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Characterization, properties and formulation of Phytosomes

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Abstract
Phytosomes are complex structures formed by the molecular complexation of plant-derived bioactive compounds with phospholipids, primarily phosphatidylcholine. This interaction leads to the formation of a unique complex wherein the phytoconstituents are embedded within the phospholipid bilayer. The resulting phytosomes exhibit improved solubility, stability, and absorption characteristics compared to free phytochemicals. The properties of phytosomes, including particle size, morphology, zeta potential, and encapsulation efficiency, play a crucial role in determining their pharmaceutical attributes. Various analytical techniques such as dynamic light scattering, transmission electron microscopy, Fourier-transform infrared spectroscopy, and differential scanning calorimetry are employed for the characterization of phytosomes. Formulation strategies for phytosomes involve the selection of appropriate phospholipids, optimization of the drug-to-lipid ratio, and incorporation of excipients to enhance stability and efficacy. Phytosomes can be formulated into various dosage forms including oral tablets, capsules, creams, and gels, catering to diverse therapeutic applications. In conclusion, phytosomes represent a promising drug delivery system for enhancing the bioavailability and therapeutic efficacy of plant-derived bioactive compounds. Further research in the characterization, formulation, and evaluation of phytosomes holds significant potential for the development of novel phytopharmaceutical formulations with enhanced therapeutic benefits.

Keywords: Phytosomes, Phyto-consitutents, Dosage, Application, Fourier-transform

Introduction
Research on the innovative drug delivery system has increased over the last few decades, and there has been a lot of focus on developing this system. The two most important criteria for a system to be considered innovative are the medication's delivery rate and how much time passes before the medicine reaches the target location at the required concentration. Unfortunately, the current method of delivering herbal medication to patients is considered antiquated and ineffective, which reduces the drug's effectiveness. Herbal medicine may benefit from the revolutionary drug delivery technology by ameliorating the herb's side effects while enhancing its efficacy. The core concept is on introducing a new way for drugs to be delivered into herbal remedies. Therefore, in order to treat increasingly severe illnesses in the future, it is crucial to include innovative drug delivery systems into Indian Ayurvedic medications.

Most phytomedicines' bioactive components are constituents of plant metabolism; examples include glycosides, terpenoids, flavonoids, and many more. But they aren't very well absorbed when used topically or when swallowed. This happens for two primary reasons: first, these phytoconstituents have a heavy molecular structure that makes simple diffusion impossible to absorb them, and second, the enterocytes' Because of how poorly they dissolve in lipids, lipid-rich tiny intestinal walls prevent them from passing through. Therefore, the transport of an appropriate amount of the active components to the intended place is crucial for the efficacy of any herbal medicine. By increasing the bioavailability of phytoconstituents, Indena's phytosome technology overcomes this obstacle. The Novel Drug Delivery System (NDDS) may decrease administration frequency and peak and valley variations, leading to improved bioavailability. Liposomes, microemulsions, Some of the drug delivery systems include microspheres, polymeric nanoparticles, and solid lipid nanoparticles (SLNs). Delivery vehicles used in phyto-formulation research. These vehicles allow for the solubilization of phytoconstituents and the sustained release of the drug.4 Ginseng (Panax ginseng), ingredients such as milk thistle, grape seed, hawthorn, green tea, and many other common herbal extracts have all been phytosomed. The Phosphatidylcholine may be directly bound to by the flavonoid and terpenoid components found in plant extracts.

Properties of Phytosome

- **Physicochemical Properties**

Natural phospholipids (e.g., soy phospholipids) and other natural substances form phytosomes. The compound is created when a solvent is mixed with standardised herb essence, which acts as a substrate, and stoichiometric quantities of phospholipid. According to the spectroscopic data, the interaction between the phospholipid and the substrate occurs because of polar head groups (such as phosphate or ammonium) create hydrogen bonds with the substrate's polar functionalities. From 50 nm to a few hundred µm, the size of the phytosome varies. Phytosomes undergo a transformation into liposomal-like structures when they meet water, adopting a micellar shape. Phytosomes work by incorporating the active ingredient into the membrane by the anchoring of the principle to the phospholipid polar head. Both the phenolic hydroxyls on flavone and the phosphate ion on phosphatidylcholine group generate hydrogen bonds in the catechin distearoyl phosphatidylcholine complex, for instance. Most phytosome complexes dissolve easily in aprotic solvents, lipids, and hardly no water at all. In addition, it is observed that they are rather unstable when exposed to alcohol. Lipophilic phytoconstituents, such as curcumin, are an exception; they reach their greatest water solubility when complexed with phospholipids.

