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FORMULATION AND INVITRO EVALUTION OF ARTEMISIA ARBORESCENS EXTRACT LOADED SOLID LIPID NANOPARTICLES

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Abstract:

The present study aimed to formulate and evaluate Artemisia arborescens-loaded Solid Lipid Nanoparticles (SLNs) to enhance drug stability, sustain drug release, and improve therapeutic efficacy. Four formulations (F1–F4) were prepared using varying concentrations of formulation components and evaluated for their physicochemical characteristics, drug-excipient compatibility, particle size, zeta potential, entrapment efficiency, in vitro drug release, and stability. The particle size of the formulations ranged from 121 to 164 nm, confirming their nanometric nature, while zeta potential values ranged from −25 to −36 mV, indicating good physical stability. Among all formulations, F3 was identified as the optimized formulation, exhibiting a particle size of 135 nm, entrapment efficiency of 81.24%, and zeta potential of −32 mV. In vitro drug release studies demonstrated a sustained release pattern over 8 hours, with F3 showing the highest cumulative drug release of 95.74%. Stability studies performed under different storage conditions for three months revealed no significant changes in the physicochemical properties or drug release profile of the optimized formulation, with drug release remaining above 92% throughout the study period.The findings of this study suggest that Artemisia arborescens-loaded SLNs represent a promising nanocarrier system capable of improving drug stability and providing sustained drug release, thereby potentially enhancing therapeutic effectiveness. The optimized formulation (F3) may serve as a suitable candidate for further in vivo investigations and future pharmaceutical applications.

Keywords: *Artemisia arborescens, Solid lipid Nano Particles, homogenizing technique, FTIR, In-vitro drug release.*

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INTRODUCTION:

Solid Lipid Nanoparticles combine the advantages of traditional colloidal carriers while minimizing their disadvantages. SLNs possess good biocompatibility, low toxicity, improved drug entrapment efficiency, enhanced penetration, and sustained drug release properties. They are especially useful for delivering herbal extracts because they protect sensitive phytoconstituents from degradation and enhance therapeutic effectiveness.

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to tradition colloidal carriers such as - emulsions, liposomes and polymeric micro and nanoparticles. Nan particles made from solid lipids are attracting major attention as novel colloidal drug carrier for intravenous applications as they have been proposed as an alternative particulate carrier system. SLN are sub-micron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid dispersed in water or in aqueous surfactant solution. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals.

MATERIALS

Artemisia arborescens was procured from Synpharma Research Labs, Hyd, Glyceryl monostearate, Tween 80, Span 20 and Methanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

Compatibility study (IR spectroscopy)

The Extract (drug) -excipients compatibility was ascertained by subjecting the extract and homogenates of extract and polymer to Infrared spectrophotometric study.

Extraction process

Fresh aerial parts or leaves of Artemisia arborescens were collected and washed with distilled water to remove impurities, followed by shade drying at room temperature for 7–10 days. The dried plant material was pulverized into coarse powder using a grinder. About 50–100 g of the powdered material was packed in a thimble and subjected to Soxhlet extraction using ethanol as the solvent for 6–8 hours until complete extraction was achieved. The obtained extract was filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was further dried and stored in an airtight container under refrigerated conditions for further formulation studies.

Method of preparation of Artemisia arborescens loaded SLNs:

Artemisia arborescens extract-loaded solid lipid nanoparticles (SLNs) were prepared by the hot homogenization method. Accurately weighed quantity of Glyceryl Monostearate (GMS) was melted at a temperature of about 70–75°C, which is above its melting point. The required quantity of Artemisia arborescens extract was dispersed uniformly in the molten lipid phase. Separately, an aqueous phase containing Tween 80 and Span 80 as surfactant was prepared and heated to the same temperature as the lipid phase. The hot aqueous phase was then added slowly to the molten lipid phase under continuous stirring to form a coarse emulsion. The obtained emulsion was subjected to high-speed homogenization using a hot homogenizer at 10,000–15,000 rpm for about 10–20minutes to reduce the particle size and obtain a nanoemulsion. The prepared nanoemulsion was then cooled to room temperature, resulting in solidification of lipid and formation of solid lipid nanoparticles. The prepared SLN dispersion was collected and stored in airtight containers at 4°C for further evaluation studies.

Table -1: Composition of Artemisia arborescens for preparation of Solid Lipid Nanoparticles

Ingredients	F1	F2	F3	F4
Artenisis arborescens(mg)	50	50	50	50
GMS (mg)	100	150	200	250
Tween 80	1%	1.5%	-	-
Span 80	-	-	1%	1.5%

Evaluation of Artemisia arborescens oil loaded nanoparticles:

Particle size:

All the prepared batches of solid lipid nanoparticles were viewed under microscope to study their size. Size of Nano particles from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanoparticles were determined.

