Phytosomes

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**ABSTRACT**

The term "phyto" and "some" refer to plants and cells, respectively. Phytosomes are small structures that resemble cells. This is a more advanced herbal formulation in which the bioactive phytocomponents of herb extracts are surrounded and bonded by lipid. The majority of bioactive ingredients, such as flavonoids and glycosides, are water soluble. The phytosome is a lipid-based vesicular delivery method that can be used to encapsulate pharmaceuticals as well as plant-derived nutraceuticals including polyphenolic substances. The phytosome, as a newly introduced food-grade delivery technology, has the potential to reduce difficulties with polyphenolic compound solubility and bioavailability, making it useful in the creation of new medicinal and food formulations. This delivery platform could help pharmaceutical companies encapsulate adequate amounts of active phytoingredients for the production of new supplements. Furthermore, phytosomes can increase polyphenolic compound bioavailability through the gastrointestinal tract while decreasing administration dosage. Furthermore, the phytosome preparation procedure is simple and can be scaled up commercially. As a promising candidate for incorporating herbal-derived polyphenolic compounds into effective cancer and other disease treatments, phytosome technology is a promising encapsulation platform for future nutraceutical nano-formulation.

**Keywords** – Phytosomes; Flavonoids; Herbal;Stability

## Introduction - Phytosomes Technology

## Phytosomes increases the absorption of lipid insoluble polar phytoconstituents orally and as well as topical route shows a better bioavailability, hence results in greater therapeutic benefit. As the absorption rate of active constituent is increased, the requirement of dose is reduced {1}.

Plant extracts flavonoid and terpenoid constituents lend themselves well to direct binding to phosphatidylcholine. Phytosomes are formed by reacting a stoichiometric amount of phospholipid (phosphatidylcholine) with a standardized extract or polyphenolic constituents (such as simple flavonoids) in a non-polar solvent. Phosphatidylcholine is a bifunctional compound with a lipophilic phosphatidyl moiety and a hydrophilic choline moiety. The phosphatidylcholine molecule's choline head specifically binds to these compounds, while the lipid soluble phosphatidyl portion, which includes the body and tail, then envelopes the choline bound material destruction by gastric secretions and gut bacteria.

As a result, the phytoconstituents form a lipid compatible molecular complex with phospholipids, known as the phyto-phospholipid complex. Specific spectroscopic techniques show that molecules are anchored to the polar choline head of the phospholipids via chemical bonds. According to precise chemical analysis, the unit phytosome is typically a flavonoid molecule linked to at least one phosphatidylcholine molecule. As a result, a small microsphere or cell is formed. The phytosome technology creates a small cell that protects the plant extract or its active constituent.

1. **Chemical Properties:**

Phytosomes are a combination of a natural product and natural phospholipids, such as soy phospholipids. A complex of this type is formed by reacting stoichiometric amounts of phospholipid and substrate in an appropriate solvent. The formation of hydrogen bonds between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functionalities of the substrate has been shown to be the main phospholipid-substrate interaction based on spectroscopic data. When treated with water, phytosomes take on a micellar shape, forming liposomal-like structures. In liposomes, the active principle is dissolved in the internal pocket or floating in the layer membrane, whereas in phytosomes, the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane, as seen in the case of the catechindistearoyl phosphatidylcholine complex, where H-bonds form.

1. **Biological properties:**

Biological Properties Phytosome are advanced forms of herbal products that are better absorbed, utilized and as a result produce better results than conventional herbal extracts the increased bioavailability of the phytosome over the non-complexed botanical derivatives has been demonstrated by pharmacokinetics studies or by pharmacodynamic tests in experimental animals and in human subjects.

1. **Characterization of phytosomes:**

The behavior of phytosomes in both physical and biological system is governed by the factors such as physical size membrane permeability; percent entrapped solutes, chemical composition as well as the quantity and purity of the starting materials. Therefore, the phytosomes are characterized for physical attributes i.e. shape, size, its distribution, percentage drug capture entrapped volume, percentage drug released and chemical composition.

* 1. **Advantages of phytosomes:**

By forming a stable complex with phospholipids, phytosomes improve bioavailability and stability profiles, and drug delivery improves absorption from the site of action in the intestinal tract when administered as a herbal constituent alone.

