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Development and Optimization of Artemisia Arborescens Extract Loaded Solid Lipid Nanoparticles

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Abstract
The aim of this study was to investigate the effectiveness of a strategy based on the development of solid lipid Nanoparticles as an innovative formulation of Artemisia arborescens with improved therapeutic efficacy. Artemisia arborescens SLNs were prepared by Solvent evaporation method using Phosphotidyl choline as lipid and Tween 80 as surfactant. The SLN formulation particle size was determined. The change of particle charge was studied by zeta potential (ZP) measurements. *In vitro* release studies of the essential oil were performed at 35°C. Data showed a high physical stability for both formulations at various storage temperatures during 2 months of investigation. The solubility of drug in different solid lipids was measured. FTIR studies indicated no interaction between drug and lipid. SLN were characterized for particle size, zeta potential, entrapment efficiency and surface morphology. *In vitro* drug release studies were performed in phosphate buffer of pH 7.4 using dialysis bag diffusion technique. The F6 formulation had shown maximum entrapment up to 90.12 % and sustained drug release for 8 h. The scanning electron microscopy and zeta potential study showed formation of good SLN dispersion. The stability study showed successful formation of stable SLNs.

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Keywords: Artemisia Arborescens, Solid Lipid Nano Particles, Solvent Evaporation Technique, FTIR, *In-vitro* Drug Release

Introduction

Solid lipid nanoparticles (SLNs) have emerged as a promising nanocarrier system to overcome these limitations associated with conventional herbal formulations. SLNs are submicron-sized colloidal carriers composed of physiologically acceptable solid lipids stabilized by surfactants. Artemisia arborescens, a medicinal plant belonging to the family Asteraceae, has been traditionally used for the treatment of infectious, inflammatory, and gastrointestinal disorders. Phytochemical investigations of A. arborescens have revealed the presence of biologically active constituents such as flavonoids, terpenoids, sesquiterpene lactones, and essential oils, which are responsible for its reported antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. However, the therapeutic application of Artemisia arborescens extract is often limited by poor aqueous solubility, chemical instability, rapid metabolism, and low bioavailability of its active phytoconstituents. Encapsulation of Artemisia arborescens extract into solid lipid nanoparticles offers a strategic approach to improve its solubility, stability, and therapeutic efficacy. Optimization of formulation variables such as lipid type and concentration, surfactant system, homogenization conditions, and drug-to-lipid ratio plays a critical role in achieving SLNs with desirable physicochemical characteristics, including small particle size, narrow size distribution, high encapsulation efficiency, and adequate physical stability. Therefore, the present research focuses on the development and optimization of Artemisia arborescens extract-loaded solid lipid nanoparticles using appropriate formulation and process parameters. The optimized SLNs are systematically characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, in-vitro release behaviour, and stability.

This study aims to establish an efficient nanocarrier system that can enhance the therapeutic potential of Artemisia arborescens extract and support its future application in pharmaceutical and biomedical fields.

Materials

Artemisia arborescens were collected from the Tirupati. HPMC Ethyl cellulose was obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

Methodology

Fourier transform infrared spectroscopy

Fourier transformed infrared spectroscopy (FTIR) analysis used for identifying the functional groups with their means of attachment thus helps assess the drug excipients interaction in terms of polymerization, cross-linking as well as drug loading in the formulation. FTIR was carried out to evaluate the interaction of excipients with the drug. For pure powdered drug KBR pellet method was used and for physical mixture, polished sodium chloride salt plates were used to check the interaction between the components of the formulation. [7]

Extraction process

Soxhlet Extraction [8]

1. Use dried, powdered Artemisia arborescens leaves (250 g).
2. Load the powder into a thimble and place it in the Soxhlet extractor.
3. Use ethanol (95%) solvent as the extracting solvent.
4. Allow extraction to proceed for 6–8 hours or until the solvent in the siphon becomes colorless.
5. Collect the extract and evaporate the solvent using a rotary evaporator.
6. Dry and store the extract in an amber vial under refrigeration (4–8°C).

