

A REVIEW ON PHYTOSOMES: NOVEL APPROACH FOR HERBAL PHYTOCHEMICALS

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ABSTRACT

The poor oral bioavailability of polyphenolic compound can be enhanced through the incorporation of them into phospholipid based self-assembled delivery system, i.e. popularly known as phytosome. "Phyto" means plants and "some" resembles a covering around/or a structure. Phytosome is generally prepared by reacting one or two moles of polyphenolic phytoconstituents and phospholipid. It may be either in the ratio of 1:1 and 1:2. By using phytosomes, one can also achieve enhanced rate and extent of the passage of lipophilic herbal constituents across lipid membrane that explains its character as a carrier as well as acid labile herbal drugs could also be protected in gastrointestinal tract. There are number of products available in the market that contains phytosomal drug delivery system such as *Ginkgo biloba*, *Silybum marianum*, and *Camellia sinensis*. The present review describes an updated overview of preparation of phytosomes, advancement in phytosomes technology, various herbal drugs for which phytosomes have been used as a carrier, its commercial availability and applications.

Keywords: Phytosomes, Novel drug delivery system, Phospholipid.

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INTRODUCTION

Most of the biologically active constituents of plants are polar or water soluble but due to the problem in absorption, restricts the utilization of these type of compounds which ultimately decreases the bioavailability.

For improvement of bioavailability, herbal products must have proper homeostasis between hydrophilic (for absorption into gastrointestinal tract fluid) and lipophilic (to cross lipid bio membrane balance) [1]. Plant preparations are widely used in traditional as well as modern medicine system. During the traditional time, various pharmacological studies have been carried out with many plants extracts and their constituents to check their therapeutic application. Over the past year, great advancement has been made for the development of novel drug delivery system (NDDS) for various plant extracts and their active constituents. Novel drug delivery such as targeted drug delivery which directly channels the active entity on the site of action and such delivery system could offer targeted and sustained release of drug so that pharmacological effect could be achieved at lower dose. The development in the area of herbal medicine started earlier to cure human diseases with lesser side effects [2].

A number of chief constituents of herbal medicine are easily soluble in water (glycoside, flavonoid); however, these constituents are bounded in their potency because they may be partially soluble or hydrophobic in nature, so when applied topically shows less therapeutic efficacy [3]. Numerous efforts have been put forward to enhance the bioavailability of such drug by formulating them to target drug delivery system such as phytosomes and liposome's are good options. The use of these techniques in formulation development process may lead to good bioavailability of herbal drugs as compare to conventional herbal extracts.

Phytosomes means herbal drug loaded in vesicles, which is available in the nano form. The phytosome provide an envelope, like coating around the active constituent of drug and due to this the chief constituent of herbal extract remains safe from degradation by digestive secretion and bacteria. Phytosome is effectively able to absorb from a water loving

environment into lipid loving environment of the cell membrane and finally reaching to blood circulation [4]. It can be used in the treatment of various fatal diseases without denaturing the active phytochemicals and enhanced bioavailability. Phytosomes are obtained by reacting phospholipid (either of natural or synthetic origin) with selected botanical constituents with an appropriate solvent, and due to their physical and chemical efficiency, these phyto-complex can be considered as a novel entity [5]. The current review highlights the future scope and emerging technologies in the field of NDDS for the benefit of herbal and traditional medicines prepared from plant origins.

PREPARATION OF PHYTOSOME

Phytosomes are generally prepared by adding accurate amount of phospholipid, i.e., Soya lecithin with herbal extracts in an aprotic solvent. Soya lecithin contains main constituent, i.e., Phosphatidylcholine which is having a dual function. Phosphatidyl part is lipophilic in nature and choline part is hydrophilic in nature. The choline part attached with hydrophilic chief active constituents, where as phosphatidyl part lipid soluble compound attached with choline bound complex. It results in the formation of lipid complex with better stability and bioavailability [6].

COMPARISON BETWEEN PHYTOSOME AND LIPOSOME

There is number of research has been carried out on phytosomes which state that phytosomes have good bioavailability, absorption, and excellent therapeutic efficacy over liposome. Comparison between phytosomes and liposomes is represented in Table 1 along with their structure in Fig. 1.

