

## A REVIEW ON GENESIS AND CHARACTERIZATION OF PHYTOSOMES

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### ABSTRACT

Phytosomes, often known as herbosomes are recently introduced in herbal formulations that exhibits better pharmacokinetic and pharmacodynamic profile than conventional botanical extracts. They are produced by a process whereby the standardized plant extract or its constituents are bound to phospholipids, mainly phosphatidylcholine producing a lipid compatible molecular complex, the major molecular building block of cell membranes and a compound miscible in both water and in oil or lipid environments. This technique makes phytosomes unique in character as potential to act as a chaperon for polyphenolics, shuttling them through biological membranes. This article apart from providing information regarding the advantages and physiochemical properties of phytosomes, gives various simple research techniques in the preparation and its optimisation. A market survey of various commercial phytosome preparations limelights this article. Apart from phytosomes this article briefs other members of the family of "Somes", which could be the area of scope for many industrialists, researchers and academicians.

**Keywords:** Flavonoids, Phytomedicines, Phytosome, bioavailability.

### INTRODUCTION

The "Somes" the cell like formulations of novel drug delivery system. There are different types of somes like Liposomes, which encapsulate water and lipid-soluble pharmacologically and cosmetically active components. Phytosomes are standardized extracts or purified fractions complexed with phospholipids for a better bioavailability and enhanced activities. and Cubosomes are bicontinuous cubic phases, consisting of two separate, continuous, but nonintersecting hydrophilic regions divided by a lipid layer that is contorted into a periodic minimal surface with zero average curvature, and Colloidosomes are solid microcapsules formed by the self-assembly of colloidal particles at the interface of emulsion droplets. "Colloidosomes," are hollow, elastic shells whose permeability and elasticity can be precisely controlled. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. Ethosomes contain phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Aquasomes - these are spherical 60300nm particles used for drug and antigen delivery. The particle core is composed of noncrystalline calcium phosphate or ceramic diamond, and is covered by a polyhydroxyl oligomeric film. Pharmacosomes are the colloidal dispersions of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar, or hexagonal aggregates, depending on the chemical structure of the drug-lipid complex. Niosomes are non-ionic surfactant vesicles and, as liposomes, are bilayered structures. etc.

Phytomedicines, complex chemical mixtures prepared from plants, have been used for health maintenance since ancient times. But many phytomedicines are limited in

their effectiveness because they are poorly absorbed when taken by mouth. The Phytosome® technology, developed by Indena S.p.A. of Italy, markedly enhances the bioavailability of select phytomedicines, by incorporating phospholipids into standardized extracts and so vastly improve their absorption and utilization<sup>1</sup>.

Over the past century, chemical and pharmacologic science established the compositions, biological activities and health giving benefits of numerous plant extracts. But often when individual components were separated from the whole there was loss of activity—the natural ingredient synergy became lost<sup>2</sup>. Standardization was developed to solve this problem. As standardized extracts became established, poor bioavailability often limited their clinical utility. Then it was discovered that complexation with certain other clinically useful nutrients substantially improved the bioavailability of such extracts. The nutrients so helpful for enhancing the absorption of other nutrients are the phospholipids.

Phospholipids are complex molecules that are used in all known life forms to make cell membranes. They are cell membrane building blocks, making up the matrix into which fit a large variety of proteins that are enzymes, transport proteins, receptors, and other biological energy converters. In humans and other higher animals the phospholipids are also employed as natural digestive aids and as carriers for both fat-miscible and water miscible nutrients.<sup>1,3</sup>

Increased bioavailability of the Phytosomes over the simpler, non complex plant extracts has been demonstrated by pharmacokinetic (tissue distribution) and activity studies, conducted in animals as well as in humans. Phytosome has an added dimension: the proven health giving activity of the phospholipids themselves.