Fig 1: Extraction process

Fig 2: Extract

Method of preparation of Artemisia arborescens loaded SLN:

Artemisia arborescens extract loaded SLN were prepared by solvent emulsification/evaporation method. The composition of all the formulations 5 mg of extract was dissolved in 10 ml methanol, and Phosphatidylcholine was dissolved in 20 ml chloroform separately; extract and lipid solutions were mixed together. The organic solvent mixture was completely evaporated at 70°C using rotary evaporator to remove of the organic solvent. Drug embedded lipid layer was then poured into 100 ml of aqueous solution containing Tween 80 surfactant and the mixture was Sonicated for 15 minutes by using Sonicator followed by homogenized for 15 minutes at different homogenization speed using high speed homogenizer. The suspension was then allowed to cool at room temperature. The suspension was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and nanoparticles was collected. [9]

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

Ingredients	F1	F2	F3	F4	F5	F6
Artemisia arborescens (mg)	5	5	5	5	5	5
Phosphatidylcholine (mg)	50	100	150	200	250	300
Tween 80 (w/v)	5	5	5	5	5	5
Methanol (ml)	10	10	10	10	10	10
Chloroform(ml)	20	20	20	20	20	20

Evaluation of Artemisia arborescens extract 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. [10]

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 SLNPs was determined by scanning electron microscopy (SEM) at 15 kV and 750 mA. [11]

Drug Encapsulation Efficiency: Lyophilized nanoparticles 50mg were dissolved in 100ml of 7.4 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. [12]

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

***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^{0}C. 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. [13]

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

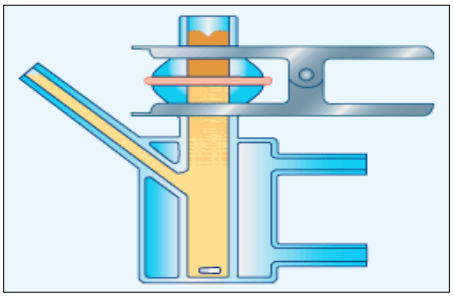

Fig 3: Franz diffusion cell

Stability studies: [14]

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^{0}C/60% RH analyzed every month for period of three months.
2. 30^{0}C/75% RH analyzed every month for period of three months.
3. 40^{0}C/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.

