

Formulation And Evaluation of Neem Extract Loaded Phytosomes for Antimicrobial Activity

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Abstract—Neem (*Azadirachta indica*) is a well-known medicinal plant possessing antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. However, the therapeutic effectiveness of neem extract is often limited due to poor stability and low bioavailability of its active phytoconstituents. The present study was aimed at the formulation and evaluation of Neem extract-loaded phytosomes to enhance the stability, entrapment efficiency, and controlled release of bioactive compounds. Neem extract-loaded phytosomes were prepared by the solvent evaporation method using soya lecithin and cholesterol as lipid carriers. The prepared formulations were evaluated for drug-excipient compatibility, particle size, zeta potential, entrapment efficiency, surface morphology, and in vitro drug release. FTIR studies confirmed the compatibility between Neem extract and formulation excipients without any significant chemical interaction.

SEM analysis revealed spherical phytosomal vesicles with uniform distribution and smooth surface morphology. The particle size of the formulations ranged from 244 to 295 nm, while zeta potential values indicated good physical stability of the phytosomal system. Entrapment efficiency was found to be in the range of 77.50% to 85.26%, with formulation F3 exhibiting the highest entrapment efficiency (85.26%). In vitro drug release studies demonstrated a sustained release profile over 8 hours, with the optimized formulation F3 showing a maximum cumulative drug release of 98.88%. The results confirmed the successful development of stable Neem extract-loaded phytosomes with improved entrapment efficiency and controlled release characteristics.

The optimized formulation exhibited superior physicochemical properties and may serve as a promising phytosomal delivery system for enhancing the therapeutic potential of Neem extract.

Index Terms—Neem extract, Phytosomes, Soya lecithin, Cholesterol, Entrapment efficiency, Controlled release, Drug delivery system.

I. INTRODUCTION

Phytosomes

Phytosomes are cell like structure Phytosomes means plant while some means cell like. Phytosomes contains the bioactive phytoconstituents of herb extracts surrounded and bound by lipid. Phytosomes are developed by incorporating standardized plant extract or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes called phytosomes and so vastly improve their absorption and bioavailability. Phytosomes are better able to transform from a hydrophilic environment into the lipid friendly environment of the enterocyte cell membrane and from there into the cell finally reaching the blood.

Phytosome have improve pharmacokinetic and pharmacological parameter which in result can advantageously be used in treatment of acute and chronic liver disease of toxic metabolic or infective origin (The Phytosome process has been applied to many popular herbal extracts including Ginkgo biloba, grape seed, hawthorn, milk thistle, green tea, and ginseng.

Phytosomes Plants are referred to as “phyto” and cells are referred to as “some”. A phytosome is a vesicular drug delivery mechanism in which lipid surrounds and binds phytoconstituents of herbal extract. When compared to ordinary herbal extract, phytosomes exhibit greater absorption, better bioavailability and

improved pharmacological and pharmacokinetic parameters because phospholipid shield vital herbal extract components from being destroyed by digestive secretions and gut bacteria. Physical size, membrane permeability, the amount of solutes that are trapped inside and chemical composition all have an impact on how phytosomes behave in physical and biological system.

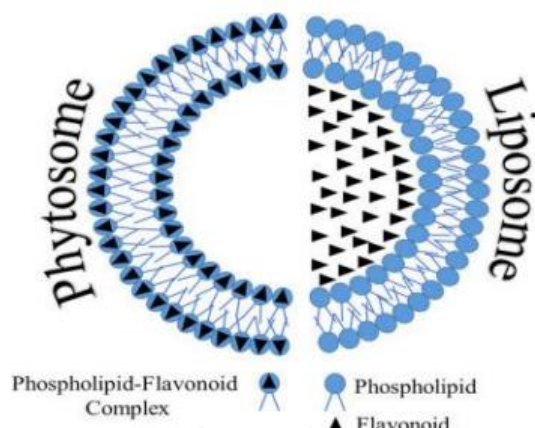


Fig-1: Structure of Phytosomes

MATERIALS

Azadirachta indica was procured from Synpharma Research Labs, Hyd. Soya lecithin, Dichloromethane, Ethanol was obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

II. METHODOLOGY

FTIR Studies

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of extract, polymer and physical mixtures of samples were weighed and mixed properly to a uniform mixture. A small quantity of the powder was compressed into a thin semi-transparent pellet by applying pressure. The IR spectrum of the pellet from 4000-400cm⁻¹ was recorded taking air as the reference and compared to study any interference.

