Indian Journal of Drugs, 2017, 5(1), 16-36 **ISSN: 2348-1684**

Review Article

PHYTOSOMES - NOVEL DRUG DELIVERY SYSTEM

Chetan K. Nimbalkar, Ketan Hatware

SVKM's NMIMS, Deemed-to-be University, School of Pharmacy and Technology Management, Shirpur Campus. Dist- Dhule, Maharashtra-425 405. India

*For Correspondence:
SVKM's NMIMS, Deemed-to-be University, School of Pharmacy and Technology Management, Shirpur Campus. Dist- Dhule, Maharashtra-425 405. India

Received: 17.01.2017
Accepted: 22.03.2017

Access this article online

Website:
www.drugresearch.in

Quick Response Code:

ABSTRACT

Novel Drug Delivery System is need of time, as it makes bioavailability, security and overall therapeutics of a drug easy-going and in the bat of an eye. In the recent days, most of the regnant maladies and nutritional disorders are treated with herbal medicines because of their less aftermath, economical and easily accessible. The potency of any herbal medication is contingent on the delivery of the effectual level of the therapeutically active constituent. But because of high polarity and poor lipophilicity, the active contents are incompletely assimilated resulting in poor bioavailability. Phytosome is the novel emerging proficiency applied to Phyto-pharmaceutical for the intensification of bioavailability and activity of botanical essences for medicinal applications. This is improved forms of herbal formulations which contain the bioactive phytoconstituents of herb essence enclosed and bound by a lipid. Phytosomes are lipid congenial molecular complexes made by the interchange of standardized plant extracts and phospholipids; mainly phosphatidylcholine in stoichiometric proportion. Phytosome demonstrated improved pharmacokinetic and pharmacodynamic response than customary botanical extracts. Phytosome technology has been effectively used to intensify the bioavailability of many best-selling herbal extracts e.g. milk thistle, Ginkgo biloba, grape seed, green tea, hawthorn, ginseng etc. These drug-phospholipid complexes can be developed in the form of solution, suspension, emulsion, syrup, lotion, gel, cream, pill, capsule, powder, granules. The objective of this review is to give emphasis on the phytosome technology along with its preparation, various properties, and characterization.

KEY WORDS: Phytosomes; Herbal Drug delivery; Phospholipids; Bioavailability; Plant Extract; Phytoconstituent.

INTRODUCTION

The furtherance in the area of herbal drug delivery embarks latterly with the intention to negotiate human maladies efficiently (Gold et al. 2000; Mukherjie 2001). The botanical preparation was commonly used in popular medicine since old times and till today the use of herbal medicines is general in most of the world's population (Cott 1995). The term "Phyto" means plant while "some" means cell-like (Mukherjee 2006). It is also known as herbosomes. The phytosome are freshly acquainted structures, which include the progressive component of herb enclosed and hold-in by phospholipids. The phytosome operation gives birth to a little cell whereby the precious plant extracts are preserved from deadening by digestive enzymes and gut bacteria. Phytosomes are healthier to shift from a hydrophilic domain into the lipid-friendly domain of the enterocyte cell membrane and from there into the cell finally accomplishing the blood (Dang 2000). Phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol are the

phospholipids used, but phosphatidylcholine is commonly utilized because of their definite remedial quality. Phosphatidylcholine is not simply a passive "bearer" for the bioactive flavonoids of the phytosomes but is itself a bioactive nutrient with the authenticated clinical effectiveness of liver disease, including alcoholic hepatic steatosis, drug-induced liver damage, and hepatitis (Pandey et al. 2010). Phytosomes are microscopic cell like framework. This is a sophisticated type of herbal formulations which consist of bioactive phytoconstituents of herb distil circumvallated and bound by a lipid. Phytosome is also documented to trigger another biochemical mechanism: 1. Accelerating the antioxidant defense systems (Townsend et al. 2004; Bordoni et al. 2002); 2. Arousal of alpha1 adrenergic stimulated glucose transport (Angeloni et al. 2007); 3. Antagonism with the production of the pro-inflammatory response function cytokines (Tedeschi et al. 2004). Most of the biologically operational elements of the herb are water soluble molecules. However, water soluble phytoconstituents (like flavonoids, tannins, terpenoids, etc.) are badly assimilated either due to their macroscopic molecular nature which cannot absorb by passive diffusion, or else due to their poor lipid solubility; gravely restricting their capability to pass through the lipid-bounding cell membranes, bring about poor bioavailability (Manach et al. 2004). Phytosomes have reinforced pharmacokinetics and pharmacological quality which can be used in the anti-inflammatory endeavor as well as in pharmaceutical and esthetic arrangement (Mascarella et al. 1993). Numerous conceptualization has been formulated to enhance the oral bioavailability, such as involvement of solubility and bioavailability ameliorator, structural alteration and framed with the lipophilic carriers (Bombardelli et al. 1998; Gupta et al. 2007; Cott 1995). The trenchancy of any herbal formulation relies on the handover of an operational amount of the active compounds. The Phytosome® technology (Singh et al. 2011), introduced by Indena S.p.A. of Italy, noticeably improve the bioavailability of selected phytomedicines, by integrating phospholipids into standard extracts and thus remarkably enhancing their absorption and practical application.

