REVIEW ARTICLE

Phytosome: A Novel Approach to Enhance the Bioavailability of Phytoconstituent

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Abstract

Novel drug delivery systems have gained significant importance because of their enhanced bioavailability and overall therapeutics. Hydrophilic nature and unique chemical structure of most of the therapeutically useful phytoconstituents which result in poor bioavailability and less absorption. The water soluble phytoconstituents have excellent bioactivity *in vitro*, but less or no action *in vivo*. Phytosome technology can overcome this problem in which the phytoconstituent is allowed to react with phospholipid. The phytoconstituent with poor lipid solubility on complexation into phytosome, can exhibit better pharmacodynamics and pharmacokinetics profile as compared to conventional herbal extract. Phytoconstituent must have good hydrophilicity which helps in dissolution in gastrointestinal fluid and hydrophobicity which helps cross lipid-rich cell membrane. Phytosome technology results in an intermolecular bonding between phospholipid, phosphatidylcholine and a single molecule of phytoconstituent. Phosphatidylcholine used in phytosome technology has also proven its clinical efficacy by acting as a carrier for fat and water miscible nutrients and natural digestive aid. Phytosomes can be used to treat acute and chronic liver failure due to improved pharmacological and pharmacokinetic property. In market, many products based on phytosome technology are available which include herbal extracts and phytochemicals with great therapeutic potential such as curcumin, ginkgo biloba, grape seed, silymarin, and many more. The present review highlights the method of preparation, properties, advantages, characterization, and applications.

Key words: Bioavailability, phosphatidylcholine, phospholipid, phytosome

INTRODUCTION

To deliver the drug at predetermined rate, during the period of treatment novel drug delivery system can be used. Uses of traditional phytomedicine since ancient times is popular to manage human disease efficiently.[1] The separation and purification of herbal extract may lead to partial loss of specific activity. Herbal products effectiveness depends on delivering an effective level of active component. However, water soluble phytoconstituent (flavonoids, terpenoids, and tannins) have poor lipid solubility due to large molecular size, which limit their ability to cross lipid-rich biological membrane, results in poor bioavailability.[2] It is a patented technology[3] developed by Indena, enhancing the bioavailability of phytoconstituent.[2,4] Phytosomes involve incorporation of water soluble phytoconstituent into phospholipids, which forms lipid compatible molecular complexes and so vastly improve their absorption and bioavailability.[5] It forms a little cell where the valuable phytoconstituent is protected from digestive secretion and gut enzyme. In phytosome, the hydrophilic phytoconstituent transits into lipid friendly environment of enterocyte cell membrane and finally reaching the blood.[6] It can be used to treat acute and chronic liver failure due to improved pharmacokinetic and pharmacological properties and therapeutically used as dietary supplement.[7] In this article, an attempt has been made to touch upon the different aspect phytosomal drug delivery system.

PHYTOSOME TECHNOLOGY

Phytosome technology had improved the absorption and bioavailability of selected phytoconstituents, by incorporating phospholipids into standardized plant

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extracts.[8] When a stoichiometric amount of phospholipid (phosphatidylcholine) reacted with standardized extract in nonpolar solvent.[3] Phytosomes form a bridge between conventional delivery system and novel delivery system and is also called as phytolipid delivery system. The word "phyto" means plant and "some" means cell-like.[9] Phosphatidylcholine is bi-functional compound, where the nature of choline moiety is hydrophilic and phosphatidyl moiety is lipophillic. In phyto-phospholipid complex, the choline head of phosphatidylcholine molecule bind to the phytoactive constituent while the lipid-soluble portion wraps the choline bound material. Hence, it produces phyto-phospholipid complex. Through spectroscopic techniques, it was analyzed that molecules are hooked through chemical bonds to the choline head of phosphatidylcholine.[8,10] For enhancement of bioavailability, greater clinical benefit assured delivery to the tissue phytosome technology has been useful.[3]

PREPARATION OF PHYTOSOMES

Phytosomes are prepared by different methods by interacting 3-2 moles natural or synthetic phospholipid, mainly phosphotidylcholine with one mole of phytoconstituent. The most preferable ratio for complexes formation between these two moieties is in the range from 0.5 to 2.0 moles.[11]

