Review Article

Phytosomes: A Novel Molecular Nano Complex Between Phytomolecule and Phospholipid as a Value added Herbal Drug Delivery System

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ABSTRACT

Phytosomes are novel form of herbal formulations which contains the bioactive phytoconstituent(s) of herb extract complexed with phospholipid to produce lipid compatible molecular complexes. Since phytoconstituents are obtained from natural resources, fewer side effects and lower phytochemical costs are added advantages for their utilization in treatment of various diseases. Unfortunately, despite the wide therapeutic potentials of poly phenolic phytoconstituents such as flavonoids, glycosides, terpenoids etc. still they suffer with poor aqueous solubility, absorption and bioavailability problems when administered orally or by topical applications. The effectiveness of any herbal product is dependent upon delivering an effective level of active compounds. The phytosome technology meets this challenge by markedly enhancing the solubility, absorption and bioavailability of the phytomedicines for better drug delivery and clinical action. It exhibit better pharmacokinetic and pharmacodynamic profile than the conventional herbal extracts.

Keywords: Phytosome, Phytoconstituents, Phospholipid, Complex, Bioavailability.

INTRODUCTION

In recent past, herbal drugs are used as promising candidates for the treatment of various diseases, fewer side effects and lower cost of phytoconstituents are value-added benefits leads to enormous usage of herbal medicines by the people. Most of the bioactive constituents of phytomedicines are plant secondary metabolites like flavonoids, glycosides, terpenoids etc.[1] which are known to possess several pharmacological activities but they are poorly absorbed when taken orally or when applied topically. Two main reasons behind the poor oral bioavailability of these phytoconstituents are the heavy molecular structure which cannot be absorbed by simple diffusion and their poor lipid solubility which limits their ability to pass across the lipid-rich outer membranes of the enterocytes of small intestine. Therefore, a large standard dose is usually required for oral dosage regimens, which limits the widespread use of phytomedicines in the pharmaceutical field. The effectiveness of any herbal product is dependent upon delivering an effective level of the active compounds. The phytosome technology developed by Indena® meets this challenge by markedly enhancing the bioavailability of phytoconstituents.[2-4]

Phytosomes

Phytosomes are the novel form of herbal formulations contains the bioactive phytoconstituent(s) of herb extract complexed with phospholipid to produce lipid compatible molecular complexes, when treated with water, these complexes form a micellar structures.[5] Phytosome is a newly introduced patented technology in which phytomolecule form complex with phospholipid by developing hydrogen bonds. They are able to transfer from the water phase external to the enterocyte lipid layer and from there into the cell, finally reaching into the blood.[6] Such a complex results from the reaction of stoichiometric amounts of phospholipid with the selected polyphenolic phytoconstituent (such as simple flavonoids) in a nonpolar solvent.[7]

On the basis of their physicochemical and spectroscopic data, it has been proved 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 functional groups of the substrate, active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane. For example, Semalty A *et al.,* showed that there is a formation of H-bonds between the phenolic hydroxyl end of the flavone moiety and the phosphate ion on the phosphatidylcholine moiety in catechin-phosphatidylcholine complex supported by ^{1}H-NMR and ^{13}C-NMR spectra of the complex with those of the pure phytomolecule.[8] The signals of fatty chain remain almost unchanged. Such evidence inferred that the too long aliphatic chains are wrapped around the active principle, producing a lipophilic envelope, which shields the polar head of the phospholipid and flavonoid molecule and enables the complex to dissolve in low polarity solvents.[9,10]

The phospholipid mainly used in the preparation of phytosomes is phosphatidylcholine which is the principal molecular building block of cell membranes miscible both in water and in oil environments, and is well absorbed when taken orally. Chemical analysis indicates that the phytosome is usually a flavonoid molecule linked with at least one phosphatidylcholine molecule. A bond is formed between these two molecules, creating a hybrid

molecule. This highly lipid-miscible hybrid bond is better suited to merge into the lipid phase of the enterocyte's outer cell membrane so they are more bioavailable as compared with conventional herbal extracts owing to their enhanced capacity to cross the lipid-rich biomembranes and finally reaches the blood. Phosphatidylcholine is not merely a passive "carrier" for the bioactive flavonoids of the phytosomes, it itself is a bioactive nutrient with documented clinical evidence of hepatoprotective activity.[11] Phosphatidylcholine is miscible in water phase and in oil/lipid phases, and is excellently absorbed when taken by mouth. Phosphatidylcholine is the principal molecular building block for cell membranes and its molecular properties make it ideal to perform its phytosomal role.[12] Pharmacokinetic and pharmacodynamic studies in experimental animals and in human subjects proved the increased bioavailability of the phytosomes over the non-complexed phytoconstituents.[13-16]

