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REVIEW

Phytosome: A Revolution in Herbal Drug Delivery System

ARPITA SINGH*, ANIL PRATAP SINGH AND NEERAJ VERMA

Goel Institute of Pharmacy & Sciences, Near Indira Canal, Faizabad Road, Lucknow-227105, India

*Corresponding author: Fax: +91 522 4077041; Tel: 0522 4006161; E-mail: arpitmohan2010@gmail.com

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The efficiency of any herbal medication is dependent on delivery of effective level of the therapeutically active compound. But severe limitations exist in their bioavailability when administered orally or by topical application. The bioavailability can be improved by the use of delivery systems, enhancing the rate and the extent of drug solubilizing into aqueous intestinal fluids and the capacity to cross the lipid rich biomembranes. Phytosomes are newly introduced herbal formulations developed to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes and so vastly improve their absorption and bioavailability. Phytosomes exhibit better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts. It is also often known as herbosomes. This article apart from providing information regarding the advantages and physiochemical properties of phytosomes gives various simple research techniques in the preparation and its optimization.

Key Words: Phytosome, Phosphatidylcholine, Phospholipid, Herbal drug delivery.

INTRODUCTION

Over the last century chemical and pharmacological studies have been performed on a lot of plant extracts to know their chemical composition and confirm the indication of traditional medicine. Most of the bioactive constituents of phytomedicines are water-soluble molecules (*e.g.* phenolics, glycosides and flavonoids). Water soluble phytoconstituents like flavonoids are poorly absorbed by simple diffusion due to their multiple ring large size molecules[1]. Their poor miscibility with oils and other lipids, severely limiting their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine[2]. Many approaches have been developed to improve the oral bioavailability, such as inclusion of solubility and bioavailability enhancers, structural modifcation and entrapment with the lipophilic carriers[3-5]. The effectiveness of any herbal product depends on delivering an effective level of the active compounds. The phytosome technology[6] developed by Indena meet these challenges by markedly enhancing the bioavailability of selected phytomedicines[7,8]. The use of phytosomes is a new advanced modern dosage formulation technology to deliver herbal products and drugs by improved absorption, producing better results than those obtained by conventional herbal extracts[9,10]. This phytosome technology is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, without compromising nutrient safety[6].

The phytosomes process produces a little cell, whereby the valuable component of the herbal extract is protected from destruction by digestive secretions and gut bacteria[11]. Phytosomes are better able to transition from a hydrophilic environment into the lipid-friendly environment of the enterocyte cell membrane and from there into the cell, finally reaching the blood[12]. The lipid- phase substances successfully employed to make flavonoids lipid -compatible are phospholipids from soya, mainly phosphatidylcholine developed by Indena. Phosphatidylcholine is miscible both in water phase and oil/lipid phases, excellently absorbed when taken by mouth. Phosphatidylcholine is the principal molecular building block for cell membranes and the molecular properties that suit phosphatidylcholine for this role also render it close to ideal for its phytosome role. Chemical analysis indicates, the unit phytosome is usually a flavonoid molecule linked with at least one phosphatidylcholine molecule.

Difference of phytosomes from liposomes: Phytosomes results from the reaction of a stoichiometric amount of the phospholipid with the selected polyphenol (like simple flavonoids) in a non polar solvent[13]. They are lipophillic substances with a definite melting point, freely soluble in non-polar solvents (in which the hydrophilic moiety was not), soluble in fats. When treated with water, they assume a micellar shape. In liposomes, the active principle is dissolved in the medium of the cavity or in the layers of the membrane, whereas in the phospholipid-

flavonoid compounds it is an integral part of the membrane. Molecules are anchored through chemical bonds to the polar head of the phospholipids, demonstrated by specific spectroscopic techniques[14,15]. Unlike phytosomes, a liposome is formed by mixing a water-soluble substance with phosphatidylcholine. No chemical bond is formed; the phosphatidylcholine molecules surround the water soluble substance. There may be hundreds or even thousands of phosphatidylcholine molecules surrounding the water-soluble compound. In contrast, with the phytosomes process the phosphatidylcholine and the individual plant components actually form 1:1 or 2:1 complex depending on the substance. This difference results in phytosome being much better absorbed than liposomes[16].