With the furtherance of natural science, the phytosomes have obtained prominence in a diverse area like pharmaceuticals, Cosmeceuticals, and Nutraceuticals in the manufacturing of distinguishable products such as solutions, emulsion, creams, lotions, gels, etc. Different industries participating in making and capitalization of phytosomal products are Indena, Jamieson natural resources, Thorne Research, Natural factors, and Natures herb (Singh et al. 2011).

BACKGROUND TO PHYTOSOME TECHNOLOGY

Phytosome technology come forth in 1989 (Bombardelli et al.1989). Settled on a histochemical measurement that, definite polyphenols had tough bonding attraction for phospholipids in their entire plant tissue, a group of Italian researchers give attention to polyphenol manufacturing known to be poorly bioavailable when taken orally. These were actually a combination of polyphenols taken out from a single plant species, and their conversion into phytosome forms remarkably increased their bioavailability. To prepare phytosomes, the polyphenol mix is chemically combined with a phospholipid preparation, consisting mainly of phosphatidylcholine (PC), which is also the important phospholipid of the cell membrane (Fig.1). The formed phytosome molecular complex is provided for improved bioavailability and efficacy, ordinarily with direct confirmation with its non-phytosome form. Phytosome technology has verified to be a breakthrough for the clinical usefulness of botanical polyphenols since better bioavailability generally impacts in improved efficacy (Kidd 2009).

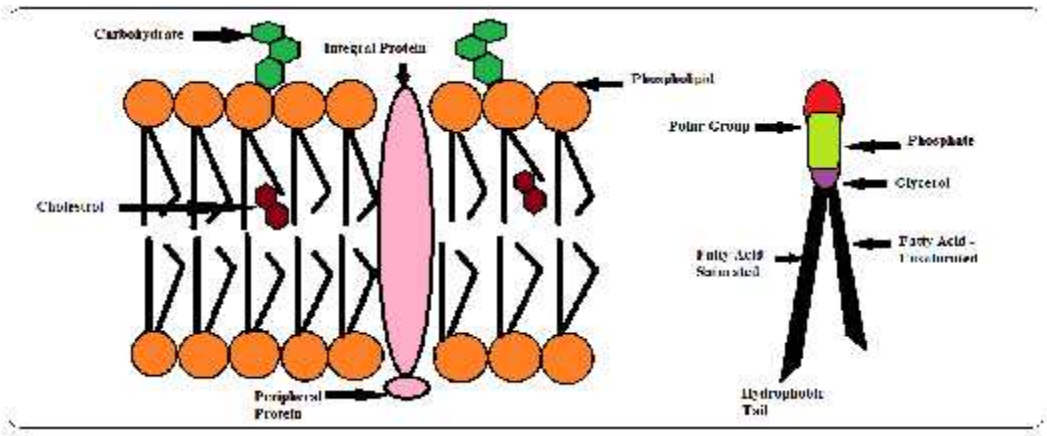


Fig. 1: Cell membranes are largely lipid phase. A double molecular layer consisting of PC and other phospholipids provides a continuous matrix into which the proteins insert.

