

## **AMPHIPHILIC DRUG DELIVERY SYSTEMS-PHYTOSOMES**

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### **ABSTRACT –**

The term “Phyto” means plant while “some” means cell-like. Phytosome is a novel drug delivery technique which contains phytoconstituents of herbal extracts. Preparation of phytosomes involve in the complexation between phytoconstituents and phospholipids especially with phosphotidylcholine which produces lipid stable molecular complexes. Phytosomes have water soluble inner layer and lipophilic outer layer. Phytosomes have high pharmacokinetic and pharmacodynamic properties. They have improved bioavailability when compared to other conventional herbal extracts. They have valuable role in pharmaceutical industries. Phytosomes can be supplied as natural digestive aids as they acts as antioxidants, hepatoprotective agents, anticancer agents and used as carriers for water soluble and lipid soluble nutrients.

**KEYWORDS** - Drug delivery, Phytosome, Phosphotidylcholine (PC), Phospholipids complex, Bioavailability, Flavonoids, Liposomes.

### **INTRODUCTION –**

Biologically active constituents of plants are mostly polar in nature and water soluble. The utilization of these compounds is restricted due to problem in absorption and decreased bioavailability. In order to improve bioavailability, plant products must maintain proper homeostasis between hydrophilic and lipophilic properties. <sup>(1)</sup>

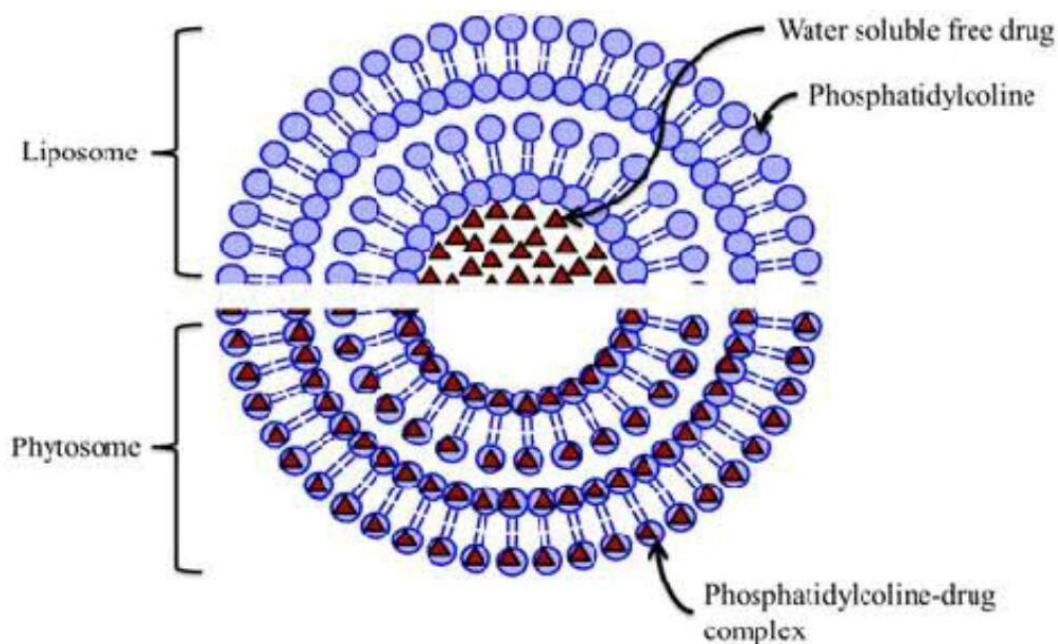
Plant products are widely used in traditional as well as modern medicine system. Recently novel drug delivery system has been developed for several active constituents. Novel drug delivery system provides targeted and sustained release of drug, this helps in achieving pharmacological effect at low dose. Herbal medicines cure human diseases with lesser side effects. <sup>(2)</sup>

Most of major constituents (flavanoides, glycosides) of plants are easily soluble in water. They show less therapeutic effect when applied topically as they are bounded in their potency due to hydrophobic nature.<sup>(3)</sup>Such drugs are formulated in targeted drug delivery systems such as phytosomes and liposomes since they are more available than conventional herbal extracts.