## Background to the phytosome Technology<sup>1,3,4,5</sup>

The poor absorption of flavonoid nutrients is likely due to two main factors. First, these are multiple-ring molecules not quite small enough to be absorbed from the intestine into the blood by simple diffusion. Nor does the intestinal lining actively absorb them, as occurs with some vitamins and minerals. Second, flavonoid molecules typically have poor miscibility with oils and other lipids. This severely limits their ability to pass across the lipid-rich outer membranes of the enterocytes, the cells that line the small intestine. The Phytosome<sup>®</sup> technology meets this challenge. Certain of the water – phase flavonoid molecules can be converted into lipid – compatible molecular complexes, aptly called phytosomes. These are better able to transition from the water phase external to the enterocyte, into the lipid phase of its outer cell membrane and from there into the cell, finally reaching the blood.

The lipid - phase substances that successfully employed to make flavonoids lipid - compatible are phospholipids from soy, mainly phosphatidylcholine (PC). Phospholipids are small lipid molecules where glycerol is bonded to two fatty acids, while the third hydroxyl, normally one of the two primary methylenes, bears a phosphate group bound to a biogenic amino or to an amino acid—thus making Phytosome<sup>®</sup>'s different from liposomes. PC is the principal molecular building block for cell membranes (Fig. 2), and the molecular properties that suit PC for this role also render it close to ideal for its Phytosome<sup>®</sup> role. PC is miscible both in the water phase and in oil/lipid phases, and is excellently absorbed when taken by mouth, and has the potential to act as a chaperon for polyphenolics, shuttling them through biological membranes. Precise chemical analysis indicates the unit phytosome is usually a flavonoid molecule linked with at least one PC molecule. A bond is formed between the two molecules to create a hybrid molecule. This hybrid is highly lipid - miscible, better suited to merge into the lipid phase of the enterocyte's outer cell membrane. Once there, it can cross the enterocyte and reach the circulating blood.

Phytosomes are not liposomes - structurally, the two are distinctly different as shown in Fig. 1H. The phytosome is a unit of a few molecules bonded together, while the liposome is an aggregate of many phospholipid molecules that can enclose other phytoactive molecules but without specifically bonding to them<sup>6,7</sup>. This difference results in phytosome being much better absorbed than liposomes showing better bioavailability. Phytosomes have also been found superior to liposomes in topical and skin care (cosmetic) products.

In liposomes, the active principles are water soluble and are hosted in the inner cavity, with little, if any, interaction taking place between the hydrophilic principle and the surrounding lipid core. Conversely, Phytosome<sup>®</sup> host their polyphenolic guest, generally little soluble both in water and in lipids, at their surface where the polar functionalities of the lipophilic guest interact via

hydrogen bonds and polar interactions with the charged phosphate head of phospholipids, forming a unique arrangement that can be evidenced by spectroscopy.

The Phytosome<sup>®</sup> formulation also increases the absorption of active ingredients when topically applied on the skin, and improves systemic bioavailability when administered orally. In water medium, a Phytosome will assume a micellar shape, forming a spherical structure, overall similar to a liposome, but with a different guest localization.

## Methods of Preparation

1. Phytosomes are novel complexes which are prepared by reacting from 3-2 moles but preferably with one mole of a natural or synthetic phospholipid, such as phosphatidylcholine, phosphatidylethanolamine or phosphatidylserine with one mole of component for example- flavolignanans, either alone or in the natural mixture in aprotic solvent such as dioxane or acetone from which complex can be isolated by precipitation with non solvent such as aliphatic hydrocarbons or lyophilization or by spray drying. In the complex formation of phytosomes the ratio between these two moieties is in the range from 0.5-2.0 moles. The most preferable ratio of phospholipid to flavonoids is 1:1<sup>8</sup>.
2. Naringenin-PC complex was prepared by taking naringenin with an equimolar concentration of phosphatidylcholine (PC). The equimolar concentration of PC and naringenin were placed in a 100 mL round bottom flask and refluxed in dichloromethane for 3 h. On concentrating the solution to 5–10 mL, 30 mL of n-hexane was added to get the complex as a precipitate followed by filtration. The precipitate was collected and placed in vacuum desiccators.<sup>9</sup>
3. The required amounts of drug and phospholipids were placed in a 100 ml round-bottom flask and dissolved in anhydrous ethanol. After ethanol was evaporated off under vacuum at 40 °C, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh. The resultant silybin-phospholipid complex was transferred into a glass bottle, flushed with nitrogen and stored in the room temperature<sup>10</sup>.

