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FORMULATION AND EVALUATION OF GINKGO BILOBA EXTRACT LOADED PHYTOSOMES FOR NEUROPROTECTIVE ACTIVITY

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Abstract:

Ginkgo biloba is a well-known medicinal plant possessing antioxidant and neuroprotective properties, but its therapeutic efficacy is limited due to poor bioavailability and low aqueous solubility. The present study was aimed at formulating and evaluating Ginkgo biloba extract loaded phytosomes to enhance its bioavailability and therapeutic efficacy. Phytosomes were prepared by the solvent evaporation method using phosphatidylcholine as the phospholipid carrier for the formation of stable phyto-phospholipid complexes.The prepared formulations were evaluated for particle size, zeta potential, entrapment efficiency, surface morphology, in-vitro drug release, and stability studies. FTIR analysis confirmed the successful formation of phytosomal complexes between the extract and phospholipids. SEM analysis revealed spherical vesicles with smooth surface morphology. Among all formulations, F4 exhibited the smallest particle size of 244 nm and highest entrapment efficiency of 85.26%, indicating improved stability and drug incorporation.In-vitro drug release studies demonstrated sustained release behavior over a period of 8 hours, with maximum drug release of 98.88% observed for formulation F4. Stability studies carried out at different storage conditions showed no significant changes in drug release and formulation characteristics, confirming the stability of the optimized phytosomal formulation. The study concluded that Ginkgo biloba extract loaded phytosomes could serve as a promising delivery system for improving bioavailability, sustained drug release, and neuroprotective activity.

Keywords: *Ginkgo biloba extract, FTIR Studies, Solvent evaporation method, In vitro drug release studies*

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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 hydrophillic enviroment 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.

MATERIALS:

Ginkgo biloba was procured from Synpharma Research Labs, Hyd, Soya lecithin, Dichloromethane, Cholesterol and ethanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

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 400-4000cm^{1} was recorded taking air as the reference and compared to study any interference.

Preparation of Ginkgo biloba extract leaf for extraction

The fresh leaves were cleaned out first with tape water to remove dusts and dirt then washed with distilled water twice. The cleaned leaves were dried in oven at 45 C for 3 to 5 days to get a constant weight dry mass. The dry leaves then powdered using grinding mill to a particle size of about 0.5 mm. The powdered dried leaves then packed in polyethylene bag and stored in sealed container at dark cool room until use.

Extraction of dried powdered Ginkgo biloba extract leaf:

The dried powdered leaves were subjected to extraction using ethanol as solvent by Soxhlet extraction method. About 50 g of powdered material was packed in a Soxhlet apparatus and extracted with 70% ethanol for 6–8 hours until complete extraction was achieved. The obtained extract was filtered and concentrated using a rotary evaporator or water bath at controlled temperature below 50°C to remove the solvent.The concentrated extract was then dried to obtain a semisolid or dry mass and stored in an airtight container under refrigerated conditions until further use for phytosome preparation.

FORMULATION DEVELOPMENT

Table-1: Formulation development of phytosomes

Ingredients	F1	F2	F3	F4
Extract (mg)	100	100	100	100
Soya lecithin(mg)	100	200	300	400
Cholesterol (mg)	50	50	50	50
Dichloromethane(ml)	10	10	10	10
Ethanol(ml)	5	5	5	5

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 Ginkgo biloba 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 (bath sonicator) for 5–10 min to reduce particle size and obtain uniform vesicles. Store the phytosomal suspension at 4 °C for further characterization.

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 zetasizer (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.

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 6.8 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°C ± 0.5°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 phosphate buffer and analysed by UV spectrophotometer.

Stability studies

The main objective of the stability testing is to provide evidence on how the quality of the drug product varies with time under the influence of temperature and humidity. The stability study for the phytosomes formulation was done as per ICH guidelines in a stability chamber for a period of 3 months.

RESULTS & DISCUSSION:

FT-IR Spectrum of Ginko biloba

FT-IR Spectra of Ginko biloba and polymers were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between Ginko biloba and polymers. It also confirmed that the stability of drug during process.