Preparation of Neem leaf for extraction

Fresh leaves of *Azadirachta indica* (Neem) were collected from healthy neem trees. The collected plant material was authenticated by a qualified botanist, and a voucher specimen was preserved for future reference. The collected leaves were washed thoroughly with distilled water to remove dust and

extraneous matter. The leaves were shade-dried at room temperature (25–30°C) for 7–10 days until a constant weight was obtained. Shade drying was preferred to prevent degradation of heat-sensitive phytoconstituents. The dried leaves were then pulverized using a mechanical grinder and passed through sieve No. 40 to obtain a uniform coarse powder.

Extraction of dried powdered Neem leaf extract:

About 100 g of powdered neem leaves were packed into a Soxhlet apparatus and extracted with 70% ethanol as the solvent. The extraction was continued for 6–8 hours until the siphon tube solvent became colorless, indicating complete extraction of phytoconstituents. The obtained extract was filtered through Whatman No.1 filter paper to remove insoluble matter. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at 40–45°C to remove the solvent. The concentrated extract was further dried in a desiccator to obtain a semisolid mass. The dried extract was weighed to determine percentage yield and stored in an airtight amber-colored container at 4°C until further use in phytosome formulation.

Percentage yield of the extract was calculated using the following equation:

$$\text{Yield (\%)} = \left(\frac{\text{Weight of dried extract}}{\text{Weight of powdered neem leaves}} \right) \times 100$$

III. FORMULATION DEVELOPMENT

Table-1: Formulation development of phytosomes

Ingredients	F1 1:1	F2 1:2	F3 1:3	F4 1:4
Extract (mg)	100	100	100	100
Soya lecithin(mg)	100	200	300	400
Cholesterol (mg)	25	25	25	25
Dichloromethane(ml)	10	10	10	10
Ethanol(ml)	10	10	10	10

Preparation of phytosomes

Dissolve the weighed amount of phosphatidylcholine in Dichloromethane in a round-bottom flask. Heat gently (30–40 °C) if needed to solubilize. Dissolve the calculated amount of neem extract in ethanol and add to the PC solution. For improved complexation, add the extract slowly while stirring. Optionally add a small amount of cholesterol to improve membrane

rigidity. Attach the flask to a rotary evaporator and evaporate the solvent under reduced pressure at 40–45 °C to form a thin lipid film on the flask wall. Keep the flask under vacuum for 30–60 min to remove residual solvent. Hydrate the thin film with phosphate buffer pH 7.4 at room temperature with gentle rotation to yield a suspension. Sonicate the suspension (probe sonicator) in an ice bath for 5–10 min (pulse mode) to reduce particle size and obtain uniform vesicles. Optionally extrude through polycarbonate membranes for size control. Store the phytosomal suspension at 4 °C for further characterization.

IV. CHARACTERIZATION

Particle Size

The particle size of the phytosomes were determined using Particle Size Analyzer (PSA) with the dynamic light scattering (DLS) method. The measurements were performed using Horiba Scientific SZ-100, with the sample diluted 10 times in aqueous medium at room temperature

Zeta-potential:

The sample was diluted with distilled water (1:100 (V/V)) and zeta potential was determined using Malvern zeta sizer (Nano ZS, Malvern Instruments, United Kingdom). Measurement was based on the electrophoretic mobility of the particles, which was converted to the zeta potential by inbuilt software based on the Helmholtz-Smoluchowski equation.

SEM analysis

The shape, surface characteristics, and size of the phytosomes were observed by scanning electron microscopy. Once again, 0.2 g of the phytosomes in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The phytosomes were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage.

Entrapment Efficiency

Centrifuge the phytosomal suspension at 15,000 rpm for 30 min. Determine free drug in the supernatant spectrophotometrically.