## Physico-chemical characterization of phytosomes

Phytosome is a complex between a natural product and natural phospholipids, like soy phospholipids. Such a complex is obtained by reaction of stoichiometric amounts of phospholipid and the substrate in an appropriate solvent. Their sizes vary between 50 nm to a few hundred  $\mu\text{m}$ . On the basis of spectroscopic data it has been shown that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functionalities of



the substrate. These phyto-phospholipid complexes are often freely soluble in aprotic solvents, moderately soluble in fats, insoluble in water and relatively unstable in alcohol. When treated with water, phytosomes assume a micellar shape forming liposome-like structures. In liposome the active principle is dissolved in the internal pocket or it is floating in the layer membrane, while in phytosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane for example in the case of the catechindistearoylphosphatidylcholine complex, in this there is the formation of H-bonds between the phenolic hydroxyl ends of the flavonoid moiety and the phosphate ion on the phosphatidylcholine moiety. Phosphatidylcholine can be deduced from the comparison of the <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of the complex with those of the pure precursors. The signals of the fatty chain remain almost unchanged. Such evidences inferred that the two long aliphatic chains are wrapped around the active principle, producing a lipophilic envelope, which shields the polar head of the phospholipid and the flavonoid molecule and enables the complex to dissolve in low polarity solvents<sup>11</sup>.

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 evaluated for their organoleptic properties i.e. shape, size, its distribution and physico-chemically characterized by UV, IR, NMR, DSC, SEM etc. Percentage drug entrapment, percentage drug release profile are also studied accordingly<sup>12</sup>.

#### Pharmaceutical scope of phytosomes:<sup>13-17</sup>

- It enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability, hence significantly greater therapeutic benefit.
- Appreciable drug entrapment.
- As the absorption of active constituent(s) is improved, its dose requirement is also reduced.
- Phosphatidylcholine used in preparation of phytosomes, besides acting as a carrier also acts as a hepatoprotective, hence giving the synergistic effect when hepatoprotective substances are employed.
- Chemical bonds are formed between phosphatidylcholine molecule and phytoconstituent, so the phytosomes show better stability profile.
- Application of phytoconstituents in form of phytosome improve their percutaneous absorption and act as functional cosmetics.

- Added nutritional benefit of phospholipids.

#### Improved bio availability, the recent research:

Recent research shows improved absorption and bioavailability with phytosomes as compared to the conventional means. Most of the phytosomal studies are focused to *Silybum marianum* (milk thistle) which contains premier liver-protectant flavonoids. The fruit of the milk thistle plant contains flavonoids known for hepatoprotective effects.<sup>18,19</sup>

Yanyu et al. prepared the silymarin phytosome and studied its pharmacokinetics in rats. In the study the bioavailability of silybin in rats was increased remarkably after oral administration of prepared silybin-phospholipid complex due to an impressive improvement of the lipophilic property of silybin-phospholipid complex and improvement of the biological effect of silybin<sup>10</sup>.

Tedesco et al. reported silymarin phytosome show better anti-hepatotoxic activity than silymarin alone and can provide protection against the toxic effects of aflatoxin B1 on performance of broiler chicks<sup>20</sup>. Busby et al reported that the use of a silymarin phytosome showed a better fetoprotectant activity from ethanol-induced behavioral deficits than uncomplexed silymarin<sup>21</sup>. Grange et al. conducted a series of studies on silymarin phytosome, containing a standardized extract from the seeds of *S. marianum*, administered orally and found that it could protect the fetus from maternally ingested ethanol<sup>22</sup>.