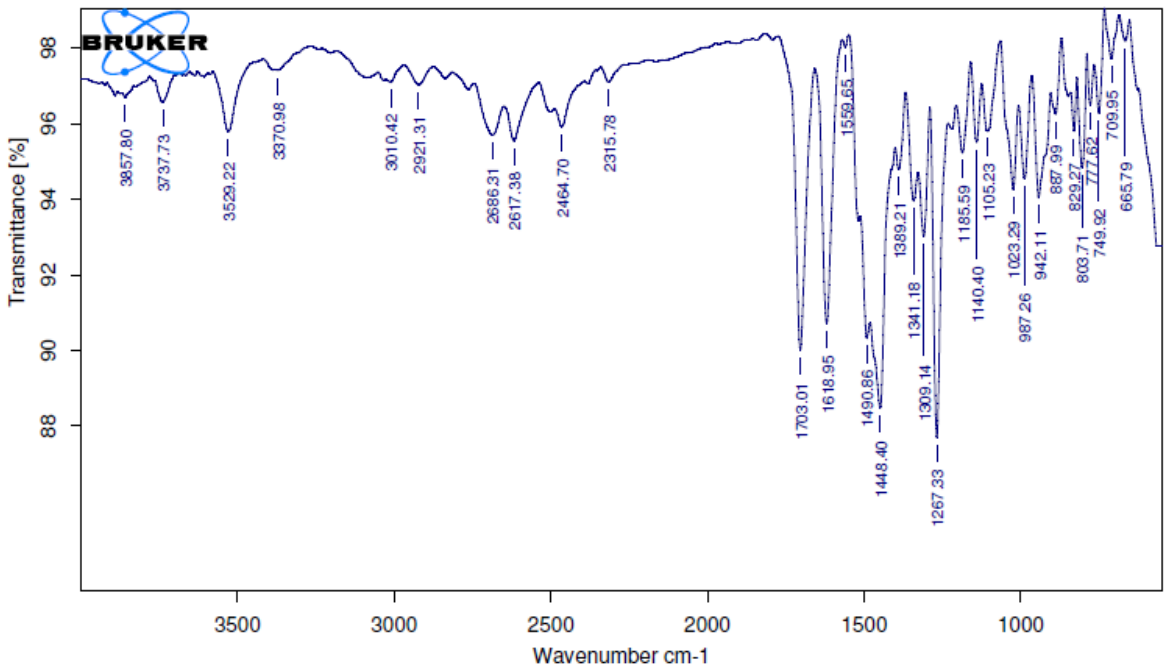

Fig-: 1 FT-IR Sample for Ginko biloba

Table-: 2 Characteristic Peaks and frequency of Ginkobiloba

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3500-3000	3120.19
2	OH Bending	3000-2500	2790.50
3	C-N stretching	1500-1000	1378.45

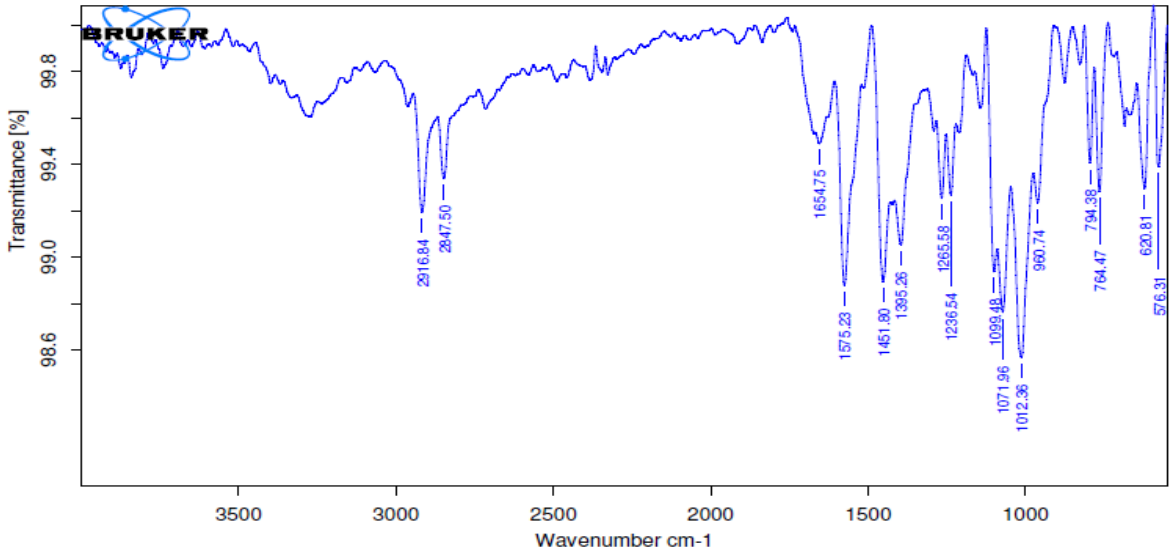

Fig-:2 FT-IR Sample for Optimaized Formulation

Table-:3 Characteristic Peaks and frequency of Optimaized Formulation

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3500-3000	3386.67
2	OH Bending	3000-2500	2891.97
3	C-N stretching	1500-1000	1202.22

Evaluation Parameters:

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. The sample was heated to -85^{0}C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of - 120^{0}c and voltage of 5kV.