- **Phytosomes in Female Reproductive System Conditions**

Six endometrial cancer patients took part in an investigation leading to the discovery of effectiveness of curcumin phytosome. During the two weeks of treatment, participants received 2 g (4,500 mg daily) of the supplement in the absence of any concomitant cancer treatment. Supplementation decreased levels of CD8 + T cell ICOS protein, monocyte counts, and MHC expression on leukocytes. The levels of cyclooxygenase-2 protein, the number of different kinds of immune cells, and T cell activation were not significantly changed (COX-2). Second, after 48 rats had their ovaries removed, each os was administered a quercetin phytosome at a dosage of 10 or 50 mg/kg. Serum glutathione, inorganic phosphorus, and calcium levels were all significantly higher after phytosome therapy compared to free quercetin at comparable concentrations. The phytosome enhanced the lipid profile and decreased blood levels of glucose, alkaline phosphatase, TNF-, acid phosphatase, and MDA in comparison to quercetin. There was also an experiment with OVCAR-3 ovarian cancer cells that used a phytosome that contained icariin. There was an increase in intracellular ROS, caspase-3 concentration, and the quantity of G2-M phase cells following incubation with phytosomes, and the cytotoxicity against ovarian cancer cells was greater with phytosomes than between 6.31 to 13.1 M when using pure icariin.

- **In Urinary Tract Dysfunctions**

Two randomised controlled studies investigated the renal system for phytosome biological effects. The primary research looked at the antiadhesive effects of cranberry extract phytosome or a similar standardised extract on urine adhesions caused by Candida albicans in 13 healthy participants. Participants took two cranberry phytosome or cranberry extract capsules daily for seven days, with urine samples collected at regular intervals. Twelve hours after administration of the phaosomal or extract, the fractions that were extracted had a comparable and significant effect on C. Albicans adherence, while the While the cranberry extract was 36 milligrammes per capsule, phytosome only contained 33 milligrammes (12 milligrammes of proanthocyanidins each capsule).

- **Effect in Wound Healing**

Patients with chronic Foot care for diabetics ulcers responded well to a therapy regimen that included These include contemporary pharmaceuticals, ginkgo biloba, and grape seed phytosome. The NHDF cells were shown to be unaffected by an aqueous Moorings oleifera leaf extract containing phytosomes up to a concentration of a concentration of 3.0 mg/mL. The formulation showed the fastest gap when compared to the extract at the same dosage, at 1 mg/mL. closure rate (94.8% after 24 hours). There were no statistically significant results with either lower or higher dosages (1.25 and 1.50 mg/mL).

- **In Musculoskeletal System**

Clinical trials involving the treatment of osteopenia were piloted. Individuals 24 weeks of treatment with a curcumin phytosome, a supplement made from the spice turmeric, was given to individuals with poor bone density who did not exhibit any other symptoms. At4, At12, and 24 weeks, we measured the density of the first finger, upper jaw, and heel. Bone density improved across the board in the group that took a single daily pill containing 1000 mg of curcumin phytosome, while there was no discernible change in the control group. Positive effects on strength and physical performance were shown in elderly persons (>65 years) with weakness when at the same dose, the same formulation was evaluated both on its own and in combination with other nutritional supplements and physical activity.

- **Symptoms and Their Impact on the Respiratory System**

The usual therapy in those suffering from mild to severe persistent asthma is a combination of beta-agonists and corticosteroids; around 32 individuals with asthma were included in a multicenter study that used this approach. After a four-week period, participants were randomised at random to either get no therapy at all or 500 mg of Boswellia serrata phytosome. Fewer inhalations were necessary for patients in the phytosome group than those in the usual medicine group. Few to mild adverse effects, including nausea and insomnia, were recorded with the phytosome therapy, which was generally well-received. A new phytosome was created by the researchers to increase the lung bioavailability of naringenin. Naringenin was successfully administered via dipalmitoyl phosphatidylcholine (DPPC), one of the primary lipids in pulmonary surfactant. Dry powder inhalation effects and related processes in rats with acute lung injury (ALI) using NPDPIs (10 mg/rat, including about 3 mg naringenin) damage were studied. Research has shown that these phytosomes may prevent lung damage in rats when administered intravenously. Results showed that NPDPIs decreased pulmonary edoemas by decreasing fluid exudation and cytokine production (namely, COX-2 and ICAM-1). In addition, NPDPI usage enhanced this, while Reducing oxidative stress in rats, naringenin and DPPC increased superoxide dismutase (SOD) activity.