### **PHYTOSOMES :**

Phyto means plant and some means cell like .Phytosomes are herbal drugs loaded in vesicles, available in nanoform. Phytosomes comprises of active constituents enclosed within envelope which prevents degradation of drug by digestive secretions and bacteria. Phytosome effectively absorbs from hydrophilic environment to lipid loving environment and finally enters to blood circulation. <sup>(4)</sup> They have improved pharmacokinetic, pharmacodynamic and pharmacological parameters. Phytosomes proven healthy activity of phospholipids. Phytosomes are also called as herbivores. Phytosomal complexes were first investigated for cosmetic applications. Phytosome is patented process and developed by “Indena” a supplier of nutraceuticals like milk thistle, ginkgo biloba, grape seed, green tea, hawthorn, ginseng etc . In Phytosomes Complex formation ratio of component and phospholipids is 2:1 and 1:1 respectively, which provides much better absorption and stability profile . Phytosomes are more stable than liposomes. On absorption of water phytosomes converts to miscellar structures like liposomes.

**Fig 1 :** Overview of phytosome and liposome :



([https://www.google.com/search?q=phytosomes&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjb45X19\\_3gAhUTb30KHemvAVoQ\\_AUIDigB&biw=1366&bih=657#imgrc=A-fbhKgTB3p2CM:](https://www.google.com/search?q=phytosomes&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjb45X19_3gAhUTb30KHemvAVoQ_AUIDigB&biw=1366&bih=657#imgrc=A-fbhKgTB3p2CM:))

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**Table: 1 -** Differences between phytosomes and liposomes:

Property	Phytosome	Liposome	References
Bonding	It is a unit of few molecules bonded together	It is an aggregate of many phospholipid molecules that encloses other phytoactive molecules without specifically bonding to them.	26
Bioavailability and Absorption	It has much better bioavailability and absorption	Its bioavailability and absorption is lesser than phytosome.	7
Arrangement of molecules	In phytosome, phospholipid (phosphatidylcholine) and an individual phytoconstituent are present in 1:1 or 2:1 ratio depending on the substance.	In liposomes, hundreds and thousands of phosphatidylcholine molecules surround the water soluble molecule.	26

**PROPERTIES OF PHYTOSOMES:**

**1) Physico-chemical properties :**

- The spectroscopic study reveals that complex between phospholipid and substrate(herbal extract) is due to formation of hydrogen bond between phosphate group and polar functional groups of substrate. <sup>(14)</sup>
- Size of phytosome ranges from 50nm -100 micrometers. <sup>(15)</sup>
- Photon correlation spectroscopy states that phytosome on hydration produces micelles like liposomes. <sup>(16)</sup>
- The phospholipid –substrate complexes are easily soluble in aprotic solvents, moderately soluble in fats and insoluble in water. <sup>(17)</sup>
- They are lipophilic substances with a definite melting point.
- When treated with water, they assume a miscellar shape, forming structures which resemble liposomes, but which exhibit fundamental differences.

## 2) Biological properties :

- Phytosomes are novel dosage forms with improved bioavailability and better absorption than conventional herbal extracts.

They also have high pharmacokinetic and pharmacodynamic activities. <sup>(17)</sup>

## PREPARATION OF PHYTOSOMES -

Phytosomes are obtained by reacting phospholipids i.e soya lecithin with herbal extracts using suitable solvents. Soya lecithin consists of phosphatidyl choline.

Choline is hydrophilic and attached to hydrophilic constituents. Phosphatidyl part is lipophilic and attached to lipid soluble compounds. Phosphatidyl part attaches to choline bound complex. It leads to formation of phyto-lipid complex with increased stability and bioavailability <sup>(6)</sup>

Some of the methods of preparation of Phytosomes are discussed below:<sup>(18)</sup>

### 1. Anti-solventprecipitationtechnique:

The specific amount of drug and soya lecithin were taken into a 100 ml round bottom flask and reflux for 2 hrs with 20 ml of dichloromethane at a temperature not more than 60 °c. Concentrate the mixture to 5-10 ml and add 20ml Hexane with continuous stirring to get the precipitate . The precipitate was strained , collected and stored in vacuum desiccators overnight. The dried precipitate is crushed in mortar and sieved through sieve no.100 meshes. Powdered complex was placed in amber coloured glass bottle and stored at room temperature.