In vitro diffusion profile

In vitro release study of the formulated phytosomes was carried out by using diffusion cell through membrane as a dialysis membrane. Diffusion cell with inner diameter 24mm was used for the study. 1 mL formulation was placed in donor compartment and freshly prepared 7.4 phosphate buffer was placed in receptor compartment. Dialysis membrane was mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that the membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. 1mL of sample was withdrawn from receiver compartment after 12 hrs and same volume of fresh medium was replaced. The withdrawn samples were diluted to 10mL in a volumetric flask with distilled water and analysed by UV spectrophotometer.

Stability studies:

To define an appropriate shelf life and suggested storage conditions, stability studies are conducted to ascertain the quality variations of a medicinal substance or medication product over time in response to environmental elements including light, humidity, and temperature. Preparation of a stability protocol outlining the quantity of batches, type of packaging, storage conditions, testing intervals, and acceptability criteria is the first step in the study, as per ICH Q1A(R2) recommendations. The batches that will be sold or used as a pilot are sealed in the designated commercial container and kept in stability chambers under specific conditions. These conditions can be long-term ($25 \pm 2^{\circ}\text{C} / 60 \pm 5\% \text{RH}$ for 12-24 months), accelerated ($40 \pm 2^{\circ}\text{C} / 75 \pm 5\% \text{RH}$ for 6 months), or intermediate ($30 \pm 2^{\circ}\text{C} / 65 \pm 5\% \text{RH}$) in nature.

V. FTIR Studies

Compatibility study (IR spectroscopy)

FTIR analysis was performed in order to study the compatibility of ingredients used in the preparation of nanoparticles, using a Shimadzu FTIR spectrophotometer (Prestige21, Shimadzu Corporation, Kyoto, Japan). Neem leaf extract and Excipients their mixture with ratio (1:1) was evaluated using FTIR spectrophotometer using potassium bromide disc technique where 1mg of the sample is

mixed with 100 mg of dry powdered KBr, the mixture is pressed into a transparent disc and was inserted in the apparatus for IR scan.

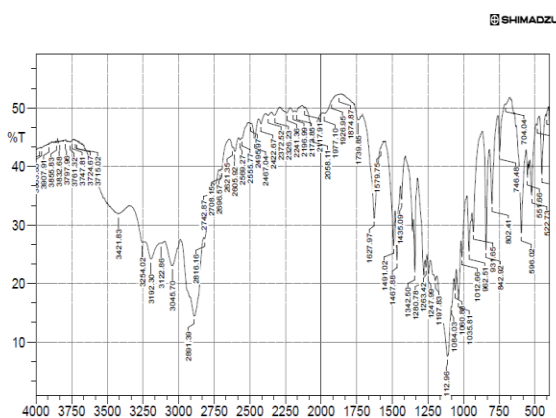


Fig-2: FT-IR Sample for Neem leaf extract

Table-2: Characteristic Peaks and frequency of Neem leaf extract

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3050-3500	3449.83
2	C-H stretching	3000-2840	2829.50
3	C= C stretching	1650-1600	1637.39
4	N-H stretching	1392-1366	1362.96

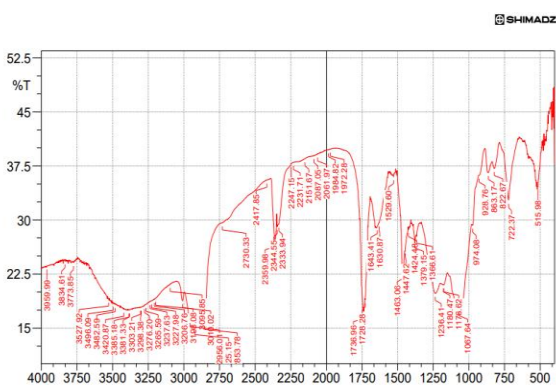


Fig-3: FT-IR Sample for Optimized formulation

Table-3: Characteristic Peaks and frequency of Optimized formulation

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	4000-3500	3834.61
2	OH Bending	3500-2750	3227.98
3	C-H stretching	1750-1250	1463.06
4	C-N stretching	1250-1000	1180.47

Determination of Vesicle morphology and Size

The morphological characteristics of formulated phytosomes were carried by using Scanning electron microscopy (SEM). A small drop of Phytosomal suspension was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. SEM images revealed spherical vesicles with smooth surfaces, confirming successful formation of phytosomes.