Characterization of Phytosomes

The size, membrane permeability, chemical composition, and proportion of entrapped solutes of phytosomes are only a few of the variables that may greatly affect their function in both biological and physical systems. The following are the criteria used to classify phytosomes:

- **Visualization**

One way to see phytosomes is with the use of scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

- **Solubility and partition coefficient**

To characterise physical mixes, active component finding the P-value for the n-octanol/water partition and the solubility of phytophospholipid complexes and active components in organic solvents or water is essential. Phytophospholipid complexes often exhibit greater hydrophilicity and better lipophilicity compared to their active components. In contrast to embelin and its physical mixtures, complexes of embelin are more soluble in n-octanol and water, as stated by Rahila.

- **Particle size and zeta potential**

Complex stability and repeatability are influenced by two important properties: size of particles and zeta potential charts. Complexes of phospholipids typically have particle sizes between fifty nanometers to one hundred millimetres. Anisha Mazumder synthesised sinigrin phytosome complexes with zeta potentials of 10.09 and 0.98 mV and average particle sizes of 153.39 nm.

- **Surface tension activity measurement**

One way to measure a solution's surface activity is using a Du Nouy ring tensiometer medication.

- **H1NMR**

To evaluate to ascertain the progression, NMR spectra are employed of complexes between phosphatidylcholine molecules and active phytoconstituents. Without the characteristic signal shape of individual molecules, the 1H NMR signal in nonpolar fluids undergoes a discernible shift beginning with atoms entangled in complex formation. There is an amplification of electrochemical signals emitted by phytoconstituents. As signal amplifiers, phospholipids are involved, and choline's N-(CH3)3 induces an up field shift in the singlet.

- **Spectroscopic evaluation**

In order to analyse the analogous response by which phytoconstituents interact with the phospholipid component, spectroscopic estimates are often utilised to build a complex between the two.

- **In vitro evaluations**

Their therapeutic activities are predicted by the physiologically energetic phytoconstituents found in phytosomes, which inform their design. You can assess the phytosomes' in vitro anti-hepatotoxic efficacy by looking at their antioxidant and free radical scavenging actions.

- **In vivo evaluations**

The therapeutic activities of the physiologically energetic phytoconstituents in the phytosomes influence their design as well. Unlike alcohol-induced hepatotoxicity, paracetamol, and thioacetamide, in vivo outcomes of organised phytosomes of animals are assessed for anti-hepatotoxic activity.

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM)

As seen in Figure 2, The surface has been examined using scanning electron microscopy (SEM). morphology and solid-state properties of various complexes. A transmission electron microscope (TEM) is a common tool for studying nanomaterials, including their crystallisation, dispersion, and size determination. While scanning electron microscopy (SEM) reveals highly crystalline forms of active compounds, these crystal structures disappear after complexation process. In TEM investigation, When gently shaken and diluted in distilled water, phyto-phospholipid complexes exhibit vesicle-like structures (Fig. 3).