Bombardelli et al. reported Silymarin phytosomes, in which silymarin (a standardized mixture of flavanolignans extracted from the fruits of *S. marianum*) was complexed with phospholipids. Phytosomes showed much higher specific activity and a longer lasting action than the single constituents, with respect to percent reduction of odema, inhibition of myeloperoxidase activity, antioxidant and free radical scavenging properties<sup>14</sup>.

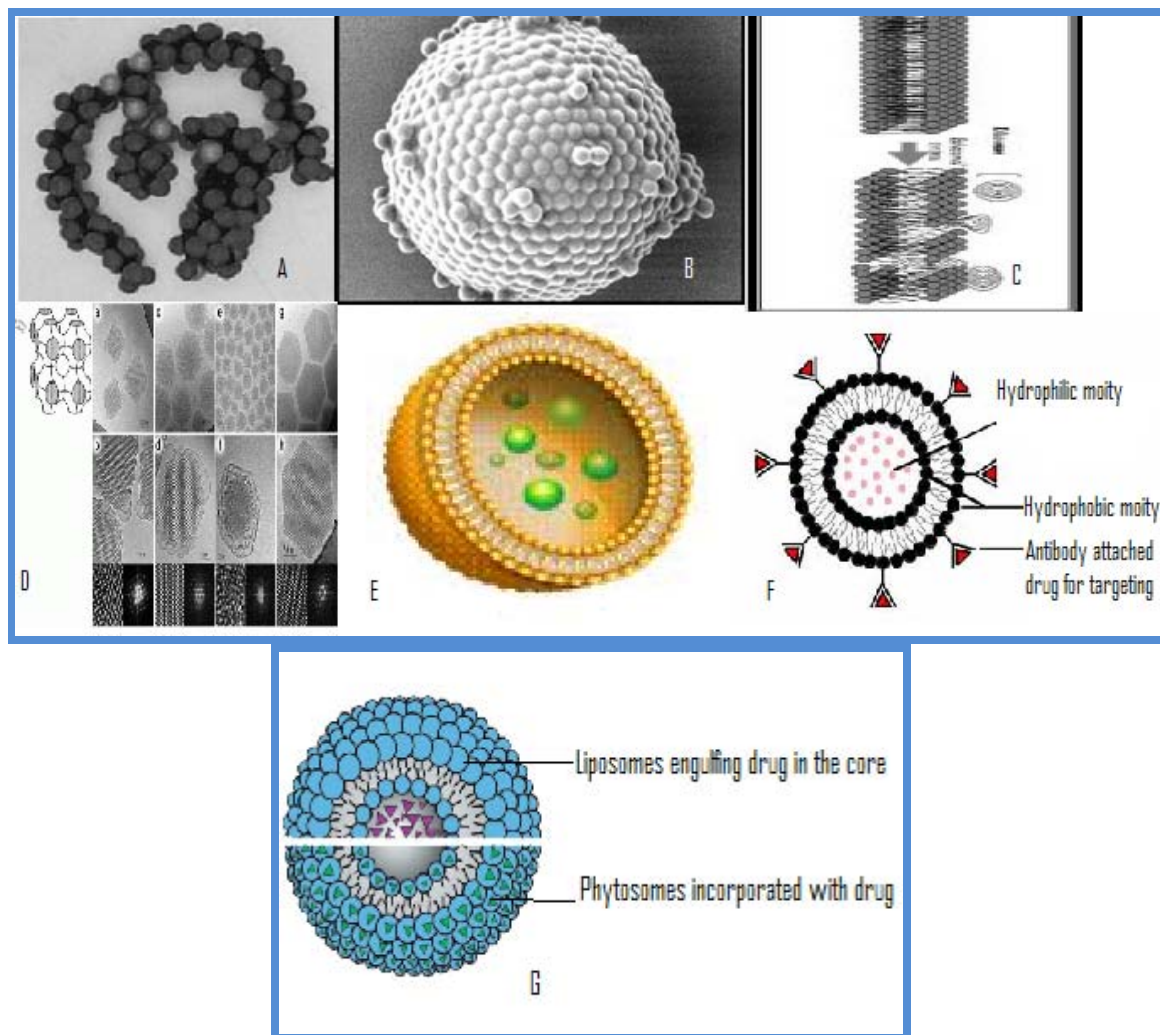
In the human subjects silybin from phytosomes effectively reaches the intended target organ, the liver. This was proven by Schandalik et al. using nine volunteer patients who had earlier undergone surgical gall bladder removal necessitated by gallstones. They received single oral doses of 120 mg silybin as silybin phytosome, and bile was accessed for silybin levels. Silybin appeared in the bile and peaked after 4 hours. In the case of phytosomal silybin, the total amount recovered in the bile after 48 hours accounted for 11 per cent of the total dose. In the case of silymarin, approximately 3 per cent of the silybin was recovered. These data demonstrate a four times greater passage through the liver for phytosomal silybin<sup>23,24</sup>.