Solvent evaporation method

A natural or synthetic phospholipid phosphotidylcholine and phytoconstituent is suspended in an appropriate solvent, further refluxed for few hours. The resultant clear mixture is being evaporated under vaccum.[12]

Salting out method

The phytoconstituent or standardized extract and phosphotidylcholine is dissolved in an aprotic solvent, such as dioxane or acetone where the solution is being stirred overnight then the formed complex is isolated from by precipitation from non-solvent like n-hexane.[13]

Lyophilization technique

Both natural or synthetic phospholipid and phytoconstituent is dissolved in different solvent and further solution containing phytoconstituent were added to a solution containing phospholipid followed by stirring till complex formation takes place. The formed complex is isolated by lyophilization.[7]

The phospholipid which are used in preparation of phytosome consist of acyl group which may be same or different in phosphatidylcholine, phosphatidylserine, phosphatidyl ethanolamine and mostly derived from palmitic, stearic, oleic, and linoleic acid.[14,15] In phytosome active principle becomes an integral part of the membrane as the active principle is anchored to the polar head of phospholipid.[6]

COMMON STAGES IN THE PREPARATION OF PHYTOSOME

Phospholipid is dissolved in organic solvent containing drug/extract (1:1)

⇩

Solution of phospholipid in organic solvent with drug/extract

⇩

Drying

⇩

Formation of thin film

⇩

Hydration

⇩

Formation of phytosomal complex

⇩

Isolation by precipitation

(Lyophilization or spay drying)

ROLE OF PHOSPHATIDYLCHOLINE IN PHYTOSOME PREPARATION

Phospholipid serves as a major moiety in composition of cellular and subcellular membrane. They are basic substance to maintain life activity. The human body uses phosphpolipid as emulsifiers and also enhances the absorption of fat-soluble substances. Furthermore, it act as surface active agent in the pleura and alveoli of lung, joints, pericardium, etc.[16] They can be extracted from egg yolk or soybeans through mechanical or chemical methods with the aid of hexane. Phosphatidylcholine has two groups mainly lipophilic, phosphatidyl group, and the choline group which is a hydrophilic moiety. Choline moiety improves memory function and aids muscle control. The choline portion binds to the herbal extract while the

phosphatidyl group covers the phytoconstituents like a cell form which further protects the active constituent from destruction from the digestive juices. Due to drug-phospholipid complex formation the bioavailability of the active constituent is increased, along with prolonged duration of action.[17]

PROPERTIES OF PHYTOSOMES

Physicochemical properties of phytosomes

Phytosome is a complex formed, on reaction between a phytoconstituent and phospholipid in a suitable solvent.

From spectroscopic analysis data it confirms, that there is a hydrogen bond formation due to main phospholipid-substrate interaction between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functionalities of the substrate. When treated with water, phytosomes assumes a micellar shape forming liposomal-like structures. For example, in the case of the catechin distearoyl-phosphatidylcholine complex, there is the formation of H-bonds between the phenolic hydroxyl ends of the phosphatidylcholine moiety. So when the 1H-NMR and 13C-NMR spectra were compared, the signals obtaining from phosphatidylcholine can be deduced with those of pure precursors from the spectra obtained. Such information conclude that the too long aliphatic chains are enwrapped the active principle, producing lipophilic envelope, which protects the polar head of the phospholipid and flavonoid molecule and modify the complex to dissolve in low polarity solvents.[18]

BIOLOGICAL PROPERTIES

Herbal extracts when developed in the form of phytosome display better absorption than conventional herbal extract which leads to an increase in bioavailability than non-complexed conventional herbal extracts which has been evaluated by pharmacokinetic studies and pharmacodynamic tests in experimental animals and human beings.[19]