PHYTOSOME TECHNOLOGY

Phytosome is also known as a Phytolipids delivery system, which forms a link between the traditional delivery system and novel delivery system (Amit et al. 2009). Novel vesicular drug delivery systems design to deliver the drug at a rate directed by the demand of the body during the course of therapy, and convey the active substance to the location of the action. Countless novel vesicular drug delivery systems have been appearing enclosing distinct paths of administration, to accomplish targeted and controlled drug delivery (Table.1). Phytosome is a novel form of botanical preparation, which includes the bioactive phytoconstituents of botanical essence complexed with phospholipids e.g. Phosphatidylcholine (Fig.2) to generate lipid aggregable molecular complexes. Water-soluble Phyto-constituent molecules (mainly polyphenols) can be transformed into lipid-compatible molecular complexes, which are termed as phytosomes. This phytosome technology is an innovative model for the pronounced amelioration of bioavailability, considerably higher clinical advantage, satisfied delivery to the tissues, and without complaining nutrient safety (Kidd 2009).

At this time, the progress of phytosomes is still in the foundation stages in republics such as India and in a foreign country. Up to now, this technology has been effective with herbal extracts for instance curcumin, ginkgo Biloba, grape seed, ginseng, milk thistle, and other goods used as widespread nutritional supplements (Venkatesan et al. 2000; Verma et al. 2013; Sharma et al. 2005). Numerous compound, which had revealed the extensive diversity of health welfares but scientists were at no time able to capture it because it broke down to effortlessly when it come in the body. This technology permitted the compound to be appropriately assimilated into the body and carried to the appropriate site. In order for the herbal formulation to have good bioavailability, they need to have a proper equilibrium among hydrophilicity and lipophilicity. In additional, they need to be able to dissolve into solutions present in the intestines besides cross biomembranes. This is why phytosomes deliver extra bioavailability than simple herbal essence (Bombardelli et al. 1991).

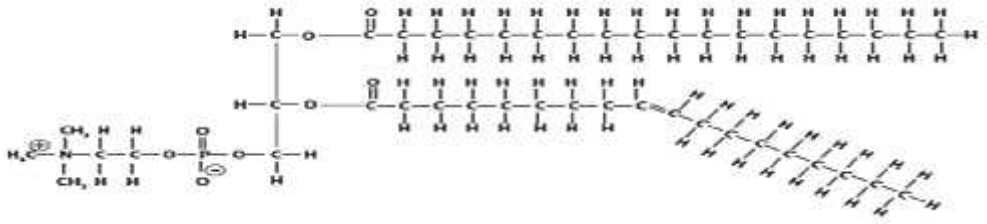


Fig. 2: Structure of Phosphatidylcholine.

Table 1: EMERGING "SOMES" AND THEIR APPLICATIONS (Kareparamban et al. 2012; Gupta et al 2011; Rathore 2012)

Vesicular system	Description	Application
Aquasomes	These are circular 60-300 nm fundamental particles utilized for the drug in addition to antigen delivery. Three layered self-assembly compositions with ceramics, carbon nanocrystalline particulate core treated with glassy cellobiose	Specific targeting, molecular shielding
Archaeosomes	Vesicles made of glycerolipids of archaea with effective auxiliary action	Poor adjuvant activity
Colloidosomes	These are solidified microcapsules made by the self-assembly of colloidal particles at the intersection of emulsion droplets and they are also vacuous, flexible shells whose permeability can be exactly managed.	Drug targeting
Cryptosomes	Lipid vesicle with surface layer made of PC and of desirable polyoxyethylene derivative from phosphatidyl ethanolamine	Ligand-mediated drug delivery.
Carbohydrosome	Carbohydrosome is methyl-2, 3-di-o-lauroyl- β-D-ribose-5 phosphocholine (DLRPC). They are novel vesicular multidimensional structures made of zwitterionic, cationic, or anionic carbohydrate-based lipids	Biological targeting of macromolecules or ligands.
Cubosomes	Cubosomes are bicontinuous cubic phases, which include two separate, uninterrupted, but non-intersecting hydrophilic domain separated by a lipid laminate that is contorted into a periodic minimal surface with zero average curvature.	Drug targeting
Discosomes	Niosomes connected with non-ionic surfactants.	Ligand-mediated drug targeting
Emulsomes	Nano-sized lipid fundamental particles made of lipid construction and a polar group.	Parenteral delivery of poorly water soluble drugs
Enzymosomes	The enzyme covalently trapped to the surface of liposomes.	Targeted delivery to tumor cells
Erythrosomes	The liposomal system in which chemically interconnected to human erythrocytes cytoskeletons is utilized to which a lipid bilayer is glazed.	Targeting of macromolecular drugs
Ethosomes	Ethosomes are lipid "Soft, malleable vesicles" representing a permeation modifier and made of phospholipid, ethanol, and water	Targeted delivery to deep skin layer
Escheriosome	These are lipoidal vesicles, composed of polar lipids extracted from Escherichia coli	Drug targeting.

