drug release of 97.17% at 24 hours.

Conclusion: The optimized Diacerin-loaded SLN formulation (F3) showed high entrapment efficiency, nanoscale size, and sustained drug release over 24 hours. It remained stable for three months under various storage conditions. These results suggest SLNs as a promising approach to enhance the oral bioavailability of Diacerin.

Keywords: Solid lipid nanoparticles, hot homogenization, particle size, entrapment efficiency, *in-vitro* release

INTRODUCTION

Lipid-based nanotechnology for encapsulation and delivery systems for active compounds can be applied in several models, including nanoliposomes, nanosuspensions, nanoemulsions, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). SLNs are the most promising lipid-based drug delivery utilized for enhancing the solubility, bioavailability and therapeutic efficacy of poorly water-soluble molecules **[1-3]**.

One of the most prevalent types of arthritis is osteoarthritis (OA). Synovitis, subchondral bone changes, and articular cartilage degradation are the hallmarks of this degenerative joint condition. It has an impact on joints, changing their cartilage in particular. The most frequently impacted joints are the knees, though the hip, ankle, shoulder, and tiny hand and foot joints may also be affected **[4]**.

Diacerein (4,5-diacetoxy-9,10-dihydro-9,10-dioxo- 2-anthracenecarboxylic acid) is novel chondroprotective/connective tissue structure modifying agent intended for the treatment of osteoarthritis **[5]**. It is a semi-synthetic anthraquinone derivative and belongs to BCS class II drug with low solubility and high permeability. It is sparingly soluble in water which results in a low oral bioavailability (35–56%). These all properties lead to poor patient compliance. In this study, Diacerein, a highly lipophilic anti-osteoarthritic drug, was used as a model drug.

MATERIALS AND METHODS

Diacerein was received as a gift sample from Dr. Reddy's Lab, Hyderabad, India. Trimyristin (TM), Tripalmitin (TP) and Tristearin (TS) were purchased from Sigma-Aldrich Chemicals, Hyderabad, India. Egg Lecithin E-80, Citric acid and Poloxamer-188 were obtained as gift samples from Hetero Labs, India. Methanol, Chloroform were of HPLC grade (Merck, Mumbai, India). Millipore Direct Q ® 3UV water was used in all the studies.

Preparation of Diacerin- SLN [6]

Diacerin-loaded SLNs were prepared using the hot homogenization followed by

ultrasonication method. The required quantities of Diacerin, lipid, and egg lecithin were dissolved in 15 mL of a 1:1 mixture of Chloroform and Methanol. The organic solvents were completely evaporated using a rotary evaporator (Heidolph, Germany). The resulting drug–lipid layer was then melted by heating to 5°C above the melting point of the lipid. The aqueous phase was prepared by dissolving Poloxamer-188 in double distilled water and heated to same temperature (based on lipid melting point) of oil phase. Then hot aqueous phase was added to the melted oil phase, and homogenization was carried out at 12000 rpm for 4 min using homogenizer (Diax 900, Germany). This gives coarse hot oil in water emulsion. To this added required quantity of citric acid, then it was subjected for ultrasonication using a 12 T probe sonicator for 20 min. After ultrasonication, the dispersion was allowed to cool to room temperature to obtain Diacerin loaded SLNs. Different concentrations of lipids were used to optimize the formulation and composition of different batches is presented in **Table 1**.

Table 1: Composition of Diacerin solid lipid nanoparticles

Ingredients (mg)	F1	F2	F3	F4	F5	F6
Diacerin	50	50	50	50	50	50
Tristearin	100	200	--	--	--	--
Tripalmitin	--	--	100	200	--	--
Trimyristin	--	--	--	--	100	200
Poloxamer-188	150	150	150	150	150	150
Egg lecithin	100	100	100	100	100	100
Citric acid	5	5	5	5	5	5
Solvent(ml)(1:1) (Chloroform: Methanol)	15	15	15	15	15	15
Purified water (ml)	15	15	15	15	15	15

Characterization of solid lipid nanoparticles

Measurement of Particle size, PDI and Zeta potential of SLN [7]

Using a Malvern Zetasizer, the particle size, polydispersity index (PDI) and zeta potential (ZP) of the prepared SLNs was measured.