PHOSPHOLIPIDS [10]

Nowadays industrially produced phospholipid delivery system has a great role and becomes more popular. The main ingredients are to achieve this goal is soya, chicken egg, etc. The main key ingredients in all this are phospholipid which comprises a glycerol unit joined with two fatty acids, and the remaining linkage is joined by a phosphate group. In phytosome preparation, the main phospholipid used as phosphatidylcholine having a great role in biological membrane and also

act as hepatoprotective. The molecular structure of Phosphatidylcholine is represented in Fig. 2.

COMMERCIAL PRODUCTS OF PHYTOSOMES [11-16]

There is numerous commercially available product based on Phytosomes are available in the market which is having great therapeutic role as compared to conventional dosage form. Some of them are listed along with their trade name, chief constituents, source, dose, and use in Table 2.

FLAVONOIDS USED IN THE PREPARATION OF PHYTOSOMES [17]

There are number of herbal chief constituents widely used in the preparation of phytosomes. Each active component having its own properties and therapeutic action. Some of important flavonoids are represented in Table 3 along with their source and molecular structure.

FUTURE PERSPECTIVE OF "SOMES" WITH THEIR APPLICATION

"Somes" are having a wide area of thrust, not only phytosome is having its property but there are some other "Somes" preparation also suggest their clinical efficacy which is represented in Table 4 along with their vesicular delivery system and application's.

PROPERTIES OF PHYTOSOMES [41,42]

Physicochemical properties

- Phytosomes are the complex between phytoconstituents and natural phospholipid, and the complex is obtained by reacting an appropriate amount of phospholipid and chief constituents in particular solvent.
- The interaction between phospholipid and substrate is due to the development of hydrogen bonds between the polar head of phospholipid and the polar functionalities of the chief constituents.
- On treatment with hydrophilic environment phytosome shows a cell-like structure like liposomes, but in a liposome, the chief constituent interacts within the internal pocket while in phytosome the chief active constituents are enveloped the polar head of phospholipid and becoming an integral part of the membrane.