ADVANTAGES

1. The absorption of lipid insoluble polar phytoconstituent is increased through oral or topical route, which results in better bioavailability and greater therapeutic benefit.
2. Dose reduction as the absorption of active constituent is improved.
3. Phosphatidylcholine is a carrier in phytosome preparation, but it also have hepatoprotective, giving synergistic effect when employed with hepatoprotective agent.
4. Drug itself in conjugation in lipid is forming vesicle; hence, the entrapment efficiency is high.
5. It shows better stability profile due to formation of chemical bond between phosphatidylcholine molecule and phytosonstituent.
6. On complex formation, a cell-like structure is produced which protects the valuable component of herbal extract is protected from digestive secretions and gut bacteria.
7. The component used in phytosome formulation have all been approved for pharmaceutical and cosmetic use, due to their improved skin penetration and lipid profile, therefore phytosome are safe.
8. The phytosome system is non-invasive, passive and can be suitable for immediate commercialization.
9. It has no large-scale drug development risk the since the toxicological profile of phytosomal component is well documented in scientific literature.
10. It is simple to manufacture, with no complicated technical investment for production of phytosome.

DIFFERENCE BETWEEN PHYTOSOME AND LIPOSOME

The distinguishing points are mentioned in Table 1 which states phytosome a better vesicular drug delivery system than liposomes with respect to bonding affinity, adsorption and bioavailablity, molecular arrangement along with the solvents used.

Table 1: Difference between Phytosome and Liposome

Property	Phytosome	Liposome
Bonding[20]	Phytosomes is a unit of few molecules bonded together by chemical bonding	In liposome, the phytoactive molecules are enclosed by an aggregate of many phospholipid molecules without specifically bonding to them
Bioavailability and absorption	Better	Lesser than phytosome
Molecules arrangement[21]	In phytosome, an individual phytoconstituent and phospholipid (PC) are present in 1:1 or 1:2 ratios depending on substance	In liposome, the water soluble molecule is surrounded by hundreds and thousands of molecules
Solvents used[22,23]	It acts with solvents having reduced dielectric constant	Liposome drug complex is formed in the presence of buffer solution or water

CHARACTERIZATION TECHNIQUES

Visualization

Transmission electron microscopy and scanning electron microscopy are used for visualization of phytosomes.[24]

Vesical size and zeta potential

Dynamic light scattering (DLS) using computerized inspection system and photon correlation spectroscopy (PCS) used to determined vesical size and zeta potential.[25]

Entrapment efficiency

Ultracentrifugation technique is used to determined entrapment efficiency.[3]

Transition temperature

Differential scanning calorimetry is used to determine transiton temperature of vesicular lipid system.[26]

Surface tension measurement

Surface tension activity can be measured by ring method in a Du Nouy ring tensiometer of the drug in aqueous solution.[27]

Vesicle stability

Assessing the size and the structure of vesicles overtime gives the idea about stability of vesicles. Structural changes are monitored by TEM and mean size is measured by DLS.[28]

Drug content

Modified high-performance liquid chromatographic method or suitable spectroscopic method used to quantify the amount of drug present.[29]

SPECTROSCOPIC EVALUATION

NMR

The NMR spectroscopic evaluation is an effective tool for structure elucidation of molecular structure. It is also helpful in knowing electron distribution in molecules along with quantum mechanical nature of bonds, based on the data the formation of the phytosomes can be concluded. To explain the spectroscopic evaluation an example is cited below.

H-NMR

Bombardelli *et al.* studied the NMR spectra of (+) cathechin, a flavanoid and its stiochiometric complex with disteroylphosphatidylcholine.[27] First, an individual 1H-NMR spectra is obtained for (+) cathechin and disteroylphosphatidylcholine which is used for comparison with the complex. In non-polar solvent, 1H-NMR signal originating from the atoms involved the formation of complex, marked change was observed, without summation of signals belonging to individual molecules. The signals obtained from flavonoids are broadened that the proton cannot relieve along with broadening of all the signals in phospholipids, while the singlet corresponding to N-(CH3)3 of choline undergo an uplift shift. The signal revealed disteroylphosphatidylcholine conceal the signal from polyphenol which suggested complex formation. On heating, the sample to 60° results in appearance of some new broad bands, which mainly corresponds to the resonance of flavanoid moiety.[30]