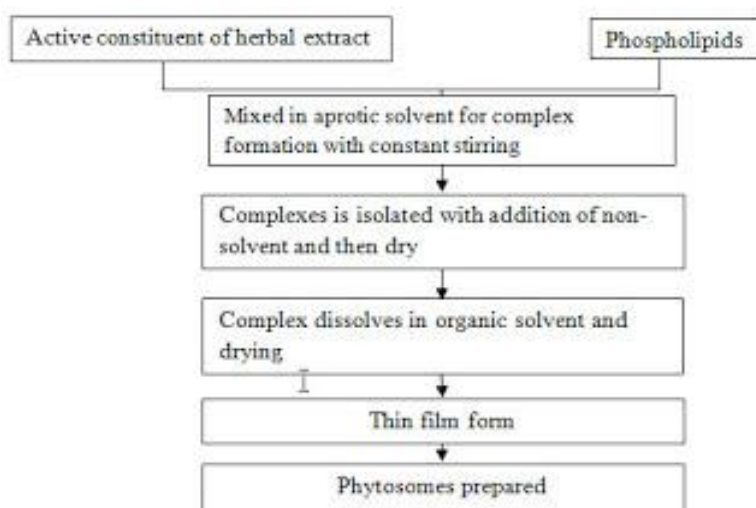
### 2. Rotaryevaporationtechnique:

The specific amount of drug and soya lecithin were dissolved in 30 ml of tetrahydrofuran in a round bottom flask and subjected to stirring for 3 hours with temperature not more than 40 °c. The sample forms a thin film to that n-hexane was added and stirred continuously by using a magnetic stirrer. The precipitate obtained was collected, placed in amber coloured glass bottle and stored at room temperature.

### 3. Solventevaporationmethod:

The specific amount of drug and soya lecithin were taken into a 100 ml round bottom flask and are refluxed with 20 ml of acetone with a temperature of 50 – 60 ° c for 2 hours. The mixture is concentrated to 5-10 ml to get the precipitate which was filtered and collected. <sup>(22)</sup> The dried precipitate phytosomes complexes was placed in amber coloured glass bottle and stored at room temperature.

**Fig 2 : PREPARATION OF PHYTOSOMES -**



#### **Selection Of Herbal Extracts -**

Herbal extracts have several pharmacological activities .Herbal extracts are selected based on their nature, availability, estimation method, stability and based on utility of already available product.

#### **Nature Of Phytoconstituents -**

Phytoconstituents are selected based on their solubility nature. Based on nature they are classified as hydrophilic (water loving) and lipophilic (lipid loving)

- ❖ Phospholipids used in preparation of phytosomes are soya phosphatidyl choline, egg phosphatidyl choline, dipalmitylphosphatidyl choline. Etc. ...
- ❖ Aprotic solvents used in phytosome preparation are Dioxane, acetone, methylene chloride.
- ❖ Nonsolvent used is n-hexane.

## Selection of dosage form for delivery of phytosomes -

Phytosomes can be formulated for both oral administration and topical administration. Orally administered phytosomes can be prepared as soft gelatin capsules, hard gelatin capsules and tablets.

- 1) **Soft gelatin capsules:** Phytosomes are formulated in form of suspensions with phyto constituents as dispersed phase and oils such as vegetable oils or semi synthetic oils as dispersion medium and this suspension is filled in to soft gelatin capsules. <sup>(8)</sup>

Eg- curcumin phytosome

- 2) **Hard gelatin capsules:** Phytosomes can be directly filled into hard gelatin capsules in powder form itself. Capsule size shouldn't increase 300 mg for low density phytosomes<sup>(12)</sup>

Eg- Ginkgo select phytosome

- 3) **Tablets:** To prepare phytosomes in form of tablets, the phytosome complex is diluted with 60-70% of excipients. Unit dosage forms can prepared from direct compression method. <sup>(8)</sup>.

Eg- Leucoselect phytosome

- 4) **Topical dosage form:** Emulsion is prepared first by using lipid solvent. Phytosome complex is incorporated in to emulsion as phyto-phospholipid complexes are dispersible in lipid solvents.<sup>(13)</sup>

Eg- Nanosized soy phytosome

**Table – 2** Commercial formulations of phytosomes available in market : (Sundaraganapathy et al., 2016)

Product	Daily dose	Indication
Grape Seed Phytosomes	50 to 100 mg	Specific for the eyes, lungs, diabetes, varicose veins, and protection against heart disease.
Green Tea Phytosomes	50 to 100 mg	Best choice for protection against cancer. Also protects against damage to cholesterol
Ginkgo BilobaPhytosomes	120 mg	Best choice for geriatric patient. Protects brain and vascular lining
SILIPHOS™	120 mg	Best choice if the liver or skin needs additional antioxidant protection
Milk Thistle Phytosomes	150 mg	Good choice when the liver or skin only needs minor support