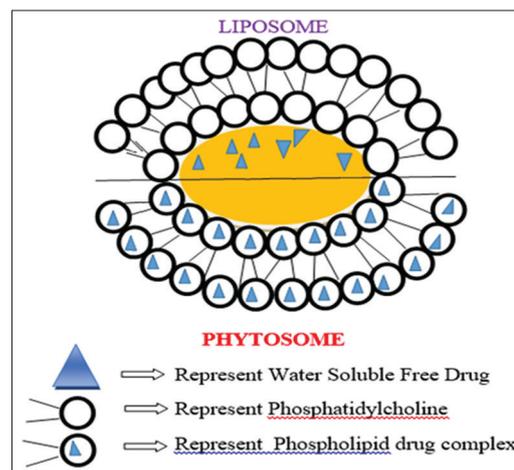


Fig. 1: Difference between phytosome and liposome

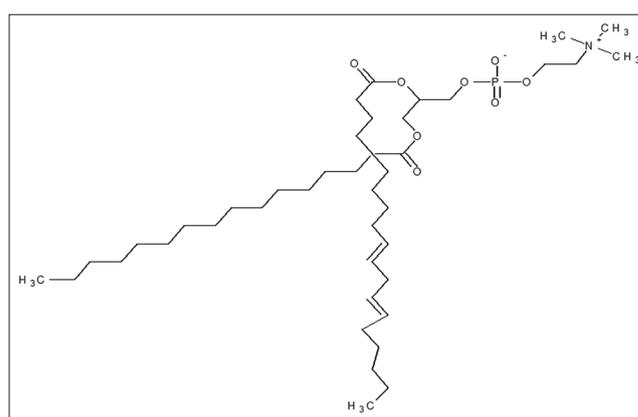


Fig. 2: Molecular structure of phosphatidylcholine

Table 1: Comparison between phytosomes and liposomes

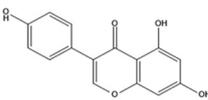
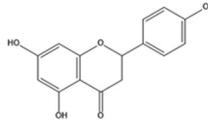
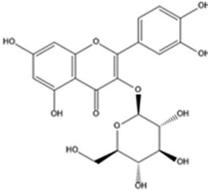
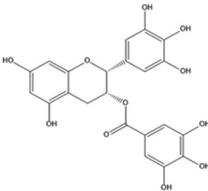
S. No.	Properties	Phytosome	Liposome	References
1.	Bonding	Associated with few molecule (mainly with Phospholipid and polyphenol extract)	Number of molecules and even they are not connected well	[7]
2.	Oral delivery	Best for oral delivery	Poor oral bioavailability	[8]
3.	Phospholipid ratio	Preferably 1:1, 1:2 ratio is preferred for its preparation	Lipid ration is increased up to 10 times than the chief active constituents	[9]

Table 2: Commercially available phytosomal product

S. No.	Trade name	Chief constituents	Source	Dose	Use
1	Centella phytosomes	Triterpine	<i>Centella asiatica</i>	-	Cicatrizing, trophodermic
2	Ginselect phytosomes	Ginsenosides	<i>Gingko biloba</i>	120 mg	Adaptogenic
3	Greenselect phytosomes	Polyphenols	<i>Camellia sinensis</i>	-	Free radical scavenging activity
4	Leucoselect	Polyphenols	<i>Vitis vinifera</i>	300 mg	Antioxidant
5	Meriva	Curcuminoids	<i>Curcuma longa</i>	200-300 mg	Anti-inflammatory
6	Silymarin	Silymarin	<i>Silybum marianum</i>	-	Antihepatotoxic
7	Oleaselect TM phytosome	Polyphenols of olive oil	<i>Olea europaea</i>	-	Anti-inflammatory, antioxidant
8	Crataegus phytosomes	Vitexin-2'-O-rhamonoside	<i>Crataegus Mexicana</i>	-	Antioxidant
9	Visnadine	Visnadine	<i>Ammi visnaga</i>	-	Circulation improver
10	Bilberry	Triterpine	<i>Vaccinium myrtilillus</i>	-	Potent antioxidant
11	Ruscogenin phytosomes	Steroid saponin	<i>Ruscus aculeatus</i>	-	Anti-inflammatory
12	PA2 phytosomes	Proanthocynidin	<i>Horse chestnut bark</i>	-	Antiwrinkles, UV protectant
13	Zanthalene phytosomes	Zanthalene	<i>Zanthoxylum bungeanum</i>	-	Soothing, anti-itching
14	Lymphaselect phytosomes	Triterpenes	<i>Melilotus officinalis</i>	-	Indicated in insomnia
15	Sabalselect phytosome	Fatty acid, sterols	<i>Serenoa repens</i>	-	Beningn prostate hyperplasia
16	Sericoside phytosome	Sericosides	<i>Terminalia sericea</i>	-	Skin improver
17	Echinacea phytosome	Echinacosides	<i>Echinacea angustifolia</i>	-	Immunomodulators, nutraceuticals
18	Rexatrol	Resveratrol	<i>Polygonum cuspidatum</i>	-	Antioxidant, antiaging

UV: Ultraviolet

Table 3: Flavonoids/chief constituents with their molecular structure which are used in the preparation of phytosomes

S. No.	Flavonoid/chief constituents	Plant name	Structure
1	Genistein	Soy tea	
2	Naringenin	Orange	
3	Isoquercetin	Onion buckwheat hyptis fasciculate	
4	EGCG	Green tea	

EGCG: Epigallocatechin gallate

Table 4: Different vesicular drug delivery system

S. No.	Vesicular system	Application	References
1.	Ethosomes	Recently introduced vesicular delivery system having ability to penetrate mammalian skin	[18-20]
2.	Sphingosomes	New vesicular system which composed of either natural or synthetic sphingolipid. These are better resistant to hydrolysis as compared to liposomes, and having better drug retention	[21-23]
3.	Ufasomes	Firstly described in 1973 by Gebicki and Hicks. Ufasomes develop vesicles having size range nano to submicron. Ufasomes shows better stability, and also shows great entrapment efficiency whether in hydrophobic/hydrophilic drugs. Ufasomes also having great role in topical delivery system	[24-28]
4.	Pharmacosomes	These are colloidal dispersion mainly consist of phospholipid. Pharmacosomes are mainly designed for improvement of entrapment efficiency of polar drugs. These are also called phytosomes	[29-32]
5.	Virosomes	These are the vesicles having size in nano and submicron range. There are number of study has been done on these virosomes which also shows good result	[33-37]
6.	Quatosomes	These are unilamellar vesicles formed by surfactant ammonium and sterol in appropriate ratio. These are also having long stability, and their morphology do not change with time	[38-40]

d. The phytosome is a combination of few molecular complex which bounded together, while the liposome is a combination of number of phospholipids which react with chief constituent but without complete bonding with them.