Zeta potential:

Zeta potential is a measure of charge present on the vesicle surface. It was determined by using phase analysis light scattering with Malvern zeta sizer at field strength of 20V/cm in distilled water and based on electrophoretic mobility of charged particles present in the nano carrier system. Charged particles were attracted to the electrode with the opposite charge when an electric field is applied.

SEM analysis:
The morphology of SLNPs was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the NPs was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.

Drug encapsulation efficiency:
Lyophilized nanoparticles 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Artemisia arborescens in nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Artemisia arborescens nanoparticles was expressed as loading capacity.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies:
The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Artemisia arborescens dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{\text{Da}}{\text{Dt}} \times 100$$

Where, Dt = Total amount of the drug
Da = The amount of drug released

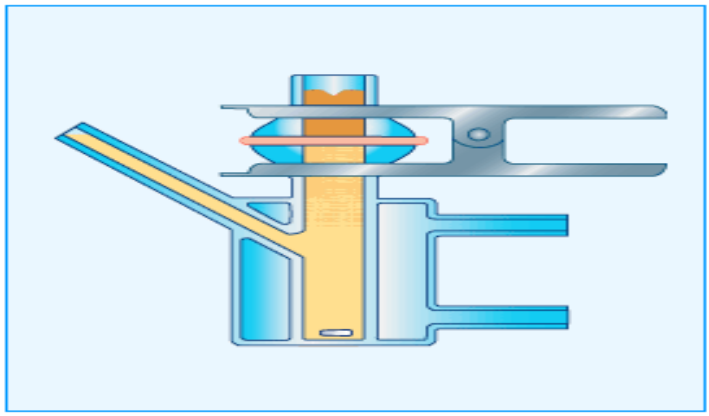

Fig-1: Franz diffusion cell

Stability studies:
Selected Formulation was subjected to stability studies as per ICH guidelines.
Following conditions were used for Stability Testing.
1. 25^0C/60% RH analyzed every month for period of three months.
2. 30^0C/75% RH analyzed every month for period of three months.
3. 40^0C/75% RH analyzed every month for period of three months.

RESULTS AND DISCUSSION:

Drug excipient compatibility studies
FTIR spectra of drug in KBr pellets at moderate scanning speed be 4000-400 cm-1 was made.

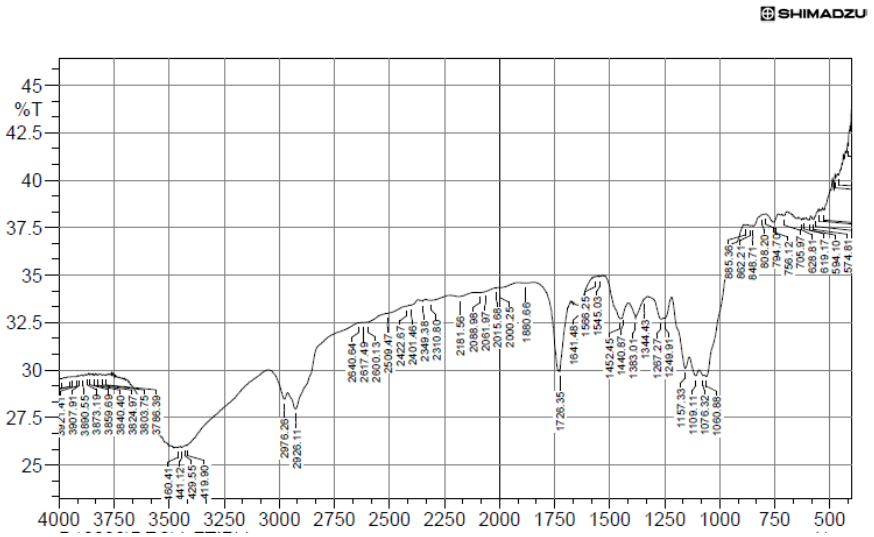

Fig-2: FTIR spectra of Artemisia arborescens

Table-2: Characteristic Peaks of Artemisia arborescens

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	4000-3500	3873.19
2	OH Bending	3000-2500	2976.26
3	C-H stretching	2500-2000	2310.80
4	C=O stretching	1500-1000	1452.45