**Table 1:** Commercial products available<sup>6,31,32</sup>:

<u>Trade name</u>	<u>Phytochemical</u>	<u>Indication</u>
18β-glycyrrhetic acid Phytosome®	18β-glycyrrhetic acid from licorice rhizome	Soothing
Centella Phytosome®	Triterpenes from Centella asiatica leaf	Cicatrizing, trophodermic
Crataegus Phytosome®	Vitexin-2''-O-rhamnoside from Hawthorn flower	Antioxidant
Escin β-sitosterol Phytosome®	Escin β-sitosterol from horse chestnut fruit	Anti-oedema
Ginkgoselect® Phytosome®	Ginkgoflavonglucosides, ginkgolides, bilobalide from Ginkgo biloba leaf	Vasokinetic
Ginselect® Phytosome®	Ginsenosides from Panax ginseng rhizome	Skin elasticity improver, adaptogenic
Ginkgo biloba Terpenes Phytosome®	Ginkgolides and bilobalide from Ginkgo biloba leaf	Soothing
Ginkgo biloba Dimeric Flavonoids Phytosome®	Dimeric flavonoids from Ginkgo biloba leaf	Lipolytic, vasokinetic
Greenselect® Phytosome®	Polyphenols from green tea leaf	Prevention of free radical-mediated tissue damages and weight management
Leucoselect® Phytosome®	Polyphenols from grape seed	Antioxidant, capillarotropic
Meriva®	Curcuminoids from turmeric rhizome	
PA <sub>2</sub> Phytosome®	Proanthocyanidin A <sub>2</sub> from horse chestnut bark	Anti-wrinkles, UV protectant
Sericoside Phytosome®	Sericoside from Terminalia sericea bark root	Anti-wrinkles
Siliphos®	Silybin from milk thistle seed	Hepatocyte protection
Silymarin Phytosome®	Silymarin from milk thistle seed	Antihepatotoxic
Virtiva®	Ginkgoflavonglucosides, ginkgolides, bilobalide from Ginkgo biloba leaf	Vasokinetic
Visnadex®	Visnadin from Amni visnaga umbel	Vasokinetic
Mirtoselect® phytosome	anthocyanosides of bilberry	potent antioxidants
Sabalselect® phytosome	saw palmetto berries	benefit non-cancerous prostate enlargement
LymphaselectTM phytosome	Melilotus officinalis	for venous disorders, including chronic venous insufficiency of the lower limbs
OleaselectTM phytosome	olive oil polyphenols.	Anti oxidant, anti inflammatory Anti hyperlipidemic.
PolinaceaTM	<i>Echinacea angustifolia</i> .	Neutraceutical, immuno modulator.