* Improves liver targeting by increasing bile salt solubility.
* The required dose is reduced as active phytoconstituent absorption improves.
* PC used in phytosome preparation, in addition to acting as a carrier, also acts as a hepatoprotective, providing a synergistic effect.
* Because of their improved skin penetration and high lipid profile, phytosomes are widely used in cosmetics.
* Herbal extracts' valuable components are protected from destruction by digestive secretions and gut bacteria.
* It ensures that the drug is delivered to the appropriate tissues.
* The nutrient safety of herbal extracts does not have to be jeopardised by delivering the herbal drug via phytosomes.
* Because of the maximum absorption of the main constituents, the dose requirement has been reduced.
* Significant improvement in drug bioavailability occurs.
* Entrapment efficiency is high and predetermined because the drug is conjugated with lipids in the formation of vesicles.
* There are no issues with drug entrapment when creating phytosomes.
* Phytosomes have a higher stability profile due to chemical bonds formed between phosphatidylcholine molecules and phytoconstituents.
* Phosphatidylcholine, which is used in the phytosomes process, both nourishes and acts as a carrier.
* Phytosomes outperform liposomes in skin care products.
* Phytosomes show a significantly higher clinical benefit.
* Phosphatidylcholine is used in the preparation of phytosomes; in addition to actingas acarrier, it also acts as a hepatoprotective, resulting in a synergistic effect.
  1. **Disadvantages of phytosomes:**

Despite the numerous benefits of phytosomes, it has been reported that phospholipids (lecithin) can induce proliferation in the MCF-7 breast cancer cell line. The leaching of phytoconstituents from the ‘some' is a significant disadvantage of phytosome. Phytosomes of Curcumin In two separate studies, Maiti et al. (2006) created phytosomes of curcumin (flavonoid from Curcuma longa, turmeric) and naringenin (flavonoid from grape fruit, Vitis vinifera). In all dose levels tested, the complex's antioxidant activity was significantly higher than pure curcumin. In another study, the developed phytosome of naringenin produced better antioxidant activity than the free compound with a longer duration of action, which could be due to a decrease in the molecule's rapid elimination from the body21.

1. **Applications of phytosomes:**
2. **Silymarin Phytosomes:**

The majority of Phytosomal research has focused on Silybum marianum (milk thistles), which contains powerful liver-protecting flavonoids. Yanyu et al. (2006) synthesized silymarin phytosomes and investigated their pharmacokinetics in rats. The bioavailability of silybin in rats was significantly increased after oral administration of silybinphospholipid complex, owing to an impressive improvement in the lipophilic properties of silybinphospholipid complex and an improvement in silybin's biological effect. Tedesco et al (2004) reported that Silymarin phytosomes have greater anti-hepatotoxic activity than silymarin alone and can protect broiler chick performance from the toxic effects of aflatoxin B1.

1. **Curcumin Phytosomes:**

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1. **Quercetin-phospholipid Phytosome:**

Complex Maiti et al. (2005) created the quercetin phospholipid Phytosomal complex using a simple and reproducible method and demonstrated that the formulation outperformed the molecule in rat liver injury caused by carbon tetrachloride.

1. **Grape seed Phytosomes:[25]**

Phytosomes made from grape seeds are comprises proanthocyanidins or Procyanidine of different molecular size complex associated with phospholipids. The properties of Procyanidine flavonoids in grape seed increases antioxidant capacity and helps in stimulation of physiological defenses in plasma, protection against induced damages in heart, atherosclerosis. In another study, rabbits were fed with diet consisting of high cholesterol diet for the period of 6 weeks, to elevate their blood cholesterol level and to induce atherosclerotic lesions in their aortas and carotid arteries. One group of rabbit fed with grape seed phytosomes in their feed for 6 weeks, followed by 4 weeks with high cholesterol diet. Less aortic plaque is developed which received conventional standardized grape seed extract. In a randomized human trial, young healthy volunteers were received grape seed phytosomes daily once for the period of 5 days. The blood samples were analysed for Total Radical-trapping Antioxidant Parameter at several time intervals during the prime day followed by on 5th day. Blood Total Radical Antioxidant Parameter levels were significantly elevated over the control, than the group received conventional standardized grape seed extract.

1. **Phytosomes of *Gingko biloba* leaves[24}:**

Studies about *Gb* phytosomes resulted in better potency compared to the conventional standardized extract from plant. When a bioavailability study is carried with healthy human volunteers the level of Gb extract (Gingko biloba leaves) constituents - flavonoids and terpenes peaked out after completion of three hours and longer persistence is observed longer period of time for 5 hours after oral administration. Phytosomal Gb extract produces a 2-4 times greater plasma concentration of terpenes than the non-Phytosomal Gb extract. The improved oral bioavailability and good tolerability is observed which makes an ideal ginkgo product for longer term treatment. Ginkgo phytosomes study in patients with peripheral vascular disorders had shown 30-60% greater improvement when compared to a conventional standardized Gb Extract. The studies were made on Gb phytosomes, were administered for 5 days in guinea pigs with broncho constriction was induced by three different agonists (histamine, PAF and Acetylcholine). The result indicated that ginkgo phytosomes can not only counteract direct broncho constriction but also have the tendency to reduce the TXA2 mediated broncho constriction of histamine. Antioxidant property is improved efficacy of ginkgo phytosomes in overcome the allergen induced broncho spasm. Studies also improved the efficacy of Gb phytosomes over the conventional standardized extract in protecting rat isolated hearts against ischemia. The results clearly says about that the phytosomes possess over the conventional preparations, thus proving it’s utility for herbal phytoconstituents.