Particle size Analysis

The particle size ranged from 244–295 nm. Formulation F3 showed the smallest particle size (244 nm), indicating better vesicle formation. Zeta potential values ranged from -20 to -25 mV, suggesting acceptable physical stability of the phytosomal system.

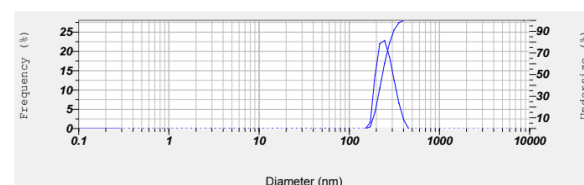


Fig-4: Particle size Analysis of Phytosomes

SEM Analysis

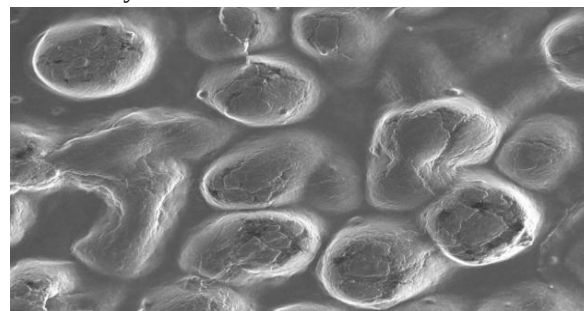


Fig-5: SEM analysis of phytosomes

Zeta potential

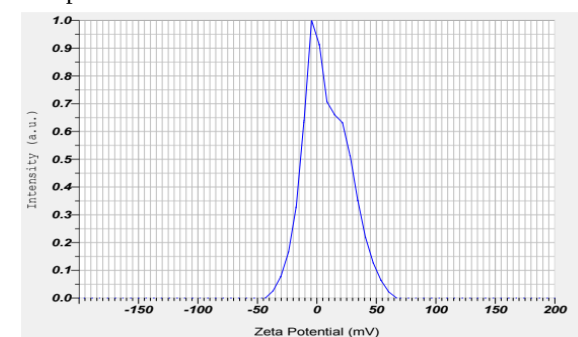


Fig-6: Zeta potential of Phytosomes

Table-4: Evaluation Studies of particle size and Zeta potential of Phytosomes

F. No	Particle size (nm)	Zeta potential(mV)
F1	259	-20
F2	262	-23
F3	244	-25
F4	295	-25

VI. EVALUATION PARAMETERS:

Entrapment Efficiency:

Table-5: Drug entrapment efficiency

F. code	Drug entrapment efficiency
F1	78.96
F2	77.50
F3	85.26
F4	81.36

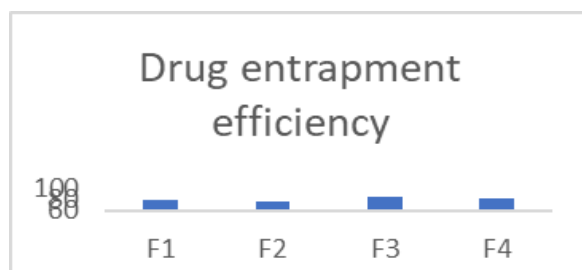


Fig-7: Drug entrapment efficiency of all formulation

Entrapment efficiency ranged from 77.50% to 85.26%. Formulation F3 exhibited the highest entrapment efficiency (85.26%), indicating efficient incorporation of Neem extract into the phospholipid matrix.

In vitro release study:

Phosphate buffer pH 7.4 was used as medium for the release studies and good linearity was observed in the plotted standard graph with a correlation coefficient of 0.998.

The drug release profiles of Phytosomes containing different ratios of synthetic polymer. The drug release profile was influenced by the phospholipid concentration used in phytosome preparation.