Phytosomes differ from liposomes

Phytosomes are not liposomes, fundamental differences exist between a phytosome and a liposome. Structurally, both are very different from each other as shown in Figure 1. Unlike phytosomes, liposomes are formed by mixing a hydrophilic or lipophilic drug with lipid. No chemical bond is formed and the lipid molecules surround the hydrophilic drug substance. There may be hundreds or even thousands of phosphatidylcholine molecules surrounding the hydrophilic drug substance. But in the phytosomes, the phosphatidylcholine and the individual plant component actually form a molecular complex either 1:1 or 1:2 depending on the substance.[17] Furthermore, in liposomes phospholipid content is five times higher than that of the phytosomes, making the delivery form not suitable for oral delivery of natural compounds. The phytosome is a unit of a few molecules and this makes a difference so that the phytosomes are much better absorbed than liposomes.

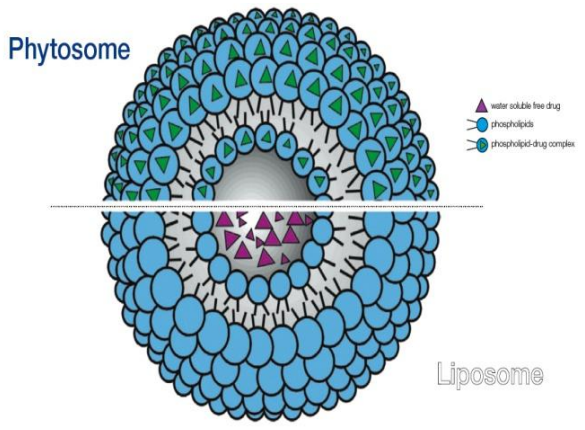

Figure 1: The molecular organization of the liposome (upper segment) versus many individual phytosomes (lower segment).

Merits of phytosomes over conventional dosage forms

The phytosomes have tremendous advantages over the conventional dosage forms. They enhance the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability followed by greater therapeutic benefits. They improve the absorption of active constituent(s) which further reduce its dose requirement. Phosphatidylcholine used in the preparation of phytosomes not only acts as a carrier but also it gives synergistic effect when hepatoprotective substances are used. Chemical bonds are formed between phosphatidylcholine molecules and phytoconstituent molecules, increases the stability profile of the formulation. Application of phytoconstituents in the form of phytosome improves their percutaneous absorption and act as functional cosmetics. The nutrient safety of the herbal extracts need not be compromised by converting the herbal drug as means of phytosomes. The phytomolecule in the phytosomes get protected from destruction by digestive secretions.[18]

Preparation Methods

Phytosomes are formulated by process in which the phytoconstituent(s) bound to the phospholipids like phosphatidylcholine (PC) through a polar end. Phytosomes are prepared by the reflex reaction between 1-3 moles (preferably with 1 mole) of a natural or synthetic phospholipid, such as phosphatidylcholine, phosphatidyl- ethanolamine or phosphatidylserine, with one mole of phytoconstituent either alone or in the natural mixture in an aprotic solvent, such as dioxane, acetone, dichloromethane etc.[19] The optimum ratio of phospholipid to phytoconstituent is 1:1 or 1:2. The complex thus formed can be isolated by precipitation with an aliphatic hydrocarbon, lyophilization or by spray drying method.

Jiang *et al.,* optimized the preparation conditions using a uniform design and step regression and prepared *herba epimedii* total flavonoid phytosomes (EFP) by means of solvent evaporation and investigated the cumulative dissolution of different ratios of EFP-PVP precipitates by means of dissolution release. The optimized preparation conditions were as follows: solvent-tetrahydrofuran, lecithin to PVP ratio 2:5, temperature 40˚C and reaction time 3 hrs.[20] Maiti *et al.,* developed the quercetin–phospholipid complex to overcome the very poor absorption of quercetin when administered orally. Phytosomes were prepared by reflexing 1 mole of quercetin with 1 mole of HSPC in 20 ml of dichloromethane till all the quercetin dissolved. The volume of the resulting solution was reduced to 2–3 ml and 10 ml of n–hexane was added to above solution to get the complex as precipitate. The complex was then filtered and dried under vacuum.[21] Freag *et al.,* developed novel diosmin loaded phytosomes inorder to improve drug dissolution and intestinal permeability. Phytosomes were prepared by solvent evaporation, salting out and lyophilisation method by using diosmin (DSN) and