Biological properties

- Phytosome increases the active absorption of active ingredients and also increase the systemically bioavailability when administered orally.
- These are the advance form of herbal products and having better efficacy as per compare to conventional herbal extract.
- Phytosome has better pharmacokinetic as compare to simple herbal drugs.

ADVANTAGE OF PHYTOSOMES [43]

- Phospholipid, i.e., phosphatidylcholine one of the valuable components of phytosome has a bifunctional activity by acting as a vehicle as well as health benefit such as hepatoprotective activity.
- The absorption of hydrophilic active constituents is increased which also increase the efficacy.

c. As the efficacy increases the dosage requirement is also reduced.

d. Phytosomes have better stability.

e. Phytosome has the ability to permeate through skin due to its lipid layer around the phytoconstituent and thus enhance the effectiveness.

f. By increasing the solubility of bile to herbal origin phytoconstituents, phytosomes enhance the liver targeting [44].

g. Phytosome increase the solubility of bile to herbal constituents [45].

h. Time period of action is increased [46-48].

The advantage of phytosome is represented in diagrammatic form in Fig. 3 [49].

MECHANISM OF PHYTOSOME TECHNOLOGY [50]

The lower absorption and bioavailability of polyphenolic constituents mainly due to two factors. These chief constituents are number of ringed molecule and are not too much small that it will absorbed by diffusion process. Second factor is that flavonoid molecule or chief constituents of polyphenols have poor solubility with lipids. These are the limitations that inhibit their absorption through biological membrane. Phytosome

technology is mainly result with complexation of polyphenols with phospholipid in 1:1 ratio or 1:2 results in the formation of phytosomal complex with lipid covering around the constituents.

PREPARATION TECHNIQUES FOR PHYTOSOMES

- Phytosome vesicles were made by thin layer rotary evaporator vacuum method. The phytosomal complex was mixed in anhydrous ethanol in 250 ml round bottom flask. The flask was attached to a rotary evaporator. The solvent will evaporate at a temperature about 60°C forming thin layer film around the flask. The film is hydrated by phosphate buffer having pH 7.4, and the lipid layer will peel off in phosphate buffer forming vesicle suspension. The phytosomal suspension was subjected to probe sonication with 60% amplitude. Phytosomal suspension will be stored in the refrigerator for 24 hrs, before characterization [51].
- Phospholipid, i.e., soya lecithin was reacted with polyphenolic extract in an equal ratio with 5 mL of dichloromethane (DCM) with stirring until evaporate. Once the DCM was evaporated 5 mL of n-hexane, was added to the thin film with stirring and left in a fume hood for complete removal of the solvent. After complete removal of n-hexane, the thin film was hydrated and sonicated for desired phytosomal complex [52].
- Weigh accurate amount of phospholipid and polyphenolic extract. Put it in 100 ml round bottom flask and reflux it with 30 mL of DCM on 60°C for 3 hrs, reduced it to 5-10 mL and add 30 mL of n-hexane with continuous stirring to get precipitate. Collect the precipitate and stored in a vacuum desiccator overnight. The dried precipitate is then passed through #100 mesh size and stored in well closed ambered colored container [53].
- Phytosomes can be prepared by reflux method. Polyphenolic extract and phospholipid were placed in 100 mL round bottom flask and refluxed in DCM for 1 hr not exceeding 40°C. The clear solution was evaporated and add 15 mL of n-hexane until a precipitate was obtained. The precipitate was taken and placed in a desiccator [54].
- Accurately, weight the quantity of phospholipid and cholesterol in round bottom flask and dissolve it in 10 mL of chloroform followed by sonication for 10 minutes using bath sonicator. Organic solvent removal can be done by subjecting it under reduced pressure in a rotary evaporator (40°C). After complete removal of the solvent thin layer is formed which is hydrated with polyphenolic extract of the drug in a rotary evaporator. Phospholipids mixture was sonicated in an ice bath for heat dissipation. Prepared phytosome were stored in an amber colored bottle [55]. The diagrammatic representation of procedure is shown in Fig. 4.