Particle size Analysis

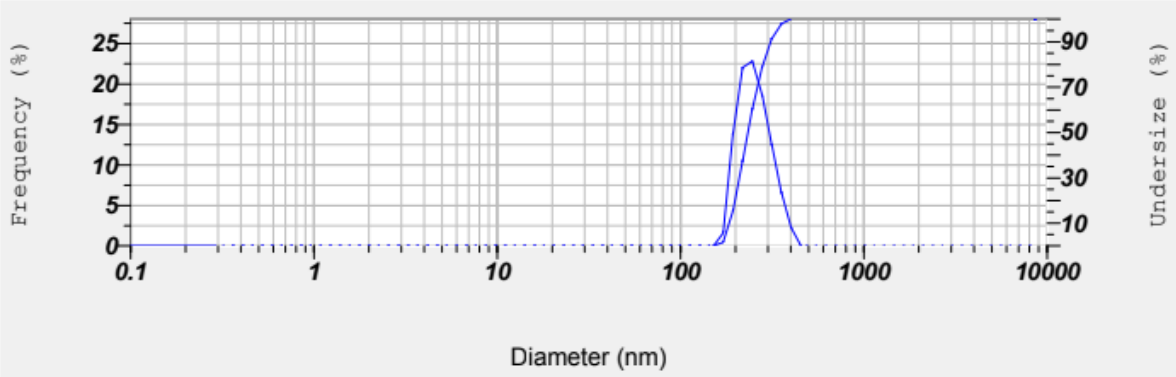

Fig-3: Particle size Analysis of Phytosomes

Discussion: SEM analysis demonstrated spherical vesicles with smooth morphology. The particle size analysis revealed vesicles in the range of 244–278 nm, which is optimal for dermal delivery and ensures uniform drug distribution. Among the formulations, F4 showed the smallest particle size (244 nm), which is expected to favor better permeation and stability.

SEM Analysis

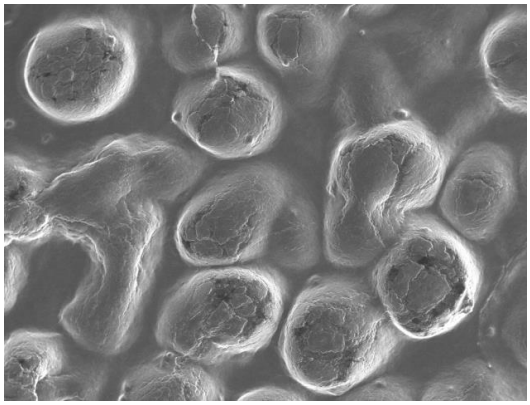

Fig-4: SEM analysis of phytosomes

Zeta potential

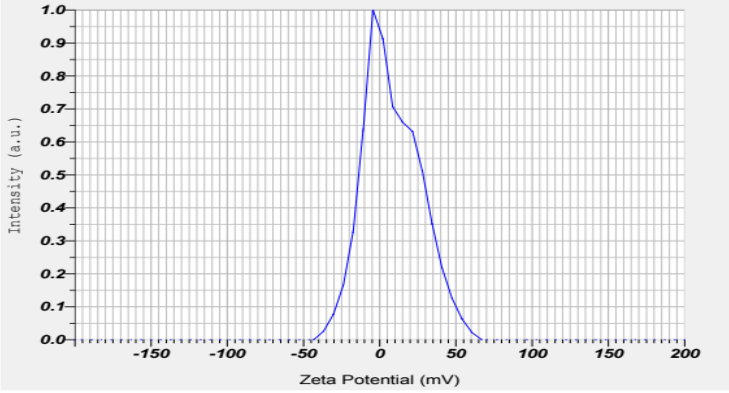

Fig-5: Zeta potential of Phytosomes

Discussion: The zeta potential values ranged between –20 to –29 mV, confirming moderate stability of phytosomal dispersions due to sufficient electrostatic repulsion. F3 (–29 mV) and F4 (–25 mV) showed the most stable vesicles, reducing the chances of aggregation during storage.

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

F. No	Particle size (nm)	Zeta potential(mV)
F1	259	-20
F2	262	-23
F3	278	-29
F4	244	-25

Entrapment Efficiency:

Table-5: Drug entrapment efficiency

F. code	Drug entrapment efficiency
F1	78.96
F2	77.50
F3	80.21
F4	85.26

Drug entrapment efficiency varied between 77.50% to 85.26%, with F4 showing the highest efficiency. Higher entrapment efficiency ensures maximum therapeutic benefit with controlled release.