**Figure 1:** The wide spectra of established “Somes”



A) Organization of the Phytosome® molecular complex B) Colloidosomes  
 C) Ethosomes D) Cubosomes E) Liposomes F) Niosomes G) Differentiation of Phytosome® and Liposome

Barzaghi et al. conducted a human study designed to assess the absorption of silybin when directly bound to phosphatidylcholine. Plasma silybin levels were determined after administration of single oral doses of silybin phytosome and a similar amount of silybin from milk thistle in healthy volunteers.

Moscarella et al. investigated in one study of 232 patients with chronic hepatitis (viral, alcohol or drug induced) treated with silybin phytosome at a dose of 120 mg either twice daily or thrice daily for up to 120 days, liver function returned to normal faster in patients taking silybin phytosome compared to a group of controls (49 treated with commercially available silymarin, 117 untreated or given placebo)<sup>26</sup>.

Grape seed phytosome is composed of oligomeric polyphenols (grape proanthocyanidins or procyanidins from grape seed extract, *Vitis vinifera*) of varying molecular size, complexed with phospholipids. The main properties of procyanidin flavonoids of grape seed are an increase in total antioxidant capacity and stimulation of

physiological antioxidant defenses of plasma, protection against ischemia/reperfusion induced damages in the heart, protective effects against atherosclerosis thereby offering marked protection for the cardiovascular system and other organs through a network of mechanisms that extend beyond their great antioxidant potency<sup>27</sup>.

In a study where rabbits were fed a high cholesterol diet for 6 weeks, to markedly elevate their blood cholesterol and induce atherosclerotic lesions in their aortas and carotid arteries. One group of rabbits received grape seed phytosome in their feed for the first 6 weeks, then 4 weeks of the high-cholesterol diet. These developed significantly less aortic plaque than did the control groups which received conventional standardized grape seed extract in similar regimen. In a randomized human trial, young healthy volunteers received grape seed phytosome once daily for 5 days. The blood TRAP (Total Radical-trapping Antioxidant Parameter) was measured at several time intervals during 1st day, then also on 5th day. Already by 30 mins after administration on 1st day, blood

TRAP levels were significantly elevated over the control which received conventional standardized grape seed extract<sup>28</sup>.

Maiti et al. developed the quercetin-phospholipid phytosomal complex by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride<sup>29</sup>.

Maiti et al. developed the phytosomes of curcumin (flavonoid from turmeric, *Curcuma longa*) and naringenin (a flavonoid from grape fruit, *Vitis vinifera*) in two different studies. The antioxidant activity of the complex was significantly higher than pure curcumin in all dose levels tested. In the other study the developed phytosome of naringenin produced better antioxidant activity than the free compound with a prolonged duration of action, which may be due to decrease in the rapid elimination of the molecule from body.

Hesperetin is a potent phytomolecule abundant in citrus fruits, such as grapefruit and oranges. In spite of several therapeutic benefits viz. antioxidant, lipid-lowering, anti-carcinogenic activities their shorter half life and lower clearance from the body restricts its use. To overcome this limitation, recently Mukerjee et al. developed a novel hesperetin phytosome by complexing hesperetin with hydrogenated phosphatidyl choline. This complex was then evaluated for antioxidant activity in CCl<sub>4</sub>-intoxicated rats along with pharmacokinetic studies. It was found that the phytosome had a sustained release property for over 24 h and enhanced antioxidant activity. Pharmacokinetic study revealed that the phytosome had higher relative bioavailability than that of parent molecule at the same dose level<sup>30</sup>.

## CONCLUSION

Phytosomes are advanced form of herbal extract that are better absorbed which results better than conventional herbal extract. Phytosomes have improved pharmacokinetic and pharmacological parameter, which in result can advantageously be used in treatment of acute liver diseases, either metabolic or infective origin. Absorption of phytosome in gastro-intestinal tract is appreciably greater resulting in increased plasma level than the individual component. As mention in the literature, Phytosomes have been therapeutically used for hepatoprotective and liver diseases. After screening and selection for phytoconstituents for therapeutics use, phytosomal drug delivery can be developed for various categories like anticancer, cardiovascular and anti-inflammatory activities.

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