Determination of entrapment efficiency [7]

Entrapment efficiency (EE) was calculated by measuring the concentration of free drug (unentrapped) in aqueous medium. Centrisart tubes with a filter membrane molecular weight cut off 20000 Da at the base of the sample recovery chamber were used to ultra-filter the aqueous medium. After placing roughly 2.5 mL of the formulation in the outer chamber and the sample recovery chamber on top of it, the mixture was centrifuged for an hour at 20,000 rpm. The aqueous phase entered the sample recovery chamber through the filter membrane, while the SLN and the

encapsulated medication stayed in the outer chamber. The amount of drug present in the aqueous phase was estimated by UV Visible Spectrophotometer at 254 nm.

Assay

To determine drug content 100 µL of the SLN formulation was taken and dissolved in Chloroform and Methanol mixture (1:1). Then further dilutions were made with the above mixture and calculated the amount of Diacerin present in formulations by UV method **[8]**.

***In-vitro* drug release studies**

In-vitro drug release studies were performed using the dialysis bag diffusion method. A dialysis membrane with a pore size of 2.4 nm (Molecular weight cutoff between 12,000–14,000 Da) was used, which was soaked overnight in double-distilled water prior to use. Two types of release media were employed: 0.1N hydrochloric acid (HCl) and phosphate buffer pH 6.8. The experimental setup consisted of two compartments: a donor and a receptor. The donor compartment contained 1 mL of the SLN dispersion. The receptor compartment comprised a 250 mL beaker filled with 100 mL of the release medium, maintained at 37 ± 0.5°C with continuous stirring.

At predetermined time intervals — namely, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours — a 2 mL sample was withdrawn from the receptor compartment and replaced with an equal volume of fresh buffer to maintain sink conditions. The collected samples were suitably diluted and analyzed using a UV-Visible spectrophotometer (model SL-150, ELICO, India) at a wavelength of 254 nm.

Stability studies

Diacerin loaded SLNs of optimized F3 were stored at room temperature, 25 ± 2°C and refrigerated temperature, 4°C for three months **[9]**. The particle size, ZP, assay and entrapment efficiency were determined at specific periods i.e., 1 day, one month and three months.

Lyophilization of SLNs

Lyophilization was employed to enhance the stability of the SLNs. The SLN dispersion was prepared using 10% w/v maltose as a cryoprotectant. The formulation was then frozen by storing it in a deep freezer at −40°C (Sanyo, Tokyo, Japan) overnight. Following freezing, the samples were transferred to a freeze-dryer (Lyodel, India), where vacuum was applied. The samples underwent various drying phases over a period of approximately 48 hours to obtain a powdered, lyophilized product **[10]**.

Solid state characterization [11-13]

Drug-excipient compatibility studies by Differential Scanning Calorimeter (DSC)

DSC analysis of pure drug, trimyristin (TM), tripalmitin (TP) and tristearin (TS), physical mixtures (PM in 1:1 ratio) were performed. The instrument was calibrated with indium. All the samples (10 mg) were heated in aluminium pans using dry nitrogen as the effluent gas. The analysis was performed within a heating range of 20–200º C and at a rate of 20º C/min.

Morphology by Scanning Electron Microscopy (SEM)

The morphology of nanoparticles was studied by Scanning Electron Microscope (SEM, Japan). Freeze dried Diacerin solid lipid nanoparticles were suitably diluted with double distilled water (1 in 100) and a drop of nanoparticle formulation was placed on sample holder and air dried. Then the sample was observed at accelerating voltage of 15000 volts.

Morphology by Transmission Electron Microscopy (TEM)

TEM observations were also performed to observe the morphology of freeze dried Diacerin-SLN following negative staining with sodium phosphotungstate solution (0.2% w/v). A thin film was made on a carbon-coated copper grid by placing a drop of SLN dispersion. Before the film was dried on the grid, it was negatively stained with phosphotungstic acid by adding a drop of the staining solution to the film; any excess solution was drained off with a filter paper. The grid was allowed to air dry, and samples were viewed under a transmission electron microscope (JEOL-Japan).