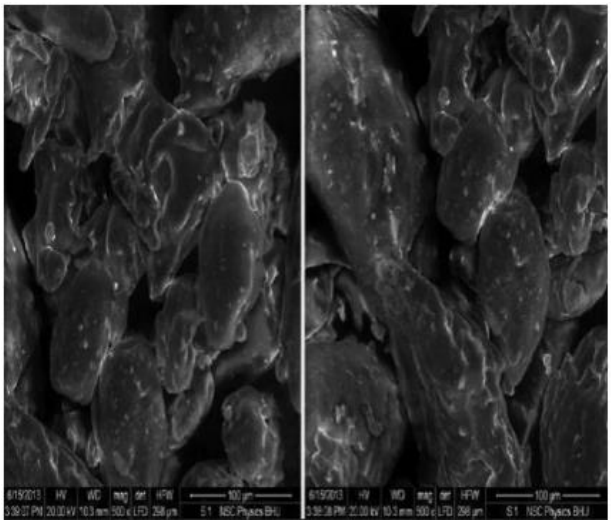

Fig 1: SEM photomicrograph of phytosome

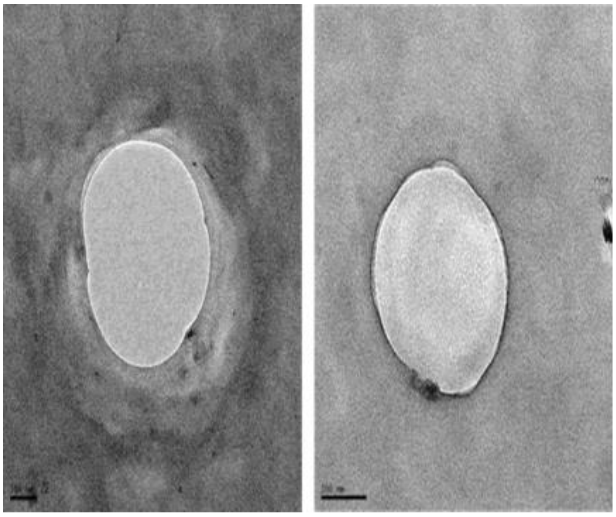

Fig 2: TEM photograph of phytosomes

- **C13NMR**

Using 13C NMR, the stoichiometric molecule containing phosphatidylcholine was unable to identify any of the phytoconstituents' carbons and the phytoconstituents kept at room temperature in C6D6. While the glycerol and choline portions' signals were amplified and some moved, the fatty

acid chains' maximum resonance remained in their original crisp line form.

Table 1: Techniques for Analysing and Characterising phytosome

Sl.No	Parameters	Techniques
1	Size and Shape	DLS, SEM, TEM, Optical microscopy, Fluorescence microscopy, AFM, Field flow fractionation, Nanoparticle tracking analysis, Scanning ion occlusion sensing, Flow Cytometry, Size-exclusion chromatography, Centrifugal sedimentation, and DSC
2	Surface charge	DLS, free-flow electrophoresis, and laser Doppler velocimetr
3	Chemical composition	FTIR, h1 NMR, GC-MS, LC-MS, DSC, TGA, and Thin-layer chromatography
4	Lamellarity and stability	31P nuclear magnetic resonance, Small-angle X-ray scattering, electron microscopy methods, DSC, TGA, DLS, and UV-Vi
5	Encapsulation efficiency	Mini-column centrifugation, HPLC, UPLC, UV-Vis, dialysis, enzymatic assays, gel electrophoresis, field flow fractionation, sample-and-separate approach, the in-situ method, and the continuous flow
6	Release behavior	Design of Experiment (DoE) with Box–Behnken desig

Abbreviations used in this context include: atomic force microscope (AFM), transmission electron microscopy, scanning electron microscopy, and dynamic light scattering. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LCMS) are two examples of nuclear magnetic resonance (NMR) spectroscopy. Thermogravimetric analysis (TGA), HPLC, and UPLC are examples of the analytical techniques used.

Method of Preparations of Phytosomes

Phospholipids used in phytosome preparation mostly come from stearic, palmitic, oleic, or linoleic acid, but can also be sourced from dermis, animal sources such as pig brain, soy lecithin, phosphatidylcholine, phosphatidyl ethanolamine, or swine. These phospholipids might have the same or different acyl groups. There are several other types of quercetin, including quercretin3, rhamnoglucoside, The following compounds are used: hyperoside, vitexine, diosmine, (+) catechin, (-) epicatechin, apigenin-7-glucoside, luteolin, 3-rhamnoside, luteolinglucoside, ginkgonetine, isoginkgonetine, bilobetine, the flavonoids that are specifically chosen.

- **Antisolvent precipitation technique**

Placing the exact amount of medication and soy the A 100 ml round-bottom flask was used for the addition of lecithin, while keeping the temperature below exceed 60°C for duration of 2 hours. Following a concentration of 5–10 ml, 20 ml of hexane is cautiously added while stirring constantly in order to get the solid. The precipitate is then via filtration, collected, and then dried in vacuum-sealed containers for night. The next step is to smash the dry precipitate using mortar and then filter it through a 100-mesh screen. Upon completion, the phytosomes complex was fine-ground and transferred to an amber-colored glass vial for storage at ambient temperature.

- **Rotary evaporation technique**

In a rotatory round-bottom flask, 30 ml of tetrahydrofuran was dissolved with the exact quantity of medication and soya lecithin. The next step is to vigorously swirl the mixture at a temperature that does not go over 40° C for three hours. The sample was thinly coated with n-hexane, which was then added while being continuously agitated with a magnetic stirrer. The amber-colored glass container containing the precipitation of phytosomes complex was put in a room-temperature storage area after collection.

- **Solvent evaporation method**

The medicine and soya lecithin were refluxed in 20 ml of acetone for two hours in a 100 ml round-bottom flask at a temperature of 50 to 60°C. The mixture is then condensed to get the precipitate. a volume of 5-10 ml. The next step was to gather and filter it. The completed process of drying the phytosome complex precipitate included transferring the mixture to a transparent amber container and storing it at room temperature.