Table-6: In vitro drug release profiles of Phytosomes (F1-F4)

Time (hr)	F ₁	F ₂	F ₃	F ₄
0	0	0	0	0
1	16.95	17.59	20.10	15.37
2	27.82	28.10	28.15	25.38
3	37.89	38.96	37.48	38.10
4	46.98	48.82	53.69	48.19
5	65.82	66.37	67.82	67.85
6	76.28	77.81	79.52	73.25
7	82.30	86.39	89.35	84.22
8	92.35	93.26	98.88	94.28

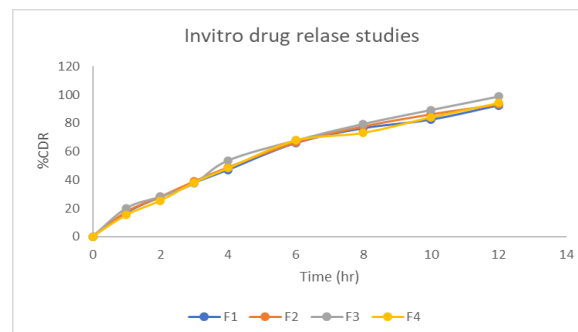


Fig-8: In vitro drug release studies of F1-F4 formulations

Formulation F3 exhibited maximum cumulative drug release of 98.88% at 12 h and was considered the optimized formulation.

Stability studies

There was no significant change in the physical appearance and drug release characteristics of the optimized phytosomal formulation (F3) during the 90-day stability study.

Table-7: Stability studies of all formulations

F.no	Parameters	Initial	1 st Month	2 nd Month	3 rd Month	Limits as per Specifications
F-3	25°C/60%RH % Release	98.88	95.55	94.58	93.68	Not less than 85 %
F-3	30°C/75% RH % Release	98.88	95.26	94.30	93.53	Not less than 85 %
F-3	40°C/75% RH % Release	98.88	95.18	94.16	93.18	Not less than 85 %

VII. CONCLUSION

The present study was carried out to formulate and evaluate Neem extract loaded phytosomes for improving the stability, entrapment efficiency, and controlled release of bioactive phytoconstituents present in neem extract. Phytosomes were successfully prepared by the solvent evaporation method using soya lecithin and cholesterol as lipid carriers. FTIR studies confirmed the presence of major functional groups associated with phytoconstituents such as flavonoids and polyphenols. The FTIR spectrum of the optimized formulation showed all characteristic peaks of the extract with only minor shifts, indicating compatibility between Neem extract and formulation excipients without any significant chemical interaction. The phytosomal formulations were successfully developed and evaluated for particle size, zeta potential, entrapment efficiency, and in vitro drug release. SEM analysis revealed that the prepared phytosomes were spherical in shape with smooth surface morphology and uniform distribution. Particle size analysis demonstrated that the formulated phytosomes were in the nanometric range (244–295 nm), which is favorable for enhanced permeation and improved therapeutic performance. Among all formulations, F3 was found to be the optimized formulation, exhibiting the smallest particle size of 244 nm and a zeta potential of –25 mV, indicating good physical stability and reduced aggregation tendency. The negative zeta potential values observed for all formulations confirmed adequate electrostatic stabilization of the vesicular system. Entrapment efficiency studies showed values ranging from 77.50% to 85.26%, indicating efficient incorporation of Neem extract within the phytosomal vesicles. Formulation F3 exhibited the highest entrapment efficiency of 85.26%, suggesting optimum lipid composition and enhanced phytoconstituent loading capacity. In vitro drug release studies carried out using phosphate buffer pH 7.4 demonstrated a sustained release pattern over a period of 8 hours. The cumulative percentage drug release of all formulations ranged from 92.35% to 98.88%. The optimized formulation F3 showed the highest drug release of 98.88% at the end of 8 hours, indicating efficient release of the encapsulated phytoconstituents from the phospholipid matrix. The sustained release behavior may contribute to prolonged antimicrobial action and improved

therapeutic efficacy. The results obtained from particle size analysis, zeta potential determination, entrapment efficiency studies, SEM analysis, FTIR studies, and in vitro drug release studies collectively indicate successful formation of stable phytosomal vesicles containing Neem extract. The optimized formulation demonstrated superior physicochemical characteristics and controlled release behavior compared to the other formulations.

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