RESULTS AND DISCUSSION

In this study, Diacerin loaded SLNs were prepared by hot homogenization followed by ultrasonication method, using three lipids, each at two different concentrations. Based on the particle size and uniformity of dispersion, the homogenization time and sonication time were optimized to 4 and 20 min respectively. As the drug is unstable in basic pH, it was important to provide acidic environment to drug[5]. Citric acid is an acidifying agent and pH modifier for many weakly acidic and weakly basic drugs. In the prepared SLNs it maintains acidic microenvironment to the drug, which protects conversion of Diacerin to rhein.

Measurement of Particle size, Polydispersity index (PDI) and Zeta potential of SLN

All the prepared formulations were analyzed in order to determine their particle size distribution, zeta potential and PDI values. The mean size of all the formulations was ranging from 179 ± 1.8 nm to 245 ± 2.6 nm **(Table 2)**. The PDI was ranging from 0.199 ± 0.001 to 0.342 ± 0.04, indicating the narrow

size distribution. The zeta potential values of SLN formulations were found to be in between -23.17 ± 1.4 mV to -28.01 ± 2.4 mV. It is currently admitted that zeta potential -30 mV is required for electro- static stabilization **[12]**.

Formulations containing Trimyristin showed lower particle sizes but the PDI was higher and ZP was lower. Formulations containing Tristearin showed lower PDI, but higher particle sizes and lower ZP. But formulations containing Tripalmitin (F3 and F4) showed relatively better size, PDI and ZP when compared to other formulations **(Table 2)**.

Table 2: Characterization of Diacerin solid lipid nanoparticles (Mean ±SD)

Formulation code	Particle size (nm)	PDI	Zeta potential (mV)	Assay (%)	Entrapment efficiency (%)
F1	232±3.21	0.232±0.002	-26.32±1.2	96.2±0.3	92.2±0.2
F2	245±2.6	0.199±0.001	-24.45±1.7	98.7±0.1	94.3±0.4
F3	179±1.8	0.315±0.003	-28.01±2.4	99.8±0.5	96.1±0.1
F4	225±1.5	0.342±0.04	-25.35±1.5	98.2±0.2	97.3±0.3
F5	182±2.1	0.258±0.01	-24.23±2.1	97.8±0.3	92.1±0.2
F6	215±1.8	0.287±0.03	-23.17±1.4	98.8±0.1	91.1±0.4

Total drug content in the SLN formulations was determined and found to be ranging from 96.2 ± 0.3% to 99.8 ± 0.5%. Entrapment efficiency of the SLN formulations were found to be 91.1 ± 0.4% to 97.3 ± 0.3%. High lipophilicity of Diacerin resulted in high entrapment efficiency of drug in triglyceride nanoparticles **(Table 2)**. High encapsulation efficiency of drug in lipid nanoparticles can cause high amount of drug to pass through the lymphatic transport, which in turn bypasses the first pass metabolism.

In vitro release of drug from Diacerin-SLNs was studied in 0.1 N HCl (pH 1.2) and pH 6.8 phosphate buffer by dialysis method. In 0.1 N HCl, the cumulative percentage of release from formulations F1-F6 was 22.84%, 29.98%, 28.83%, 37.30%, 34.51%, and 48.87% respectively for a period of 24 hours. In pH 6.8 phosphate buffer, the cumulative % of release from formulations F1-F6 was 56.56%, 49.45%, 97.17%, 55.96%, 53.03% and 59.19% respectively in 24 hours **(Figure 4)**. The drug released from Diacerin-SLNs slowly in 0.1 N HCl medium when compared to that in pH 6.8 phosphate buffer, which could be due to the solubility difference of drug in these media. Formulation F3 showed maximum release of 97.17% in pH 6.8 phosphate buffer during 24 hours. In comparison, F3 formulation exhibited reasonably good particle size, better PDI, high zeta potential value and the higher entrapment efficiency with release of drug from the lipid matrix in pH 6.8 phosphate buffer, hence it was considered as the optimized formulation.

Since the aqueous SLN dispersion is liable to physical and chemical stability problems, lyophilization was considered to increase the stability of SLNs for extended period of time. The optimized (F3) SLN formulation was freeze dried with 10% maltose and resulted in SLN powder. Upon reconstitution, increase in size, PDI and zeta potential were noticed. Due to removal of water in freeze drying process, particle attractive forces would increase; this might be reason for increase in the particle size of the SLNs formulation **[14-16]**.