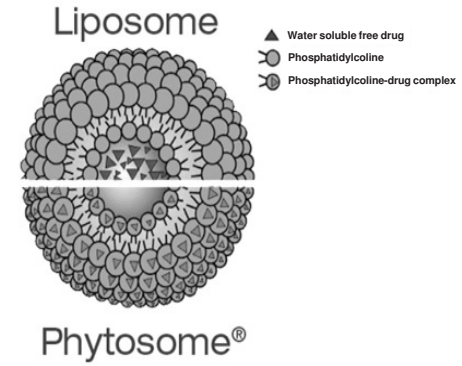

Fig. 1. Structural difference between liposome and phytosome

Method of preparation: Phytosomes are novel complexes which are prepared by reacting 3-2 moles, preferably with one mole of a natural or synthetic phospholipid, such as phosphatidylcholine, phosphatidylethanolamine or phosphatidyiserine 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 of 0.5-2.0 moles. The most preferable ratio of phospholipid to flavonoids is 1:1[12]. In the phytosome preparations, phospholipids are selected from the group consisting of soya lecithin, from bovine or swine brain or dermis, phosphatidylcholine, phosphatidylethanolamine, mostly derived from palmitic acid, stearic acid, oleic acid and linoleic acid. Selection of flavonoids are done from the group consisting of quercetin, kaempferol, quercretin-3, rhamnoglucoside, quercetin-3-rhamnoside, hyperoside, vitexine, diosmine, 3-rhamnoside, (+)catechin, (-)epicatechin, apigenin-7-glucoside, luteolin, luteolinglucoside, ginkgonetine, isoginkgonetine and bilobetine. Some liposomal drugs complex operates in the presence of water or buffer solution whereas phytosomes operate with the solvent having a reduced dielectric constant. Starting material of component like flavonoids are insoluble in chloroform, ethyl ether or benzene. They become extremely soluble in these solvents after forming phytosomes. This chemical and physical property change is due to the formation of a true stable complex[5]. 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 as reported by Jain[17].

Jiang *et al.*[19] have optimized the preparation conditions using a uniform design and step regression and have prepared Herba Epimedii total favonoid phytosomes (EFP) by means of solvent evaporation, investigated the cumulative dissolution of differentratios of EFP-PVP precipitates by means of dissolution release. The optimized preparation conditions are as follows: solvent-tetrahydrofuran, lecithin to PVP ratio-2.5, temperature-40 ºC and reaction time-3 h. The oil/water apparent partition coeffcient of icariin was enhanced more than 4-fold by phospholipid. The cumulative dissolution of herba epimedii favonoids of the EFP-PVP precipitate was signifcantly higher than that of its physical mixture and a herba epimedii extract tablet. Yanyu *et al.*[20] prepared a silybin-phospholipid complex using ethanol as a reaction medium. Silybin and phospholipids were resolved into the medium, after the organic solvent was removed under vacuum condition and a silybin-phospholipid complex was formed.

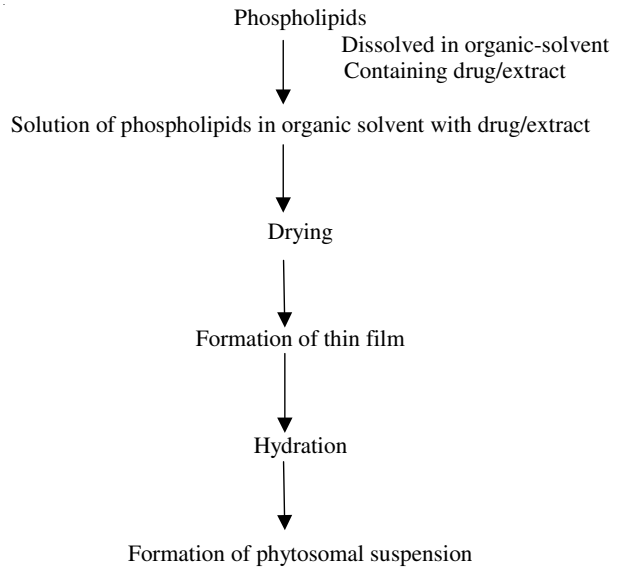

Fig. 2: Common stages for preparation of phytosomes[18]

Characterization and evaluation of phytosomes: The behaviour of phytosomes in both physical and biological systems is governed by factors such as the physical size, membrane permeability, percentage of entrapped solutes, and chemical composition as well as the quantity and purity of the starting materials. Phytosomes can be characterized in terms of their physical attributes *i.e.* shape, size, distribution, percentage drug captured, entrapped volume, percentage drug released and chemical composition[20].