CHARACTERIZATION TECHNIQUES OF PHYTOSOME [56-58]

Differential scanning calorimetry

Drug polyphenolic extract, phosphatidylcholine, a physical mixture of drug extract and Phosphatidylcholine, and drug-phospholipid complex were placed in an aluminum cell and heated to a temperature of 50-250°C/minutes from 0 to 400°C in the atmosphere of nitrogen.

Scanning electron microscopy (SEM)

SEM was used to determine the size of the particle and its appearance. Dry sample was placed on electron microscope brass stub coated with gold in an ion sputter. Random scanning of the complex at 100.

Transition electron microscopy (TEM)

TEM was used to characterize the size of phytosomal vesicles with 1000 magnification.

Drug entrapment and loading capacity

Drug phytosomal complex was centrifuged at 10000 rpm for 90 minutes at 4°C to separate phytosome from the untrapped drug. The concentration of free drug can be measured by doing ultraviolet spectroscopy. The percentage drug entrapment can be calculated as given formula:

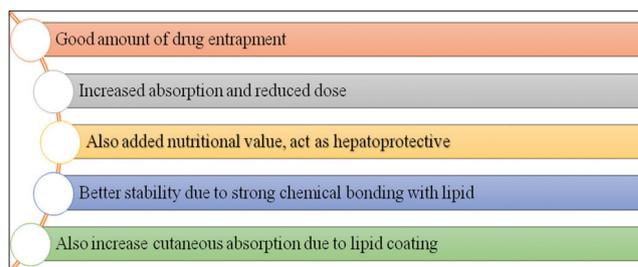


Fig. 3: Advantage of phytosome

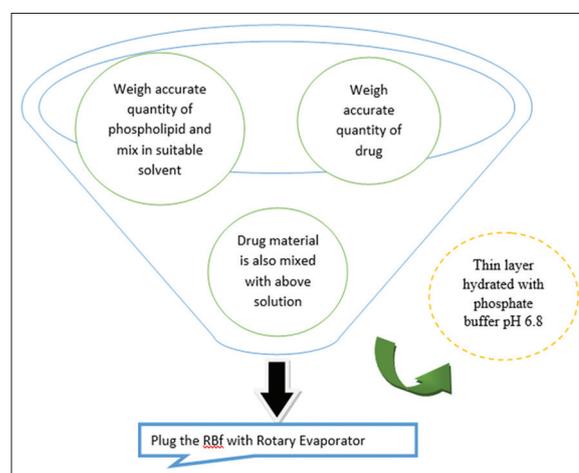


Fig. 4: Preparation method for phytosomes

$$\text{Entrapment efficiency (\%)} = \frac{\text{Weight of total drug} - \text{weight of free drug}}{\text{Weight of total drug}} \times 100$$

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis will be done for checking the structure as well as chemical stability of drug, phospholipid. The phytosomal drug will be crushed with potassium bromide to obtain pellets at 600 kg/cm² pressure. Scanning will be done between the ranges of 4000-400 cm⁻¹.

Size analysis and zeta potential

Malvern Zetasizer is used to check the particle size and zeta size of phytosomal complex. Argon laser is used for this particle size and zeta sizer characterization.

In vitro and in vivo evaluations

In vitro and in vivo evaluation will depend on the properties of the drug, their chief phytoconstituents bounded by phospholipid layer and on the bases of that particular animal model is selected for its evaluation.

ADVANCES IN PHYTOSOME TECHNOLOGY

There are number of research articles reveals the importance of phytosomal delivery system over conventional herbal extract. Advances in phytosomal delivery system are as follows:

- Bacopaside well-known chief constituents present in *Bacopa monnieri* plant having anti-amnesic activity. This study is an attempt to prepare phytosome from bacopaside and its in vivo evaluation on rodents. There is remarkably great change in the therapeutic efficacy of the compound prepared by phospholipid as compare to simple *B. monnieri* extract [59].
- Another study also reveals that there is the preparation of berberine phospholipid complex solid dispersion, which not only increase the solubility of the compound but also increase its flow ability and dissolution rate for industrial production [60].