Drug-excipient compatibility studies by Differential Scanning Calorimeter (DSC)

The compatibility status of the lipids in the SLN formulation was investigated by differential scanning calorimetry (DSC) and was based on the fact that different lipids possessed different melting points and enthalpies. DSC thermograms of pure drug, lipids, physical mixtures are shown in **Figure 1A-1E**.

The DSC thermogram of pure Diacerin showed a sharp endothermic peak at 224º C with high enthalpy. DSC was also conducted for lipids, Trimyristin, Tripalmitin and Tristearin and showed an endothermic peak at 56º C, 64º C and 72 ºC respectively. The physical mixture of drug and lipid exhibited the same endothermic peak at 225º C and concluded that no interaction was found between the drug and excipients.

SEM studies were conducted on optimized formulation (F3) and the study revealed that the particles possessed smooth surface and spherical in shape **(Figure 2)**. TEM studies demonstrated that particle shape was nearly spherical and in nanometer size **(Figure 3)**.

The stability of the optimized SLN formulation (F3) was ascertained by monitoring the particle size, ZP, assay and entrapment efficiency of Diacerin after storage at room temperature and refrigerated temperature (25 ± 2°C and 4°C) for a period of 3 months. No drastic increase in particle size, ZP, assay and entrapment efficiency was observed when stored at refrigerated temperature and room temperature for a period of 3 months **(Table 3)**.