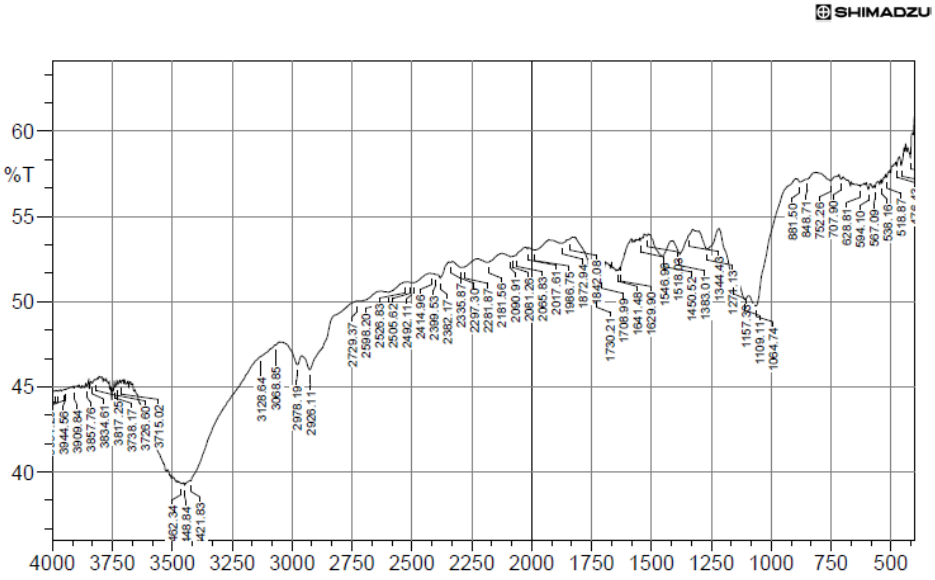

Fig-3: FTIR Spectra of physical mixture of drug and excipients

Table-3: Characteristic Peaks and frequency of physical mixture of drug and excipients

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	4000-3500	3857.76
2	OH Bending	3000-2500	2729.19
3	C-H stretching	2500-2000	2335.92
4	C=O stretching	1500-1000	1450.52

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of Pure drug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks were obtained as above and as they were in official limits (±100 cm^{-1}) the drug is compatible with excipients.

Scanning Electron Microscopy:

The surface characteristic of prepared crystal was studied by SEM (ZEISS Electron Microscope, EVO MA 15). Powder samples was mounted onto aluminum stub using double sided adhesive tape and sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode.

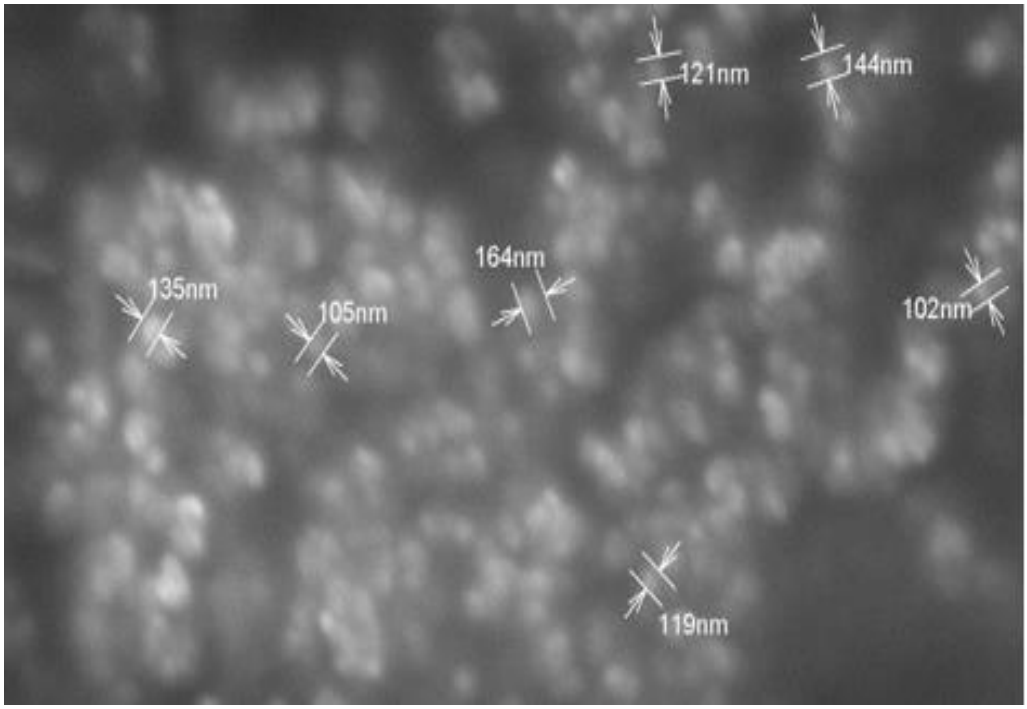

Fig-4: SEM Analysis of SLNs

Determination of Zeta potential:

Zeta potential is a measure of charge present on the vesicle surface. It was determined by using phase analysis light scattering with Malvern zetasizer at field strength of 20V/cm in distilled water and based on electrophoretic mobility of charged particles present in the nanocarriers. Charged particles were attracted to the electrode with the opposite charge when an electric field is applied.