Table 5: Innovation in Phytosome with Patent title and Patent number

S. No.	Patent title	Description of innovation	Patent no.	References
1	Phospholipid complexes of olive fruits or leaves extracts having improved bioavailability	Having improved bioavailability	EP/1844785	[72]
2	Compositions comprising <i>Gingko biloba</i> derivatives for the treatment of asthmatic and allergic conditions	Useful for asthma and allergic condition	EP1813280	[73]
3	Treatment of skin and wound repair with thymosin beta-4	Composition of thymosin for treatment of skin	US/2007/0015698	[74]
4	Soluble isoflavone compositions	Exhibit improved solubility	WO/2004/045541	[75]
5	Phospholipid curcumin complex and piperine as chemosensitizing agent	Treatment of drug resistant	EP2228062 A1	[76]
6	Fatty acid monoesters of sorbityl furfural and composition for cosmetic and dermatological use	Fatty acid monoester of sorbityl furfural selected from two different series of compounds in which side chain is linear alkyl radical optionally containing at least one ethylenic unsaturation	EP1690862	[77]
7	Cosmetic and dermatological compositions for the treatment of aging and photodamaged skin	Cosmetic or dermatological composition for topical treatment	EP1640041	[78]
8	Complex of saponin with phospholipid and pharmaceutical and cosmetic compositions containing them	High lipophilic and improved bioavailability and suitable for use in pharmaceutical cosmetic compositions	EP0283713	[79]
9	An antioxidant preparation based on plant extract for the treatment of aging or photodamaged skin	Used in circulation problems, arteriosclerosis and high blood pressure	EP1214084	[80]

- c. Another research state that there is the preparation of sinigrin phytosome. The study was carried out for *in vitro* wound healing capacity and the result is also appreciable as compare to sinigrin alone [61].
- d. One research reported silymarin phytosomes with better antihepatotoxic activity as compare to silymarin alone and also having great role for the protection against B1 aflatoxin on broiler chicks [62].
- e. The phytosomes from standardized extract of seeds of *S. marianum* have administered orally which is having great effect on foetus from maternally ingested alcohol [64].
- f. One clinical research reveals that the study of 232 patient with chronic hepatitis when treated with silybin phytosome at a dose of 120 mg twice or thrice a day up to 120 days having great role for recovery of liver function [63].
- g. Grape seed phytosome also having great role in ischemia induced damage in the heart, also having protective against atherosclerosis. The main chief constituents responsible for this is proanthocyanidins/procyanidins [64].
- h. *Camellia sinensis* or the extract of green tea when incorporated in phytosomes having improved oral bioavailability as compared to uncomplexed green tea extract. Epigallocatechin 3-o-gallate is the main active constituents present in green tea [65-67].
- i. Further clinical trial suggested that phytosomes of green tea free from caffeine also having a significant effect on anti obesity and antioxidant activity. Its also having effect on low-density lipoprotein [68-70].
- j. Quercetin phytosomal complex reveals the better therapeutic property in rat liver injury induced by carbon tetra chloride [71].

SOME PATENTED TECHNOLOGY OF PHYTOSOME

There is numerous work has been done for commercialization of Phytosome, out of them few patents technology are representing in Table 5 along with their patent title, description of innovation and patent number.

CONCLUSION

Herbal products always have great concern of denaturation and bioavailability. There is so many novel approaches are available in the form NDDS. Despite these approaches liposomes and phytosomes are most suitable novel approaches for herbal drugs to overcome this kind of problems. These delivery systems have improved the

pharmacotherapeutics and pharmacokinetics of herbal drugs. This kind of delivery systems is also utilized in the field of nutraceuticals and cosmeceuticals for improving therapeutic effect and permeability in the skin. The formation of phytosomes are simple and reproducible a part of that phospholipids used in the preparation of phytosomes have their own beneficial effects in the body.

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