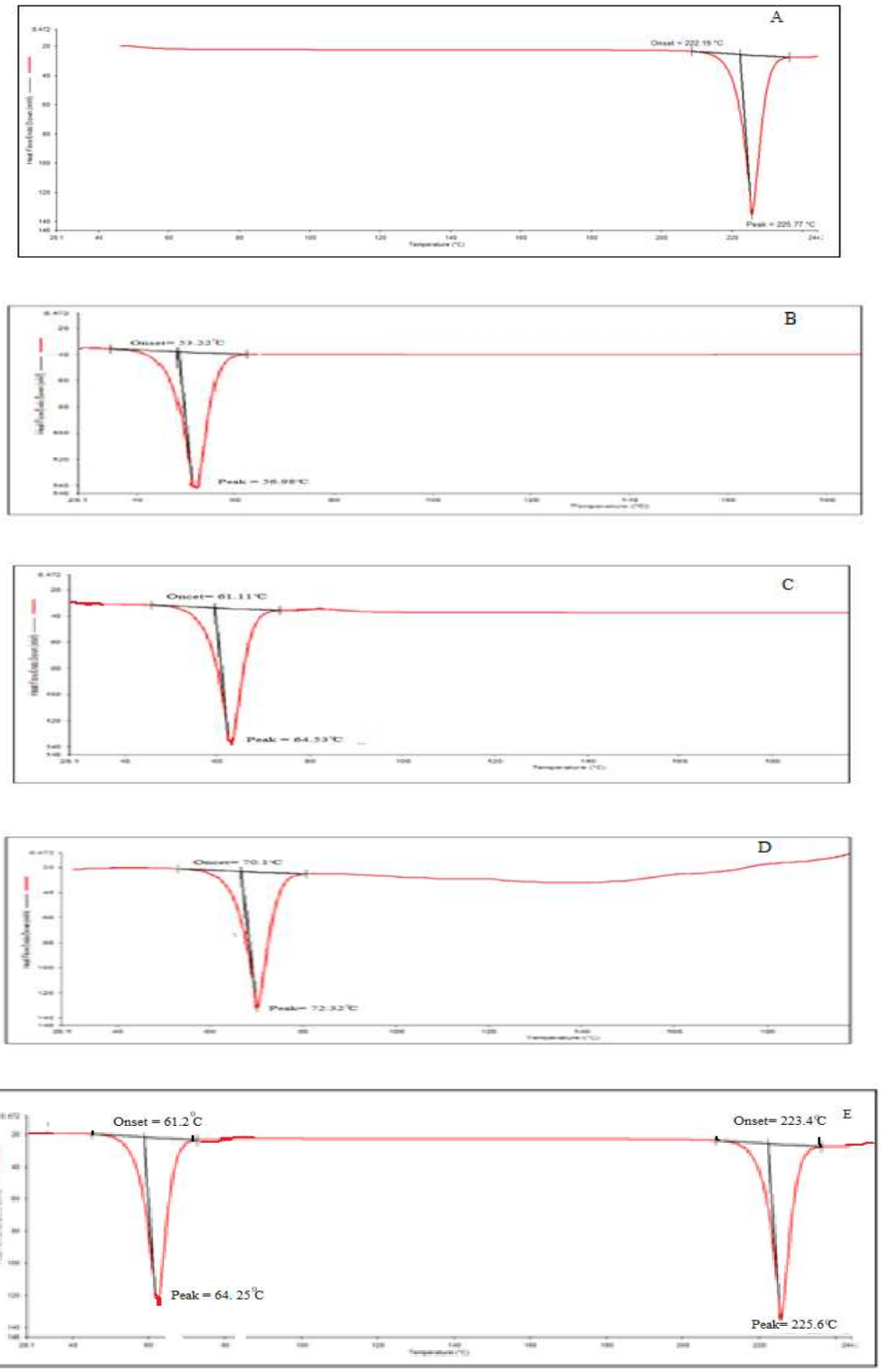

Figure 1: A) DSC thermogram for pure drug B) DSC thermogram for Trimyristin C) DSC thermogram for Tripalmitin D) DSC thermogram for Tristearin E) DSC thermogram for Physical mixture

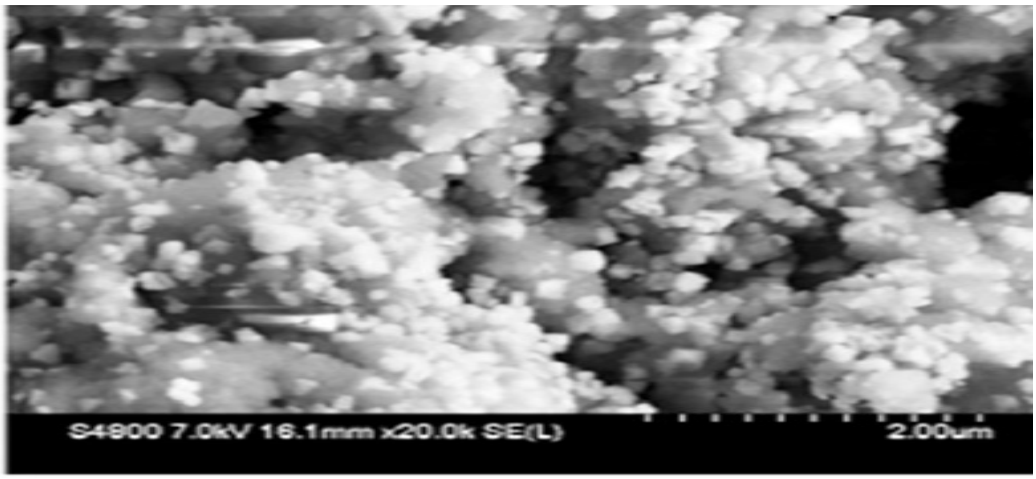

Figure 2: SEM image of lyophilized SLN optimized formulation (F3)

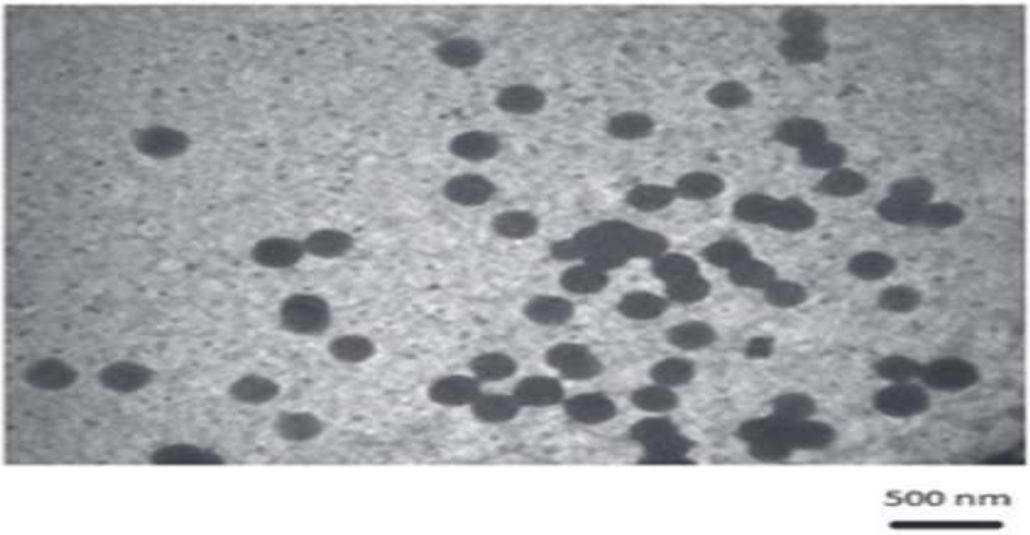

Figure 3: TEM of Diacerin Freeze dried SLN (F3)