soyabean phosphatidyl choline (SPC) in molar ratio 1:1 (F1), 1:2 (F2) and 1:4 (F3) and compared. For the solvent evaporation method, DSN (0.1% weight/ volume) and SPC (Lipoid® S100; Lipoid Co) were suspended in a dioxane-methanol mixture (7:3), refluxed for 5 hrs, then the solvent was evaporated under vacuum. For the salting out method, DSN (0.1% weight/volume) and SPC were dissolved in a mixture of DMSO, dehydrated ethanol, and chloroform (2:2:3) to reach a final volume of 35 mL. The solution was then stirred on a magnetic stirrer overnight then n-hexane (75 mL) was added until precipitation occurred. For the lyophilization technique, DSN was completely dissolved in DMSO. The resulting DSN solution (2.5% weight/volume) was added to the solution of SPC dissolved in t-butylalchol (1.5% weight/volume) followed by stirring for 3 hrs on a magnetic stirrer until complex formation. The complex was then isolated by lyophilization.[22]

Phytosome Formulations

Phytosome complexes can be formulated for oral as well as topical administration. Some possible phytosomal formulations are as follows,

Soft gelatin capsules

Soft gelatin capsules represent an ideal solution to formulate phytosome complexes. The phytosome complex can be dispersed in oily vehicles to obtain suspensions to be filled in soft gelatin capsules. Vegetable or semi-synthetic oils can be used to this purpose. Indena® recommend a granulometry of 100% <200 µm to best perform capsule production. According to Indena® experience, not all the phytosome complexes behave in the same way when dispersed in oily vehicles and when the oily suspension is filled in the soft gelatin capsules; for this reason preliminary feasibility trials should be performed to select the most suitable vehicle. Example: Ginkgoselect® Phytosome®

Hard gelatin capsules

The Phytosome complex can be formulated in hard gelatin capsules as well. A direct volumetric filling process (without precompression) can be applied, even if the apparently low density of the phytosome complex seems to limit the maximum amount of powder that can be filled into a capsule (usually not more than 300 mg for a size 0 capsule). With a piston tamp capsule filling process, however, it is possible to increase the amount of powder which can be filled in a capsule, but precompression might affect the disintegration time. Indena® recommends monitoring the related parameters during product/ process development. A preliminary dry granulation process is advisable define the best manufacturing process. Example: Ginkgoselect® Phytosome®

Tablets

Dry granulation represents the ideal manufacturing process to obtain tablets with higher unitary doses and with suitable technological and biopharmaceutical properties. However, due to the limited flowability, potential stickiness and low apparent density of the phytosome complex, a direct compression process can be applied only for low unitary doses; note that whenever a direct compression process is applied, the phytosome complex should be diluted with 60-70% of excipients to optimize its technological properties and to obtain tablets with appropriate technological and biopharmaceutical characteristics. On the other hand, wet granulation should be avoided due to the negative effect of water and heat (granulation/ drying) on the stability of the phospholipid complex. Example: Leucoselect® Phytosome®

Topical dosage forms

The phytosome complex can be formulated topically as well. The ideal process to incorporate the phytosome complex in emulsion is by dispersing the phospholipidic complex in a small amount of the lipid phase and add it to the already created emulsion at low temperatures (not higher than 40°C). The phytosome complexes are dispersible in the main lipidic solvents employed in topical formulations. In case of formulations containing a limited amount of lipids, the phytosome complex might also by dispersed into the watery phase, and again added to the final formulation at temperature lower than 40°C. Example: Escin/ß-Sytosterol Phytosome®, Glycyrrhetinic acid Phytosome®.