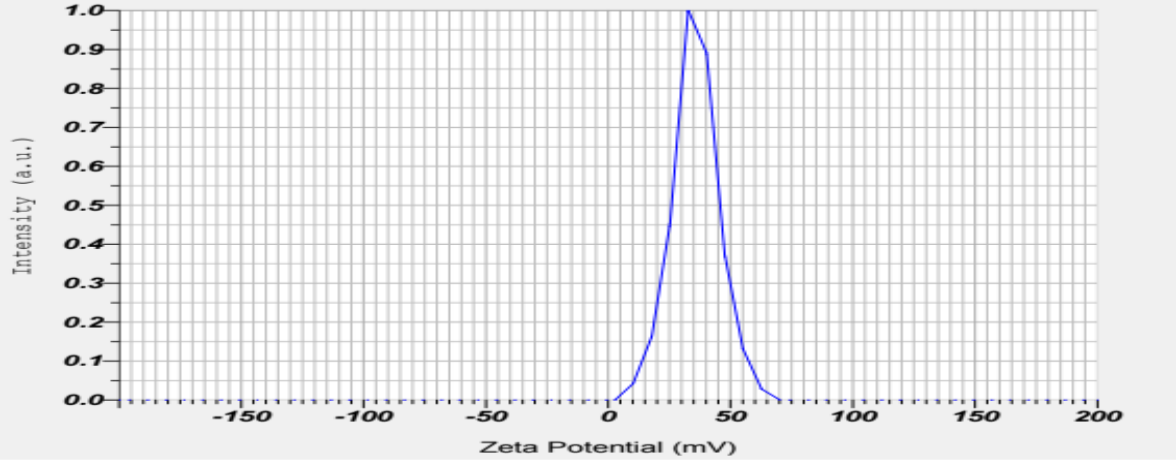

Fig-5: Zeta potential of optimized formulation

Zeta potential

The addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized Artemisia arborescens nanoparticles formulation was measured and found to -32 mv. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable.

Particle size

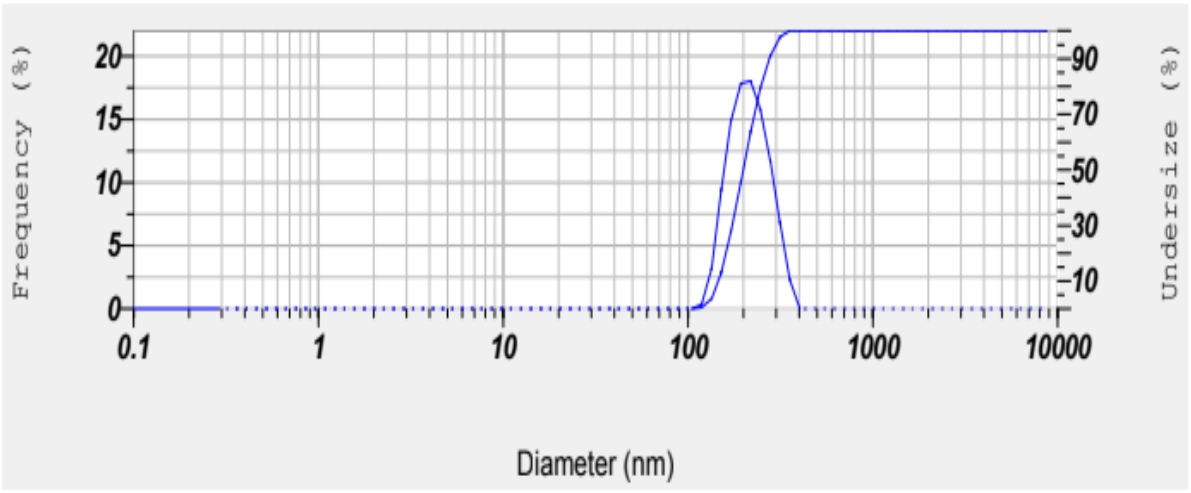

Fig-6: Particle size of optimized formulations

Characterization of Solid lipid nanoparticles of Artemisia arborescens

Table-8: Evaluation Studies of SLN particle size

F. no	Particle size (nm)	Entrapment Efficiency (%)	Zeta Potential(mV)
F1	121	75.63	-28
F2	144	79.81	-25
F3	135	81.24	-32
F4	164	78.68	-36