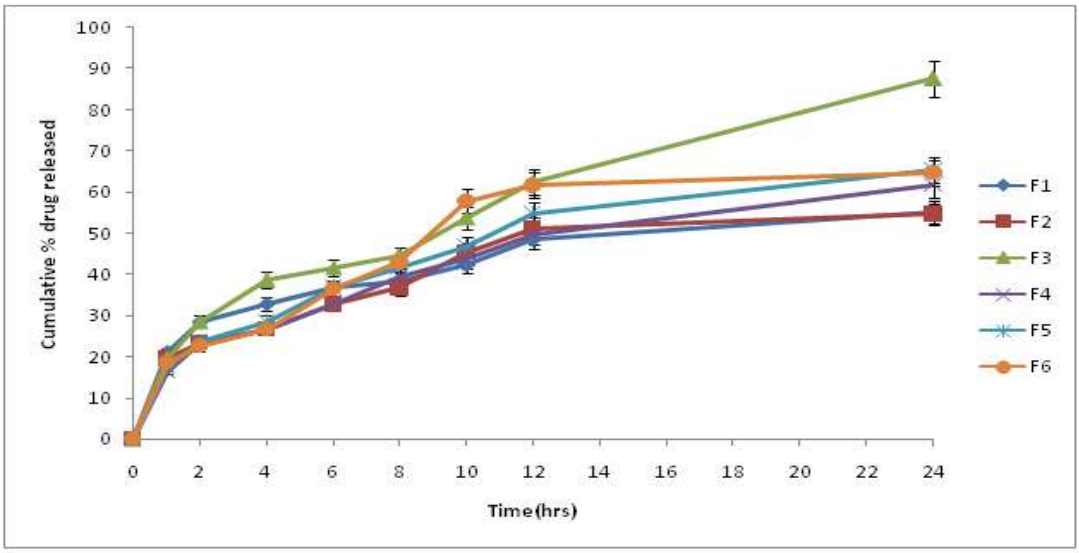

Figure 4: Cumulative % drug released from Diacerin SLN formulations (F1-F6) (Mean ±SD)

Table 3: Accelerated stability studies of optimized formulation (F3)

Storage condition	Duration	Particle size (nm)	Zeta potential (mV)	Assay (%)	Entrapment efficiency (%)
Room temperature (25 ± 2°C)	0	179±1.8	-28.01±2.4	99.8±0.5	96.1 ± 0.1
	1 month	179±1.5	-28.01±1.3	99.6±0.2	96.2 ± 0.2
	2 months	178±1.7	-28.21±1.2	99.7±0.3	95.3±0.3
	3 months	179±1.3	-29.01±1.2	99.5±0.1	96.1 ± 0.1
Refrigerator temperature (4°C)	0	179±1.8	-28.01±2.4	99.8±0.5	96.1 ± 0.1
	1 month	178±1.6	-28.21±1.2	98.7±0.3	96.3±0.3
	2 months	178±1.7	-28.13±1.2	99.2±0.1	95.6±0.2
	3 months	178±1.5	-28.11±1.2	99.5±0.2	95.3±0.4

CONCLUSION

The study successfully developed and optimized Diacerin-loaded SLNs to overcome the limitations of poor solubility and low oral bioavailability associated with Diacerin. Among the formulations, F3—formulated with Tripalmitin—demonstrated optimal characteristics, including high entrapment efficiency, nanoscale particle size, and sustained drug release over 24 hours. The formulation remained physically stable under both room temperature and refrigerated conditions for three months. These findings indicate that SLNs, particularly the optimized F3 formulation, offer a promising delivery system for enhancing the therapeutic efficacy and oral bioavailability of Diacerin in the treatment of osteoarthritis.

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