## ADVANTAGES :

- ✓ The bioavailability and absorption in intestinal tract of herbal extract increases due to complexation with phytochemical <sup>(9)</sup>
- ✓ Phytosomes facilitates absorption of water soluble compounds to lipid soluble compounds. <sup>(9)</sup>
- ✓ Phytosomes can also be used for pharmaceutical and cosmetic purpose. <sup>(12)</sup>
- ✓ Drug of the phytosome can easily penetrate through the skin for transdermal delivery. <sup>(7)</sup>
- ✓ Phytosomes consists of hepatoprotective agents so provides synergistic effect for liver protection. <sup>(10)</sup>
- ✓ Phosphatidyl choline is vital part of cell membrane and acts as carrier and also nourishes skin<sup>(5)</sup>
- ✓ They are highly stable due formation of chemical bonds between Phosphatidyl choline and substrate. <sup>(11)</sup>
- ✓ Due to high absorption of phytosome dose requirement is less. High therapeutic effect can be achieved with small doses.
- ✓ Phytosomes are easy to manufacture and it doesn't require any complicated equipment.

## DISADVANTAGE :

- Phytoconstituent is rapidly eliminated from the phytosome<sup>(8,11)</sup>

## CHARACTERIZATION AND EVALUATION TECHNIQUES OF PHYTOSOMES -

Physical characteristics like shape, size, drug release, drug distribution are used. Methods used for evaluation of characteristics are Melting point determination method, Infrared spectroscopy, Thin layer chromatography (TLC), NMR spectroscopy, Diffraction analysis, scanning electron microscopy (SEM), transmission electron microscopy, differential scanning calorimetry (DSC), photon correlation spectroscopy, percentage drug entrapment. <sup>(19)</sup>

### Characterization techniques: <sup>(25)</sup>

- 1) **Vesicle size and zeta potential** : Particle size and the zeta potential can be estimated through computerised inspection system by DLS. <sup>(15)</sup>
- 2) **Transition temperature**- Differential scanning calorimetry is used to determine transition temperature of vesicular lipid system.
- 3) **Surface tension** – Surface tension of a drug dissolved in aqueous solution is determined by Dunoy ring method.
- 4) **Entrapment efficiency**- Entrapment ability of drug in Phytosomes can be determined by ultra centrifugation technique. <sup>(15)</sup>
- 5) **Drug content**- High performance liquid chromatography technique is used to determine drug content of phytosomes. Other spectroscopic methods may also be used. <sup>(11)</sup>

**6) Stability studies-** The optimised formulation of phytosomes was placed in humidity chamber for 2 months for stability studies. After 2 months the weight variation, hardness, friability, disintegration and percentage drug content of phytosomal formulation were evaluated.

#### Spectroscopic evaluations:

- 1) **Scanning electron microscopy (SEM)** - SEM is used to determine particle size and shape. Here, brass stub of electron microscope is coated with gold, on which dry sample is placed and observed under 100X. If any special bulging appears on powder Phytosomes are confirmed. <sup>(1,19)</sup>
- 2) **Transmission electron microscopy (TEM)** - Entrapment and distribution of drug with in phospholipid is investigated from TEM study. It is also used to determine particle size. <sup>(1,19)</sup>
- 3) **Photon correlation microscopy (PCS)** - PCS is used to determine particle size and also for confirmation of vesicles from hydrolysis. PCS proves that phytosomes on hydrolysis produces unilamellar liposomal structures.
- 4) **Fourier transform infrared spectroscopy (FTIR) analysis-** To examine structure and chemical stability of drug and phospholipid. The phytosome is converted to pellets by treating with potassium bromide.

**Table 3 :** Commercial products of phytosomes:

Phytosomes	Phytoconstituents	Therapeutic applications
Silybin Phytosome	Silybin from Silybum marianum	Hepatoprotective, antioxidant for liver and skin
Ginkgo Phytosome	24% Ginkgo flavanods	Protects brain and vascular linings, anti skin ageing.
Ginseng tea phytosome	37.5% ginsenosides	Nutraceuticals, immunomodulator
Green tea phytosome	Epigallocatechin	Nutraceutical, systemic antioxidant, anticancer
Grape Seed Phytosome	Procyanidins	Nutraceutical, systemic antioxidant, cardioprotective
Hawthorn Phytosome	Flavonoids	Nutraceutical, cardio-protective and Antihypertensive
Olive oil Phytosome	Polyphenols	Antioxidant, anti-inflammatory, antihyperlipidemic
Echinacea Phytosome	Echinacosides	Nutraceutical, immunomodulator

Centella Phytosome	Terpenes	Vein and skin disorders.
Palmetto berries Phytosome	Fatty acids, alcohols and sterols	Noncancerous-prostate enlargement.