Entrapment efficiency

The drug entrapment efficiency of all 4 formulations was evaluated. From the F3 formulation showed maximum drug entrapment efficiency 81.24% compared to other formulations. The zeta potential or the change on the surface of colloidal particles in

Artemisia arborescens solid lipid nanoparticles was measured by electrophoretic light scattering mode using zeta sizer nano ZS. The particle charge of Artemisia arborescens SLNs were quantified at 25º C. The samples were diluted approximately with the deionized water for the measurements of particle size.

In vitro drug release studies

Table-9: In vitro drug release studies of all formulations

Time (hrs)	F1	F2	F3	F4
0	14.69	13.67	12.69	14.27
1	26.38	25.69	27.14	25.14
2	32.49	33.47	35.58	34.93
3	43.97	45.69	46.91	42.25
4	55.69	56.74	57.84	57.84
5	64.58	66.98	67.89	69.82
6	70.20	72.39	73.65	71.54
7	83.49	84.67	85.91	81.35
8	92.67	93.58	95.74	94.51

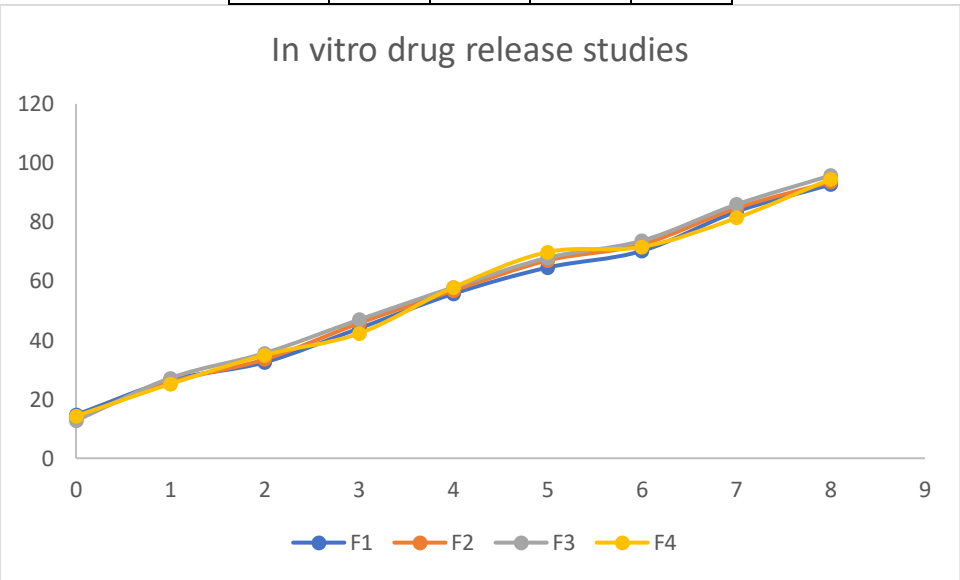

Fig-7: In vitro drug release studies of (F1-F4) formulations

The drug release studies of all formulations of Artemisia arborescensSLNs were conducted by means of diffusion apparatus for a time period of 8 hrs. From the drug release studies as depicted in Figure, the results showed that F-3 formulation showed maximum drug release rate of 95.74 % within 8 hrs.

Stability studies

There was no significant change in physical and chemical properties of the solid lipid nanoparticles of formulation F-3 after 90 days. Parameters quantified at various time intervals were shown.

Table-10: Stability studies of all formulations

F.no	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-3	25^0C/60%RH % Release	95.74	94.85	93.68	92.25	Not less than 85 %
F-3	30^0C/75% RH % Release	95.74	94.17	93.57	92.17	Not less than 85 %
F-3	40^0C/75% RH % Release	95.74	94.27	93.46	92.06	Not less than 85 %

CONCLUSION:

The present study successfully formulated and evaluated Artemisia arborescens-loaded Solid Lipid Nanoparticles (SLNs). FTIR studies confirmed drug–excipient compatibility, while SEM analysis revealed spherical nanoparticles with good stability. Among all formulations, F3 was identified as the optimized formulation, exhibiting a particle size of 135 nm, entrapment efficiency of 81.24%, and maximum cumulative drug release of 95.74% over 8 hours. Stability studies demonstrated that the optimized formulation remained stable for three months without significant changes in its properties. These findings indicate that the optimized SLN formulation is a promising carrier for sustained drug delivery and warrants further in vivo investigation.

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