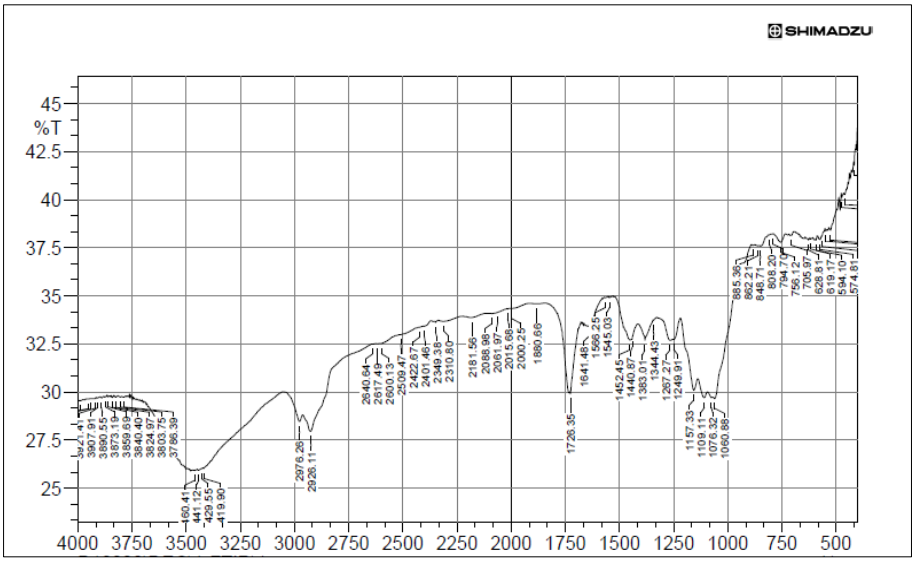

Fig 4: FTIR spectra of Artemisia arborescent

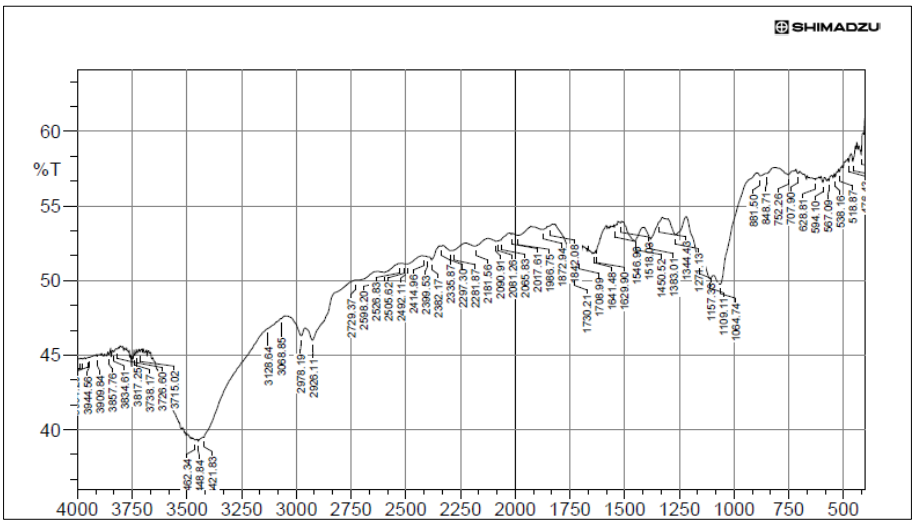

Fig 5: FTIR Spectra of physical mixture of drug and excipients

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 was 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 aluminium 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.

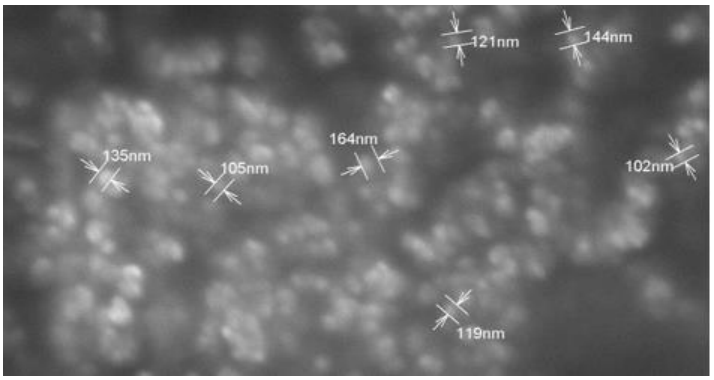

Fig 6: SEM Analysis of nanoparticles

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 nanocarriersystem. Charged particles were attracted to the electrode with the opposite charge when an electric field is applied.

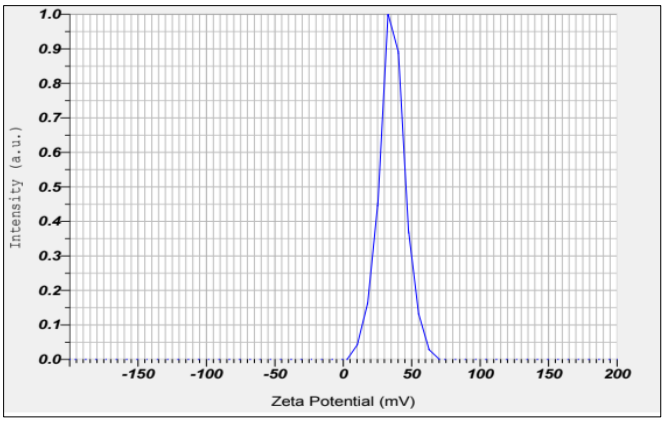

Fig 7: Zeta potential of optimized formulation

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 -28 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

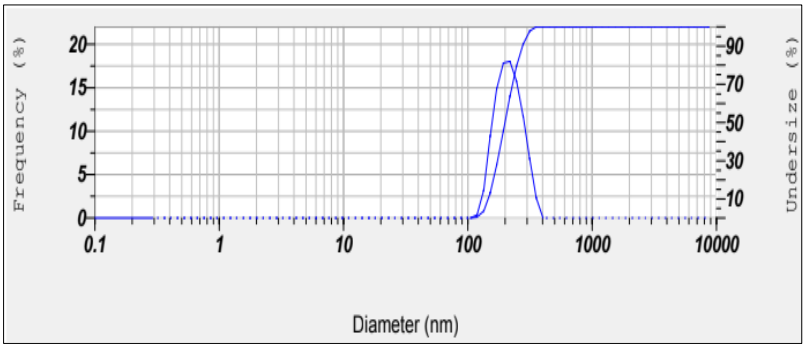

Fig 8: Particle size of optimized formulations

In general, particle size was with a diameter of < 164 nm. The surfaces of the nanoparticles were smooth.

Characterization of Solid lipid nanoparticles of Artemisia arborescens

Table 2: Evaluation Studies of SLN particle size

F. no	Particle size (nm)	Entrapment Efficiency (%)	Zeta Potential(mV)
F1	172	85.10	-27
F2	160	84.52	-25
F3	128	86.50	-22
F4	137	88.18	-30
F5	156	85.67	-27
F6	164	90.12	-28

Entrapment efficiency

The drug entrapment efficiency of all 6 formulations was evaluated. From the F6 formulation showed maximum drug entrapment efficiency 90.12 % 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 zetasizer nano ZS. The particle charge of Artemisia arborescent SLNs were quantified at 25^{o} C. The samples were diluted approximately with the deionized water for the measurements of particle size.