Different characterization techniques used for phytosomes

Visualization: Visualization of phytosomes can be achieved using transmission electron microscopy and by scanning electron microscopy[21].

Vesicle size and zeta potential: The particle size and zeta potential can be determined by dynamic light scattering using a computerized inspection system and photon correlation spectroscopy[22].

Entrapment efficiency: The entrapment efficiency of a drug by phytosomes can be measured by the ultracentrifugation technique[23].

Transition temperature: The transition temperature of the vesicular lipid systems can be determined by differential scanning calorimetry[24].

Surface tension activity measurement: The surface tension activity of the drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer[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 dynamic light scattering and structural changes are monitored by transmission electron microscopy[26].

Drug content: The amount of drug can be quantified by a modified high performance liquid chromatographic method or by a suitable spectroscopic method[27].

Spectroscopic evaluations

To confirm the formation of a complex or to study the reciprocal interaction between the phytoconstituent and the phospholipids, the following spectroscopic methods are used[28].

^{1}H-NMR: The NMR spectra of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine have been studied by Bombardelli *et al*.[29]. In nonpolar solvents, there is a marked change of the ^{1}H-NMR signal originating from the atoms involved in the formation of the complex, without any summation of the signal peculiar to the individual molecules. The signals from the protons belonging to the favonoids are to be broadened that the proton cannot be relieved. In phospholipids, there is broadening of all the signals while the singlet corresponding to the N-$(CH_3)_3$ of choline undergoes an uplift shift.

^{13}C-NMR: In the ^{13}C-NMR spectrum of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine, particularly when recorded at room temperature, all the flavonoid carbons are clearly invisible. The signals corresponding to the glycerol and choline portion of the lipid (between 60-80 ppm) are broadened and some are shifted, while most of the resonances of the fatty acid chains retain their original sharp line shape.

FTIR: The formation of the complex can be also 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 microdispersed in water or when incorporated in very simple cosmetic gels.

***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[28]. For example, *in vitro* antihepatotoxic activity can be assessed by the antioxidant and free radical scavenging activity of the phytosomes. For assessing antihepatotoxic activity *in vivo*, the effect of prepared phytosomes on animals against thioacetamide, paracetamol, alcohol induced hepatoxicity can be examined[30,31]. Skin sensitization and tolerability studies of glycyrrhetinic acid-phytosome® ointment, a commercial product, describe the *in vivo* safety evaluation methodology[32]. Filburn *et al*.[33] studied the bioavailability of a silybin-phosphatidylcholine complex in dog models to examine the pharmacokinetic parameters of this new complexed form.

Chemical properties of phytosomes: Phytosomes 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. On the basis of spectroscopic data it has been concluded 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. When treated with water, phytosomes assumes a micellar shape forming liposomial-like structures, liposomes 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, there is the formation of *H*-bonds between the phenolic hydroxyls of the flavones moiety and the phosphate ion on the phosphatidylcholine side. This can be deduced from the comparison of the NMR of the complex with those of the pure precursors. The signals of the fatty chain are almost unchanged. Such evidences inferred that the two long aliphatics chains are wrapped around the active principle, producing a lipophilic envelope, which shields the polar head of the phospholipid and the flavonoid[30].

Phosphatidylcholine: It is a bifunctional compound miscible both in water and in oil environments and is well absorbed when taken by mouth. This itself a bioactive nutrient with documented clinical efficacy for liver disease, including alcoholic hepatic steatosis, drug-induced liver damage and hepatitis. Phosphatidylcholine is present in egg yolk, brain tissue and a wide variety of animal fat and plant oils[34].

Biological properties of phytosomes: Phytosomes are advanced forms of herbal products as they are better absorbed, utilized and 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[24].

Merits of phytosomes

Phytosomes have the following merits[25-27]:

(1) It enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability, hence significantly greater therapeutic benefit.

(2) Appreciable drug entrapment.

(3) As the absorption of active constituent(s) is improved, its dose requirement is also reduced.

(4) 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.

(5) Chemical bonds are formed between phosphatidylcholine molecule and phytoconstituent, so the phytosomes show better stability profile.

(6) Appilcation of phytoconstituents in form of phytosome improve their percutaneous absorption and act as functional cosmetics.

(7). Added nutritional benefit of phospholipids.