1. **Phytosomes of green tea:**

Green tea leaves (*Thea sinensis*) is characterized due to the presence of polyphenolic compound epigallocatechin 3-O-gallate26. These phytoconstituents are potent modulators in many biochemical process which is linked to the breakdown of homeostasis in many chronic-degenerative diseases like cancer, atherosclerosis etc., Green tea also furnishes many beneficial activities like antioxidant, anticarcinogenic, antimutagenic etc., but the demerit with these polyphenols are poor bioavailability. The polyphenols complexion derived from green tea strongly improvised oral bioavailability is observed. The study was made on absorption of Phytosomal preparation in healthy human volunteersalong with non-complexed green tea extrac. In the study period of 6 hours the plasma concentration of total flavonoids was more than doubled when compared between the Phytosomal and nonphytosomal was measured as Total Radical Trapping Antioxidant Parameter. The peak antioxidant effect was a 20% enhancement and it showed that the phytosomes formulation had about double the total antioxidant effect.

* + - 1. **Phytosomes containing dosage forms**

The prepared Phytosomes administered via orally or topically in order to achieve the best results in the form of bioavailability. It is needed to study about dissolution and disintegration parameters for the developed dosage forms. Some of the examples were listed below:

1. **Soft gelatin capsules:**

A granulometry of 100 % < 200 μm in the suspension form vegetable or semi-synthetic oils is recommended.

1. **Hard gelatin capsules:**

NMT 300 mg in size 0 capsule, without recompression method is used to fill the hard gelatins.

1. **Tablets:**

Dry granulation is preferred than Wet granulation to avoid adverse effects of phospholipids, represents the ideal manufacturing process to obtain tablets with higher unitary doses.

**D. Topical dosage forms:**

The emulsion were used for this purpose to ensure the good result from the complex of phospholipids.

1. **Preparation of Phytosomes**

Phytosomes are prepared by various methods by interacting 3-2 moles natural or synthetic phospholipid, mainly phosphatidiccholine with one mole of phytoconstituents (Saha et al., 2013). The preferable ratio for the complex formation is in the range from 0.5 to 2.0 moles.

**Solvent evaporation method**

The particular quantity of drug, polymer and phospholipids can be taken into a spherical bottom flask and reflux with specific solvent at a temperature 50-60ºc for 2 hr. The mixture may be concentrated to 5 – 10 ml to get the precipitate which can be filtered and collected. The dried precipitate phytosome loaded can be placed in amber coloured glass bottle and stored at room temperature29.

1. **Rotary evaporation technique**

The specific amount of drug and soya lecithin were dissolved in 30 ml of tetrahydrofuran in a rotary round bottom flask followed by stirring for 3 hours at a temperature not exceeding 40oC. Sample Thin film was obtained when n-hexane was added and continuously stirred with the help of magnetic stirrer. The precipitate is collected and stored in amber coloured glass bottle at room temperature.

1. **Anti-solvent precipitation technique**

The desired quantity of drug and soya lecithin was taken in a 100 ml round bottom flask. Refluxed with 20 ml of dichloromethane at a temperature not exceeding 60oC for 2 h. Further it is concentrated. 20 ml of hexane was added with continuous stirring results in precipitation. Precipitation is filtered, collected and stored in vacuum desiccators. The dried precipitate is crushed in mortar and sieved by using Sieve no. 100. Powdered complex was placed in amber colored glass bottle and stored at room temperature.

1. **Salting Out method**

The phytoconstituent and phosphatidecholine is made dissolved in an aprotic solvents like dioxane or acetone. Later the solution is continuously stirred to form the complex, later isolated from by precipitation from non-solvents like n-hexane.

1. **Lyophilization Technique**

Both the natural or synthetic originated phospholipid and phytoconstituent were dissolved in different solvents. Further solution containing phytoconstituent were added to a phospholipid solution. Stirred to get a complex further the complex separated out by lyophilization.

The phospholipids used in phytosomes preparation consist of acyl group which is different in phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and mostly derived from palmitic, stearic, oleic, and linoleic acid. In phytosome active, principle is an integral part of the membrane because the active principle is in connection with the polar head of phospholipid.