***In vitro* Drug Release Studies**

Table 3: *In vitro* drug release studies of all formulations

Time (hrs)	F1	F2	F3	F4	F5	F6
0	24.69	23.67	22.69	24.27	26.69	24.27
1	36.38	35.69	37.14	35.14	37.14	35.14
2	42.49	43.47	45.58	44.93	47.58	44.93
3	53.97	55.69	56.91	52.25	56.91	52.25
4	65.69	66.74	67.84	67.84	67.84	67.84
5	74.58	76.98	77.89	79.82	77.89	79.82
6	80.20	82.39	83.65	81.54	85.65	81.54
7	93.49	94.67	95.91	93.35	96.90	93.35

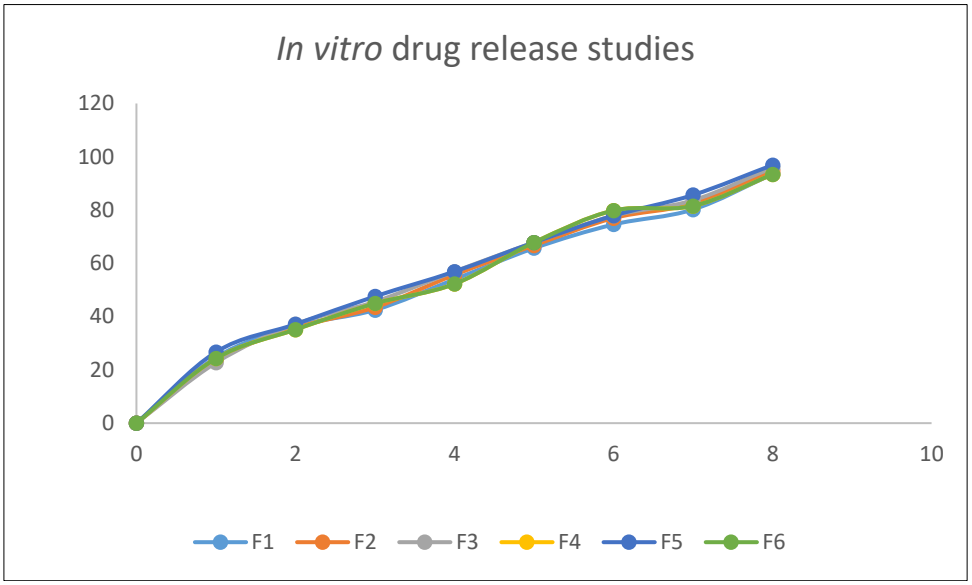

Fig 9: *In vitro* drug release studies of (F1-F6) formulations

The drug release studies of all formulations of Artemisia arborescens SLNs 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 3 formulation showed maximum drug release rate of 96.90 % within 8 hrs.

Stability Studies

There was no significant change in physical and chemical properties of the solid lipid nanoparticles of formulation F-6 after 90 days. Parameters quantified at various time intervals were shown.

Table 4: Stability studies of all formulations

F.no	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-6	25^0C/60%RH % Release	96.30	95.55	94.58	93.68	Not less than 85 %
F-6	30^0C/75% RH % Release	96.30	95.26	94.30	93.53	Not less than 85 %
F-6	40^0C/75% RH % Release	96.30	95.18	94.16	93.18	Not less than 85 %

Conclusion

The current study suggested a unique Artemisia arborescens solid lipid nanoparticle formulation for regulated release. A drug encapsulation effectiveness of up to 90.12 % has been attained in this study. Artemisia arborescens solid lipid nanoparticles containing soy lecithin were created using the Solvent evaporation method, then the particle size was decreased by sonication formulation using solid lipid nanoparticles performed well in terms of medication content and encapsulation effectiveness. This shows that the formulation procedure was suitable and reproducible in nature, and it provided a good yield. The formulation with the best encapsulation efficiency was (F-6). It was discovered that the percentage of encapsulation efficiency along with the soy lecithin concentration. According to the method described, permeation studies with dialysis membrane were conducted. The *in vitro* drug release profiles of all the formulations indicated an initial burst effect, followed by a gradual drug release. The formulations demonstrated good drug release from the lipid. These solid lipid nanoparticles contained more Artemisia arborescens and released it more quickly.

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