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. It was cleared from the release profiles of formulations, that the drug release was governed by polymer nature and content.

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	27.82	28.10	24.97	28.15
2	37.89	38.96	35.16	37.48
3	46.98	48.82	49.87	53.69
4	76.28	77.81	75.98	79.52
6	82.30	86.39	82.10	89.35
8	92.35	93.26	96.37	98.88

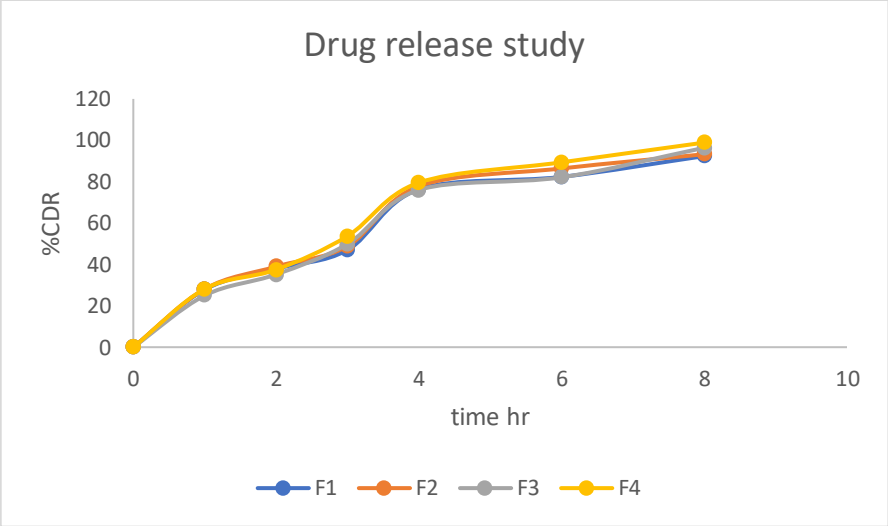

Fig-6: In vitro drug release studies of F1-F4 formulations

Drug release profiles showed sustained release over 8 hours, with maximum release of 98.88% observed for F4. The release was dependent on the polymer composition, with optimized ratios providing controlled and prolonged drug delivery.

Stability Studies :

Table-7: Results of stability studies of optimized formulation F-4

	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	**25^{0}C/60%RH % Release**	98.88	97.85	95.58	94.37	**Not less than 85 %**
F-4	**30^{0}C/75% RH % Release**	98.88	97.52	95.26	94.22	**Not less than 85 %**
F-4	**40^{0}C/75% RH % Release**	98.88	97.43	94.13	94.32	**Not less than 85 %**

CONCLUSION:

The present study successfully formulated and evaluated a phytosomes containing Ginkgo biloba Extract with the aim of enhancing its bioavailability therapeutic efficacy. The phytosomes were prepared using phosphatidylcholine, demonstrating good entrapment efficiency, optimal particle size, and stable zeta potential, indicating a well-established complex between the Ginkgo biloba Extract and phospholipids. The present study focuses on the formulation and evalution of Ginkgo biloba Extract loaded phytosomes to enhance its therapeutic efficacy and bioavailability.Characterization through FTIR confirmed successful phytosome formation. phytosomes of Ginkgo biloba Extract were prepared using the Solvent evaporation method with phosphatidylcholine as a carrier, forming stable Phyto complexes. SEM analysis demonstrated spherical vesicles with smooth morphology. Among the formulations, F4 showed the smallest particle size (244 nm), which is expected to favor better permeation and stability. In vitro drug release studies revealed a sustained release profile. Drug entrapment efficiency varied between 77.50% to 85.26%, with F4 showing the highest efficiency. Higher entrapment efficiency ensures maximum therapeutic benefit with controlled release. Drug release profiles showed sustained release over 8 hours, with maximum release of 98.88% observed for F4. The release was dependent on the polymer composition, with optimized ratios providing controlled and prolonged drug delivery. Stability testing at different storage conditions (25 °C/60% RH, 30 °C/75% RH, and 40 °C/75% RH) over 3 months demonstrated no significant reduction in drug release (remaining above 94%). This indicates that the optimized phytosomes is physically and chemically stable under accelerated storage conditions. Overall, the ginkgo biloba extract phytosomes offers a promising natural formulation for topical application, potentially providing enhanced bioavailability therapeutic efficacy and neuroprotective activity.

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