13C-NMR

Similar the 13C-NMR analysis was carried for (+) catechin, disteroylphosphatidylcholine and (+) catechin-disteroylphosphatidylcholine complex. In C6D6, at room temperature spectrum of (+) catechin and its stiochiometric complex with disteroylphosphatidylcholine when recorded, all the flavanoid carbons were hidden. The signals representing to the glycerol and choline portion of the lipid are broadened and some are shifted, while most of the resonance of the fatty acid chains retains their original sharp line shape. On heating to 60°, and all the signal belonging to the flavanoid moiety reappear, although still, they are overlapping and broad.[30]

FTIR

IR spectroscopy confirms the complex formation by comparing the spectrum of the complex with the spectrum of the individual component and their physical mixture. For the control of the stability of the phytosome, when microdispersed in water or when incorporated into simple cosmetic gel FTIR spectroscopy is an important tool. By comparing the spectra of the complex in solid form with the spectra in micro dispersion in water after lyophilization, at different times, stability can be confirmed.[31]

IN VIVO EVALUATION

Experimental models are chosen on the basis of anticipated therapeutic activity of the plant constituent in phytosome for *in vivo* and *in vitro* examination. For example, examination of antihepatotoxic activity can be assessed by antioxidant or free radical scavenging property of phytosome. The *in vivo* anti-hepatotoxic studies on animals through the effect of phytosome on alcohol induced or paracetamol-induced hepatotoxicity.[32]

APPLICATIONS AND RESEARCH DONE ON PHYTOSOME

Clinical usefulness of oral supplement, a combination product containing alpha-lipoic acid, curumin phytosome, and B group vitamins by Giorgi *et al*.[33] It was carried out in 180 patients with carpel tunnel symdrome (CTS). The treatment was associated with good compliance and high level of satisfaction; it resulted into clinical usefulness of this supplement before and after surgery in CTS patients.

Hepatoprotective effect of curcumin, Silybin Phytosome(®) and α-R-lipoic acid was evaluated against thioacetamide by Ali *et al.* in Rats. Liver's Serological analysis and histopathological results showed an increase hepatic level of malondialdehyde, gluthathione depletion, macrophage activation also IL-6 and tumor necrosis factor-α decreased which indicates inhibition of oxidative stress and anti-inflamattory activity.[34]

The relative absorption of standardized curcuminoid mixture and its corresponding lecithin formulation (Meriva) was examined by Cuomo *et al*.[35] in a randomised, double blind cross-over design human study. It resulted into improved absoption and better plasma profile than unformulated curcuminoid mixture.

Complex formation of curumin with phosphatidylcholine was compared with curcumin in topical formulation by Gupta, Dixit.[36] On comparision of the activity, it showed that the high amount of curcumin in topical preparation did not provide better bioavailability, whereas phytosomes due to their amphiphilic nature of the complex enhances the water and lipid miscibility of curcumin.

The beneficial effect of Greenselect Phytosome®, a proprietary lecithin formulation was evaluated by Gianni *et al*.[37] It was carried out in 50 asymtomatic participants for metabolic syndrome factors and with increased plasma oxidative stress. The results was compared to control (lifestyle, dietary changes alone), Greenselect Phytosome® was found to be effective for waist/weight change. It leads to the relevance of multiple factor involved in metabolic syndrome and improving the beneficial effects of lifestyle and dietary changes and improved health profile.

Preparation of silymarin (SM) phytosome and its pharmacokinetic studies is carried out in rats by Yanyu *et al*.[13] The study consequence into increased bioavailability of Silybin after oral administration owing to improved lipophilic property of Silybin-phospholipid complex and increased biological effect of Silybin.

A standardized mixture of flavolignans was extracted from fruit of *S. marianum* from which SM phytosomes was prepared, studied by Bombardelli *et al*.[30] Higher specific activity and long-lasting action than single component were shown by SM phytosomes with respect to percent reduction of edema, antioxidant, and free redical scavenging properties, inhibition of myeloperoxidase activity.