Evaluation of Phytosomes

Phytosomes can be characterized in terms of their physical attributes i.e. particle size, distribution, surface charge, drug entrapment, percentage drug released and chemical composition to know the behavior of the phytosomes in physical and biological systems. The followings are different charcaterization techniques used to characterize the phytosomes,

Surface morphology

Surface morphology of phytosomes can be observed using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).[23]

Particle size and surface charge

The particle size, size distribution as poly dispersity index (PDI) and surface charge as zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS).[24]

Entrapment efficiency

The drug entrapment efficiency in phytosomes can be measured by the ultracentrifugation technique. [24]

Transition temperature

The thermal behavior off the phytosomes can be determined by differential scanning calorimetry (DSC).[25]

Vesicle stability

The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. The mean size is measured by DLS and structural changes are monitored by TEM.[26]

Drug content

The amount of drug can be quantified by a modified high performance liquid chromatographic method or by a suitable spectroscopic method.[26]

Spectroscopic evaluation

Formation of complex between phytomolecule and phospholipid moiety as well as the corresponding interactions between the two can be studied by the following spectroscopic evaluations,[8,16,22]

^{1}H-NMR: The complex formation between the active phytoconstituents and the phosphatidylcholine molecule can be estimated by this method. Bombardelli *et al.,* studied the NMR spectra of phytosome complex in nonpolar solvents. There was a marked change in ^{1}H-NMR signal originating from atoms involved in the formation of complex, without any summation of the signal peculiar to individual molecules. The signals from protons belonging to the phytoconstituents are broadened. In phospholipids there is broadening of signals while the singlet corresponding to the N-$(CH_3)_3$ of choline undergoes an upfield shift.

^{13}C-NMR: In the ^{13}C NMR of the phytoconstituents and the stoichiometric complex with the phosphatidylcholine when recorded in C_6D_6 at room temperature all the phytoconstituents carbons were invisible. The signals corresponding to the glycerol and choline portion are broadened and some are shifted, while most of the resonance of the fatty acid chains retains their original sharp line shape.

FTIR: The formation of the complex also can be confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. FTIR spectroscopy is also a useful tool for the control of the stability of phytosomes when micro-dispersed in water or when incorporated in very simple cosmetic gels. From a practical point of view, the stability can be confirmed by comparing the spectrum of the complex in solid form (phytosomes) with the spectrum of its micro-dispersion in water after lyophilization, at different times. In the case of simple formulations, it is necessary to subtract the spectrum of the excipients (blank) from the spectrum of the cosmetic form at different times, comparing the remaining spectrum of the complex itself.

***In vitro* and *in vivo* evaluations**

Models of *in vitro* and *in vivo* evaluations are selected on the basis of the expected therapeutic activity of the biologically active phytoconstituents present in the phytosomes. For example, *in vitro* antihepatotoxic activity can be assessed by the antioxidant and free radical scavenging activity of the phytosomes.[27] For assessing antihepatotoxic activity *in vivo*, the effect of phytosomes in animals (wistar or albino rat model) against thioacetamide, carbon tetrachloride, paracetamol or alcohol induced hepatoxicity can be examined.[21] Bioavailability and PK studies of phytosomes conducted in rat models and also on humans.[28]

Some Patented Technologies Related to Phytosomes

There are a number of innovative processes and formulation research studies in the field of phytosomes carried out by academic scientists as well as in industrial laboratories. Some patents for phytosomes and other related technologies along with their applications and innovations are listed in Table 1.

Table 1: Some patented technologies related to phytosomes.

Title of patent	Innovation	Patent number
Phospholipid complexes of olive fruits or leaf extracts having improved bioavailability	Phospholipids complexes of olive fruits or leaf extracts or compositions having improved bioavailability.	EP/1844785
Compositions comprising *ginko biloba* derivatives for the treatment of asthmatic and allergic conditions	Compositions containing fractions deriving from *ginkgo biloba*, useful for the treatment of asthmatic and allergic conditions.	EP1813280
Fatty acid monoesters of sorbityl furfural and compositions for cosmetic and dermatological use	Fatty acid monoesters of sorbityl furfural selected from two diff series of compounds in which side chain is a linear or branched C_3 -C_{19} alkyl radical optionally containing at least one ethylenic unsaturation.	EP1690862
Cosmetic and dermatological composition for the treatment of aging or photodamaged skin	Composition for topical treatment of the skin comprises a substance that stimulates collagen synthesis and a substance that enhances the interaction between extracellular matrix and fibroblasts Cosmetic or dermatological composition for topical treatment.	EP1640041
Treatment of skin and wound repair with thymosin β4.	Compositions and methods for treatment of skin utilizing thymosin β4.	US/2007/0015698