1. **Mechanical Dispersion method**

This method involves with the concept that the lipids were made dissolved in organic solvent which were brought in contact with aqueous phase containing the drug. Initially, phytosomes were made dissolved in diethyl ether which later slowly injected in an aqueous solution containing the phytoconstituents to be encapsulated. There by removal of the organic solvent under reduced pressure leads to the formation of phytophospholipid complex. Novel methods for phospholipid complex preparation includes Super Critical Fluids (SCF), which include Gas Anti-Solvent technique (GAS) compressed anti solvent process (PCA), Supercritical Anti Solvent method (SAS).

1. **Different additives – Phytosomes Formulation**

**Phospholipids like** Phosphatidyl choline from the sources like Soya, egg, Dipalmityl & Distearyl phosphatidyl choline.

**Aprotic solvents like**Dioxane, Acetone, Methylene chloride

**Non solvent like** n-hexane and non-solvent i.e. aliphatic hydrocarbon

**Alcohols**

**V. Characterization techniques of Phytosomes**

* 1. **Visualization Method**

To visualize the phytosomes TEM (Transmission Electron Microscopy) and SEM (Scanning Electron Microscopy) are used.

1. **Transition temperature**

Inorder to determine the transition temperature of vesicular lipid system a DSC (Differential Scanning Calorimetry).

1. **Surface tension measurement**

Ring method in a Du Nouy Ring Tensiometer is choosen for the determination of Surface of drug in aqueous solution.

1. **Vesicle stability**

Assessment of vesicle size and structure overtime tells about the stability of vesicles. If any structural changes occurs it can be monitored by TEM and mean size is measured by DLS.

1. **Scanning electron microscopy (SEM)**

SEM (Scanning Electron Microscopy) is used for the determination of average particle size distribution and the surface morphology in the complexes. The dry sample which has to be analysed is to be placed on Scanning Electron Microscope with the help of brass stub and made coated with gold in ion sputter. Thereby the digital images of phytosome complex were taken by random scanning of brass stub at Magnification power of about 1000, 5000, 10000 and 30000X.

1. **Entrapment efficiency**

The Entrapment Efficiency of phytosomal formulation can be determined by Ultra Centrifugation.

1. **Evaluation of Phytosomes**

Spectroscopic evaluation is carried to confirm the formation of a complex or to study the interaction between the phytoconstituent and phospholipids by the following spectroscopic methods.

1. **1H-NMR:**

Bombardelli et al studied the NMR spectra of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine. In non polar solvents, a marked change of the 1H-NMR signal originated because of atoms which are involved in complex formation, without any signal peculiar to molecules individually. The signals from the protons which is belonged to flavonoids are broadened, that the proton cannot be relieved. In phospholipids, the signals were broadened while the singlet corresponding to the N-(CH3)3 of choline undergone uplift shift. The sample is heated at 60˚C resulted in formation of broad bands, which is related mainly to the resonance of the flavonoid moiety.

1. **13C-NMR:**

In the spectrum of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine, in C6D6 at room temperature, all the flavonoid carbons were unclear with poor visibility. The signals related to glycerol and choline portion of the lipid esp., between 60–80 ppm were broadened and some were shifted, while resonances of the fattyacid chains retained with original sharp line. When heated at 60˚C, all the signals related to flavonoid moiety were reappeared even though they were very broad and overlapping.

1. **FTIR:**

The complex formation is confirmed by IR spectroscopy by comparing the spectrum of the complex with individual components spectrum along with their mechanical mixtures. It is an useful tool to control the phytosomes stability when they were micro-dispersed in water or incorporated in simple gels. In practical approach phytosomes stability can be assessed by comparing the spectrum of the complex with the spectrum of its micro-dispersion in water after lyophilization, at different times. Like in simple formulations, it is needed to exempt the spectrum of the excipients from the spectrum of the cosmetic form at different times, to compare the remaining spectrum of the complex itself.[55]

1. **Evaluation by In vitro & In vivo Method:**

Choosing of In-vitro and In-vivo evaluation method to select the phytoconstituents for desired therapeutic activity present in the phytosome is important. For instance! Invitro anti-hepatotoxic activity can be analysed by examining the free radical scavenging activity of phytosome. Skin sensitization and tolerability studies of glycyrrhetinic acid phytosome ointment, a commercial product, describe the in-vivo safety evaluation methodology2.

1. **Marketed Formulations**

Many marketed formulations were introduced into the market for the treatment of various diseases .some of the examples are Leucoselect® phytosomes which is used as sysytemic anti- oxidant and is the best choice for the most people under the age of 50.Green select® phytosome it is used as the best choice for protection against cancer. Silybin phytosome is the best choice if the liver needs additional anti – oxidant protection. Sabalselect ® phytosome it enhances immune function in response to a toxic challenge.

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