The antioxidant effect of grape seed extract was evaluated by single-blinded cross-over, placebo-controlled trial in 20 participants with coronary artery disease. The patients received 300 mg of grape extract for 5 days. No effect was observed on serum Vitamin C and E but on 5th day the total antioxidant activity was increased from 408.1 ± 22.9 to 453.3 ± 453.3 micromol/l Trolox equal to 1 h postdose.[38]

Sinigrin, a glucosinolates was complexed with phosphatidylcholine and was compared with sinigrin for its wound healing property by Mazumder *et al.* The wound healing and cytotoxic effect was analyzed on A-375 and HaCaT cells, where sinigrin phytosomes exhibit 71% wound closure than sinigrin alone after 42 h and this approach can be explored for wound treatment in case of cancer.[39]

SM, phosholipid complex was prepared by Zeng *et al.* and then, converted into microporous osmotic pump tablets (MPOP). The results reveal the dissolution rate for SM complex was higher than sylimarin alone and release rate for SMMPOP was 85% along with zero order releases profile, which represents its potential application for sustained release formulation.[40]

A standardized pomegranate extract from *Punica granatum* Linn converted to herbosomes, by Vora *et al*.[41] Better antioxidant and hepatoprotective activity were shown by enzyme levels of liver glutathione system, where the rise in the levels of enzyme of liver glutathione system was prevented and also from histopathological examination of liver.

Diomisin, a phlebotonic flavonoid possessed solubility problem which results in poor bioavailablity, so phospholipid complex was prepared by May Freag *et al*.[12] where the prepared phytosomes shows 80% of diomisin was permeated through rats intestine which resulted in enhanced dissolution and permeation characteristics through oxygenated rats intestine and increased drug delivery.

Preparation of berberine phytosome and its pharmacokinetic studies were prepared by Fei Yu *et al*.[42] The study results in increased oral bioavailability of Berberine after oral administration due to improved lipophilic property of berberine-phospholipid complex and improvement of antidiabetic effect of berberine.

A standardized aqueous extract from *Tecomella undulata* stem bark and lectihin was used to formulate phytosomes by Nagpal *et al*.[43] where the prepared phytosomes shows an improvement in bioavailablity without causing structural modification of the phytoconstituents.

Hyaluronic acid phytosomes loaded with L-carnosine was prepared by Abdelkader *et al*.[44] in different molar ratios

by solvent evaporation technique, where the prepared phytosomes showed *ex vivo* transcorneal permeation of L-carnosine loaded phytosome without affecting human corneal cell viability.

Quercitin phytosome was studied for its anti-itching effect in humans by Maramaldi *et al*.[45] was induced by ultraviolet radiation or with histamine solutions. The prepared quercetin phytosome was formulated into 1% cream and 1% dexchlorpheniramine was a positive control. Results for reduction in erythema was measured along with decrease in mean wheal diameter where the findings proved quercetin as skin protective agent against itching and inflammation also increased the hydration of skin.

Quercetin phytosomes were prepared by Minaei *et al*.[46] to enhance the delivery of doxorubicin, a chemotherapuetic agent against MCF-7 cells. MTT assay confirms an increase in permeability of the chemotherapuetic agent to the MCF-7 breast cancer cells with an increase in percentage of apoptosis 40.11 ± 7 to 72-58 ± 7.13 ($P < 0.05$).

Abutilon indicum and Piper longum phytosomes were prepared by Sharma and Sahu[47] to evaluate its hepatoprotective effect in CCl_4 induced animal model agent. The phytosomal formulation showed potent and significant hepatoprotective effect than high dose of combined extract.

Echinacoside possess low bioavailability, so its phytosome were prepared by Li *et al*.[48] the results obtained after preparation of phytosome by solvent evaporation method showed 2.82 fold increase in intestinal absorption rate with an 3.39 fold increase in permeability coefficient and also the pharmacokinetic parameters (Tmax = 1.500 h, Cmax = 3.170 mg/mL, AUC0-∞ = 9.375 mg/L h and AUC0-24 = 7.712 mg/ L h).