Soluble isoflavone compositions	Isoflavone compositions exhibiting improved solubility (Example: light transmittance), taste, color, texture characteristics and methods for making.	WO/2004/045541
An anti-oxidant preparation based on plant extracts for the treatment of circulation and adiposity problems	Preparation based on plant extracts which has an anti-oxidant effect and is particularly useful in treatment of circulation problems such as phlebitis, varicose veins, arteriosclerosis, haemorrhoids and high blood pressure.	EP1214084
Complexes of saponins with phospholipids and pharmaceutical and cosmetic compositions containing them	Complexes of saponins with natural or synthetic phospholipids have high lipophilic and improved bioavailability and are suitable for use as active principle in pharmaceutical, dermatologic and cosmetic compositions.	EP0283713

Applications of Phytosomes

Some marketed phytosome formulations with their active constituents, the daily dose and specific indications are given in Table 2.

Table 2: Therapeutic applications of some phytosomes with their dose.

Phytosomes	Phytoconstituent complexed with pc	Daily dosage	Indication
Leucoselect® phytosome	Procyanidolic oligomers (PCOs) from grape seeds	50–100mg	Systemic antioxidant, specific. Best choice for most people under age of fifty. Also specific for the eyes, lungs, diabetes, varicose veins, and protection against heart disease.
Greenselect® phytosome	Epigallocatechin 3-O-gallate from camelia sinensis (Green tea)	50–100mg	Systemic antioxidant. Best choice for protection against cancer and damage to cholesterol.
Ginkgoselect® phytosome	24 % ginkgo flavono glycosides From Ginkgo biloba	120mg	Best choice for most people over the age of 50. Protects brain and vascular lining.
Silybin phytosome	Silybin from silymarin (milk thistle)	120mg	Best choice if the liver or skin needs additional antioxidant protection.
Siliphos™ milk thistle phytosome	Silybin from silymarin	150mg	Good choice for liver or skin support.
Hawthorn phytosome	Flavonoids	100mg	Best choice in heart disease.
Panax ginseng phytosome	37.5% ginsenosides from roots of Panax ginseng	150mg	As a Food Product.
Glycyrrhiza phytosome	18-beta glycyrrhetinic acid	-	Anti-inflammatory Activity.
Mirtoselect® phytosome	Anthocyanosides from an extract of Bilberry	-	These improve capillary tone, reduce abnormal blood vessel permeability & are potent antioxidants. They hold great potential for the management of retinal blood vessel problems and venous insufficiency.
Sabalselect® phytosome	An extract of saw palmet to Berries through supercritical CO_2 (carbon dioxide) extraction	-	It delivers fatty acids, alcohols and sterols that benefit prostate health. Also beneficial for non-cancerous prostate enlargement
Polinacea™ phytosome	Echinacosides and a unique high-molecular weight Polysaccharide from Echinacea angustifolia	-	It enhances immune function in response to a toxic challenge.
Oleaselect™ phytosome	Polyphenols from olive oil	-	As potent antioxidants, inhibit harmful oxidation of LDL cholesterol, and also have anti-inflammatory activity.
Lymphaselect™ phytosome	A standardized extract of melilotus officinalis	-	Indicated for venous disorders, including chronic venous insufficiency of the lower limbs.

CONCLUSION

The phytoconstiuents such as flavonoids, glycosides, terpenoids etc. have been found to possess great beneficial pharmacological activities to treat various diseases. But due to certain lacunae, especially the phenolic compounds, their phenolic nature affects the oral absorption and bioavailability. These aspects constitute a hindrance against the widespread use of these phytoconstituents in the pharmaceutical field. These hindrances can be tackled by formulating an appropriate drug delivery system. Phospholipid-based drug delivery system has been found promising for better and effective delivery of natural drug and can enhance the rate and extent of drug absorption across the lipoidal biomembrane. Phytosomes, as a value added formulation offered a successful pathway towards the utilization of these phytoconstituents, with improved bioavailability through the skin or gastrointestinal tract. They have distinctive advantages over other several drawbacks of conventional formulations. The formulation methodology for phytosome is simple, reproducible and can be easily upgraded to a commercial scale. As far as potential of the phytosome technology is concerned, it has a great future for use in formulation technology and applications of hydrophilic plant compounds. The phytosome technology may open a new avenue in the field of herbal drug research by delivering the active phytoconstituent in the formulation effectively in controlled release manner with increased bioavailability and improved bioactivity.

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