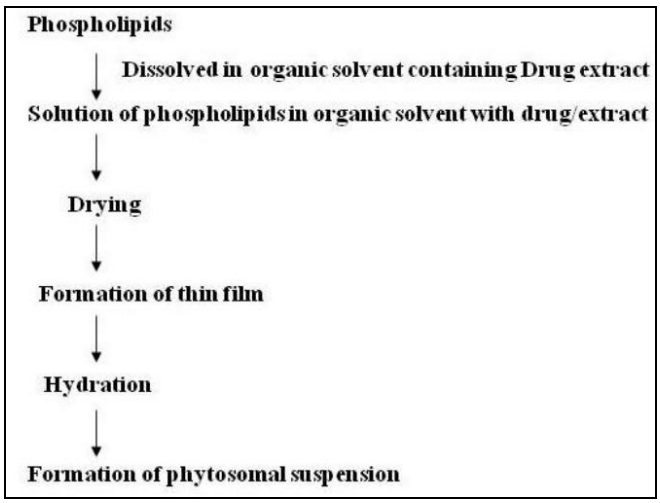

Fig 3: Steps of Phytosome Preparation

Formulations of Phytosomes

Both topical and oral formulations of phytosomes are possible.

- **Soft gelatin capsules**

One common method for creating phytosome complexes is using soft gelatin capsules. Suspensions of the phytosome complex are filled into soft gelatin capsules by dispersing them in oily carriers. Oils derived from plants or manmade materials may be used for this task. According to Indena, for optimum capsule manufacture, a granulometry of 100% < 200 µm should be used. Based on their expertise, Indena found that not all phytosome complexes behaved similarly when placed in oily vehicles or when the oily solution was packed into soft gelatin capsules. As a result, early feasibility studies were conducted to determine the best

vehicle to use. Ginkgoselect Phytosome is an example of

- **Hard gelatin capsules**

Hard gelatin capsules are another possible formulation for the Phytosome complex. There is no need to utilise precompression when using a direct volumetric filling technique; this is true even if the phytosome complex seems to have a low density, which limits the quantity of powder that may be mixed with the capsule (size 0 capsules typically do not contain more than 300 mg). Although precompression may impact the disintegration time, increasing the quantity of powder that fills a capsule using a piston tamp method is possible. It is recommended by Indena® to keep an eye on the relevant parameters when developing products or processes. Take Ginkgoselect® Phytosome as an example.

- **Tablets**

Tablets with high unitary dosages, biopharmaceutical characteristics, and appropriate technology are best produced via the dry granulation production technique. Unfortunately, the phytosome complex has poor The direct compression procedure is limited to low unitary dosages because to its possible stickiness, low apparent density, and flow ability. To get the most out of it and get tablets with the right biopharmaceutical and technological properties, dilute the phytosome complex with 60–70% excipients whenever you use the direct compression method. Water and heat (granulation/drying) have a detrimental impact on the stability of the phospholipid complex; hence it is best to avoid wet granulation. Leucoselect® Phytosome is one such example.

- **Topical dosage forms**

A topical formulation of the phytosome complex is also possible. When the pre-made emulsion is introduced to a little quantity of the lipid phase containing the phospholipidic complex at low temperatures (below 40°C), the phytosome is formed complex may be most effectively included in the emulsion. Topical formulations primarily employ lipidic solvents, which are soluble in phytosome complexes. At temperatures below 40°C, The phytosome complex may potentially dissolve in the aqueous phase as well, which is useful for formulations with minimal lipid content. It can then be added back to the final formulation. Illustration: Phytosome® Glycyrrhetinic acid and Phytosome® Escin/ßSytosterol.

Conclusion

The characterization, properties, and formulation of phytosomes present a fascinating avenue in the field of pharmaceutical science and drug delivery. Through the molecular complexation of plant-derived bioactive compounds with phospholipids, phytosomes offer a promising strategy to enhance the bioavailability and therapeutic efficacy of phytochemicals. The characterization of phytosomes is essential to understand their physicochemical properties, including particle size, morphology, and encapsulation efficiency. Advanced analytical techniques provide valuable insights into the structural integrity and stability of phytosome formulations, guiding formulation optimization and quality control processes. Formulation strategies play a crucial role in tailoring phytosome-based formulations for specific therapeutic applications. Optimization of the drug-to-lipid ratio, selection of appropriate excipients, and incorporation into various dosage forms enable the development of tailored formulations with desired release profiles and pharmacokinetic profiles. Overall, phytosomes represent a promising platform for the delivery of plant-derived bioactive compounds, offering opportunities to overcome the challenges associated with poor solubility and bioavailability. Continued research and innovation in the characterization, properties, and formulation of phytosomes hold immense potential for the development of novel phytopharmaceuticals with enhanced therapeutic benefits and clinical utility.

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