A randomized placebo-controlled trial was carried on Greenselect Phytosome® in 20 obese women for weight maintenance after weight loss program. Twenty obese women in one group, one treated with 150 mg/dose of Greenselect Phytosome® and 15 mg/dose of pure piperine (GSP group), second cohort of women were given placebo (P group). A remarkable weight reduction due to lifestyle intervention was observed (−6.2 ± 2.6 in GSP group vs. −4.8 ± 3.1% in P group). The percentage of weight maintenance after deliberate weight loss in obese women was ≥5% in the GSP group than in the P group.[49]

A randomized clinical trial was carried out on 32 patients with mild to severe persistent asthma and there compliance for inhaled corticosteroids and long-acting beta-agonists was verified. The patients have been randomized to receive Casperome® 500 mg/day (Boswellia Phytosome) for a duration of 4 weeks. The results when analyzed showed a decrease in number of inhalations required to the patients than who did not received Casperome® therapy.[50]

A pilot study on curcumin phytosome Meriva® (1 g/day) was administered to 25 diabetic patients. An evidential decrease in the edema score ($P < 0.05$) and an improvement in the venoarteriolar response ($P < 0.05$) was determined in participants using Meriva®, it was found to be well tolerated for the control or management of diabetic microangiopathy.[51]

Furthermore, Meriva® was tested in patients suffering from osteoarthritis to determine its safety and efficacy. Improvement was seen in clinical end points such as Western Ontario and McMaster Universities score, treadmill walking performance and Karnofsky Performance Scale Index when compared to control group. Superior efficacy and safety contributes toward management of osteoarthritis.[52]

A clinical trial was carried out for Greenselect Phytosome® in obese patients ($n = 100$) which compared was compared with green tea extract. After the treatment significant weight loss and reduced body mass index (14 kg weight loss in Greenselect Phytosome® than 5 kg loss in diet only group), reduced waistline. Safety and efficacy were observed along with absence of adverse effect which proved as an effective tool in weight management.[53]

A Phase I trial was carried out for Silybin phtyosome in participants suffering from prostate cancer. The participants received oral treatment for 4 weeks 2.5-20 g daily, in three-divided doses. From the trial, the dose recommended for phase two trail was 13 g, also an adverse event asymptomatic liver toxicity was commonly seen.[54]

SM, a hepatoprotective agent was evaluated in 21 broiler chicks against the toxic effects of alflatoxin B1. As per serum biochemistry decrease in alanine amino transferase was observed in chicks treated with alflatoxin B1 (0.8 mg/kg of feed).[55]

A double blind, randomized cross-over trial was carried out on Leucoselect Phytosome in a cohort of heavy smokers on low-density lipoprotein susceptibility to oxidation. During trial, no adverse effect was observed along with good compliance. As per oxidative indices, the concentration of thiobarbituric acid reactive substances was significantly reduced in cohort of participants taking Leucoselect Phytosome.[56]

Raynaud's disease (RD) is associated with a condition systemic sclerosis. Therefore, a double-blind placebo-controlled trial was carried out on RD patients to study the effectiveness of ginkgo biloba extract (Seredrin), on severity and duration of attacks. Frequency of attacks was reduced significantly in case of treatment, a reduction of 56% (13.2 ± 16.5 reduced to 5.8 ± 8.3) than placebo, 27%. Hence, it was proved effective in reducing the incidence of Raynaud's attack per week in RD patients.[57]

CONCLUSION

Phytosome is an innovative form of formulation for herbal extract which shows better absorption than conventional herbal extract. This review is an attempt to present a concise profile regarding phytosomes benefit, chemical properties, physical characteristics, method of preparation, and applications. The poor bioavailability and absorption of water soluble phytoconstituent which could be overcome by phytosome technology as it provides optimum delivery of active phytoconstituent. The absorption of phytosome through skin and gastrointestinal tract is increased due to improved lipid solubility, which enable them to cross biological membrane, resulting in enhanced bioavailability. It requires less doses than conventional herbal extract due to increased bioavailability. Furhermore, phytosomes are superior to liposomes due to their stability profile. Phytosome technology has great approach for use in formulation technology and application of hydrophilic plant extract.

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