Applications of phytosomes: Most of the phytosomal studies are focused to *Silybum marianum,* which contains premier liver-protectant flavonoids. The fruit of the milk thistle plant (*S. marianum*, family steraceae) contains flavonoids known for hepatoprotective effects. Silymarin has been shown positive effects in treating liver diseases of various kinds, including hepatitis, cirrhosis, fatty infiltration of the liver (chemical and alcohol induced fatty liver) and inflammation of the bile duct. The antioxidant capacity of silymarin substantially boosts the liver's resistance to toxic insults[35]. Silymarin primarily contains three flavonoids of the flavonol subclass (having a fully saturated c-ring). Silybin predominates, followed by silydianin and silychristin. Silybin is actually a flavonolignan, probably produced within the plant by the combination of a flavonol with a coniferyl alcohol. It is now known that silybin is the most potent of the three[36]. Silybin protects the liver by conserving glutathione in the parenchymal cells[35], while phosphatidylcholine helps repair and replace cell membranes[37]. These constituents likely offer the synergistic benefit of sparing liver cells from destruction. In its native form within the milk thistle fruit, silybin occurs primarily complexed with sugars as a flavonyl glycoside or flavonolignan. Silybin has been extensively researched and found to have impressive bioactivity, *albeit* limited by poor bioavailability. Busby *et al.*[38] reported that the use of a silymarin phytosome showed a better fetoprotectant activity from ethanol-induced behavioural deficits than uncomplexed silymarin. Yanyu e*t al.*[39] prepared the silymarin phytosome and studied its pharmacokinetics in rats. 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. Bombardelli *et al.*[7] 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 components, with respect to per cent reduction of odema, inhibition of myeloperoxidase activity, antioxidant and free radical scavenging properties. Maiti *et al.*[40] developed the quercetin-phospholipids complex by simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetra-chloride. Recently Maiti *et al.*[41,42] developed the phytosomes of curcumin and naringenin in two different studies. In first study phytosome of curcumin was developed to overcome the limitation of absorption and to investigate the protective effect of curcumin phospholipid complex on carbon tetrachloride induced acute liver damage in rats. The complex showed enhanced aqueous or *n*-octanol solubility. The antioxidant activity of the complex was significantly higher than pure curcumin in all dose levels tested. In second study the developed phytosome of naringenin produced better antioxidant activity than the free compound with a prolonged duration of action, which may be helpful in reducing the fast elimination of the molecule from body. Hesperetin is a potent phytomolecule of citrus fruits, such as grapes and oranges. It has variety of therapeutic benefits *viz*. antioxidant, lipid-lowering, anticarcinogenic activities, shorter half life and lower clearance from the body restricts its use. To overcome this limitation, recently Mukerjee *et al.*[43] developed a novel hesperetin phytosome by complexing hesperetin with hydrogenated phosphatidylcholine. This complex was then evaluated for antioxidant activity in CCl_4 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. Studies have shown ginkgo phytosome (prepared from the standardized extract of *Ginkgo biloba* leaves) produced better results compared to the conventional standardized extract from the plant (GBE, 24 % ginkgo flavone glycoside and 6 % terpene lactones). In a bioavailability study conducted with healthy human volunteers the levels of GBE constituents (flavonoids and terpenes) from the phytosomal form peaked after 3 h and persisted longer for at least 5 h after oral administration. It was found that the phytosomal GBE produced a 2-4 times greater plasma concentration of terpenes than did the non-phytosomal GBE. Its improved oral bioavailability and good tolerability makes it the ideal ginkgo product for long term treatment[44].

Conclusion

In recent times, the emerging technology of drug delivery and drug targeting is also being applied to phytopharmaceuticals. Plant extracts or mainly polar phytoconstituents like flavonoids, terpenoids, tannins, xanthones when complexed with phospholipids like phosphatidylcholine give rise to a new drug delivery technology called phytosome, showing much better absorption profile following oral administration owing to improved lipid solubility which enables them to cross the biological membrane. This means more amount of active constituent becomes present at the site of action (liver, brain, heart, kidney, *etc.*) at similar or less dose as compared to the conventional plant extract or phytomolecule. Hence, the therapeutic action becomes enhanced, more detectable and prolonged. Thorough study of literature reveals that several plant extracts (crude, partially purified or fractionated) are reported to possess different significant pharmacological or health promoting properties like cardiovascular, antiinflammatory, immunomodulator, anticancer, antidiabetic etc for prophylactic and health purposes as nutraceuticals, in due course.

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