## APPLICATIONS :

### 1. Antioxidant property :

Silybum maritimum produces a phytosome silypide. S. Moscarella et al 1993 reported that silypide prevents the oxidation of liver, induced by paracetamol (high doses) and CC14 in rats.

### Mechanism of action-

Oxidation is inhibited by inhibition of lipid per oxidation by reacting with oxygen species.

- In hepatitis B virus and hepatitis C virus with hepatocyte necrosis continuous usage of silypide for 2 months reduces liver function.
- Serum malondialdehyde levels of patients are also remarkably reduced (36%) after usage of silypide for 2 months.<sup>(20)</sup>

### 2) Anticancer property :

Chemical constituents of medicinal plants like flavones, flavanoids, isoflavones, catechins, isocatechins, coumarin, anthocyanins, lignins possess anti oxidant property which leads to anticancer property . The present existing therapies for cancer like chemotherapy, radiotherapy have several side effects like cardiac toxicity, neurotoxicity, renal toxicity, pulmonary toxicity, myelo suppression..So plant products are formulated as phytosomes and due to bipolar moiety solubility, permeability are improved which leads to increase its potency as an anticancer agent.

S. Shahili et al 2013 investigated anti proliferative activity methanolic Terminalia arjuna bark and its phytosome in human breastfeeding cancer through MTT assay by comparing with quercetin and its phytosome. IC50 values of extract and its phytosomes are found to be 25 micrograms and 15 micrograms respectively, which indicates that they show more antiproliferic activity than free drug.

D. Gallo et al 2003 reported that silypide used to treat human ovarian cancer. According to HPLC report silybin levels in tumour cells are found to be 7.0mg/ml and 183.5ng/g. treatment with silybin reduces concentration of VEGF but not the levels of VEGF in tumour cells<sup>(21)</sup>

### 3) Wound healing :

Sinigrin is one of major glucosinolate found in Brassicaceae family. Wound healing activity of Sinigrin was studied by A. Muzumder et al. 2016. It shows 71% wound closure in 42 hrs. But Sinigrin-phytosome complex is efficient in complete (100%) wound healing in 42 hrs. It shows less toxicity towards Ha Ca T cells.



S.Lakshmidivi et al.2012 reported that ethanolic extract of *Wrightia arborea* leaves can heal the wound upto 65.63%. Whereas *Wrightia arborea* phytosomes shows 90.40% wound healing. <sup>(22)</sup>

#### 4) Hepato protective :

*Ginkgo biloba* belongs to ginkgoaceae family. It possess hepatoprotective, anti anesthetic, cardioprotective, anti diabetic activities. About 1.1% of adults receiving rifampicin treatment are prone to clinical hepatitis. S. R.Naik et al 2008 investigated hepatoprotective activity of ginkgo select phytosomes. Rifampicin induced hepatotoxicity in rats. Present studies evaluated that rifampicin induced hepatic damage may be linked to its free radical scavenging activity and antioxidant activity.

Mangiferin shows improved scavenging activity on Diphenyl-1-picrylhydrazyl radical which leads to stimulation of liver regeneration. Mangiferin phytosomes shows reduced levels of serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase, bilirubin, malonyl dehydrogenase, and also improved levels of decreased super oxidizedismutase, glutathione, catalase albumin, glutathione peroxidase etc., when compared to normal mangiferin and standard drug Silymarin.

#### 5) Transdermal application :

Plant products are used in management of inflammation, but have stability and bioavailability problems. Some of the phytosomes may have hydrophobic nature and so they are inefficient to enter circulatory system. Such drugs are formulated in form of phytosomes as the phytosomes technique increases the hydrophilicity of lipophilic drugs and lipophilicity of hydrophilic drugs to cross biological membrane. And also bioavailability can be increased. Phytosomes can also be applied topically for cosmetic purpose <sup>(24)</sup>

#### 6) Anti- diabetic activity :

Phytoformulation, *Allium cepa*-phospholipid complex (ACP) shows anti diabetic activity in streptomycin induced rats. Treatment with ACE (*Allium cepa* extract), ACP glucose tolerance in normal animals significantly improved. In Streptozocin induced diabetic rats, a single dose of ACE and ACP shows reduced SG levels compared to basal levels. Administration of both the doses of ACE and ACP for fifteen days shows greater percentage reduction in glycemia and restored to near normal value of all tested lipid parameters. <sup>(23)</sup>

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