Genosomes	Synthetic macromolecular complex utilized in the transfer of functional gene.	Cell-specific gene transfer
Hemosomes	Liposomes which consist of hemoglobin made by, immobilizing hemoglobin with polymerizable phospholipid.	High capacity oxygen carrying system
Layersome	The layersomes are a multilayered structure, coated with biocompatible polyelectrolytes in order to strengthen their structure.	Oral administration and incorporation in biomaterials.
Marinosome	Marinosomes are liposomes made of natural marine lipid essence containing a high proportion of polyunsaturated fatty acids like eicosapentaenoic acid and docosahexaenoic acid.	Treating inflammatory skin disorders.
Photosomes	Liposomes containing photolyase, which lose the ingredient through the membrane by photo triggered charges.	Photodynamic therapy
Pharmacosomes	Pharmacosomes are the amphiphilic structure of drugs with lipids. The drugs conjugated by hydrogen bonds to lipids. Represented as ultrafine vesicular, micellar, or hexagonal aggregates.	Hydrophilic and lipophilic drugs delivery to improve their solubility, bioavailability and minimize the gastrointestinal toxicity of various drugs.
Proteosomes	A multisubunit enzyme which is high in molecular weight, which includes catalytic activity.	Better catalytic activity, turnover than non-associated enzymes
Subtilosome	Subtilosomes are made from phospholipids separated from Bacillus subtilis.	A novel potential carrier system used in drug delivery.
Transfersome	Transfersomes are ultra deformable, self-optimized aggregates for transdermal application, which contain a mixture of lipids and biocompatible membrane softeners.	Systemic delivery of therapeutically meaningful amounts of macromolecules.
Ufasomes	Vesicles surrounded by fatty acids squeezed out from long chain fatty acids by mechanical agitation in the presence of buffer solution.	Ligand-mediated drug targeting
Vesosomes	Nested bilayer made of bilayers surrounding an aqueous center, which contains unilamellar vesicle.	Multiple compartments of Vesosomes give better protection to the interior content of serum Virosomes
Virosomes	Liposomes pointed with virus glycoprotein's, added in the liposomal bilayer based on retrovirus-based lipids.	Immunological adjuvant

PROPERTIES OF PHYTOSOMES

Following are some of the vital properties of phytosomes:

physicochemical properties

1. Phytosome is made by reaction of the stoichiometric quantity of phospholipid with the standardized herb essence as substrate. The spectroscopic information discloses that the phospholipid substrate interplay is because of the generation of hydrogen bond in between the polar head (i.e., phosphate and ammonium group) and the polar functionalities of the substrate (Tripathy et al. 2013).
2. The magnitude of Phytosome changes from 50 nm to a few hundred μm (Amit et al. 2013).
3. Phytosome, when reacted with water, gets converted into a micellar shape resembling liposome and photon correlation spectroscopy (PCS) disclose this liposomal organization acquired by phytosome (Jain 2005).
4. The H1NMR and C13 NMR data concluded that the fatty chain responsible for unchanged signals both in free phospholipid and in the complex, which shows that long aliphatic chains are clothed around the active principle generating lipophilic envelope (Dayan et al. 2000)
5. The complexes are generally freely soluble in aprotic solvents, moderately soluble in fats, insoluble in water and comparatively unstable in alcohol. But the phytosomes of certain lipophilic phytoconstituents like curcumin has proved maximum water solubility upon complexation with phospholipid (Maffei et al. 1994).

biological properties

Phytosome is innovative complexes which show improved absorption; hence they reduce bioavailability problem and shows excellent outcomes than traditional formulation, which has been proved by pharmacokinetic studies or else by pharmacodynamic tests in experimental animals in addition to human subjects (Maffei et al. 1994).

Table 2: DEFERENCE BETWEEN PHYTOSOME AND LIPOSOME (Sharma et al. 2005)

Phytosome	Liposome
Phytosome is a unit of a molecule bonded together.	Liposome is an aggregate of many phospholipid molecules that can enclose other phytoactive molecules, but without definitely conjugating to them.
Phytosome complex can passably be related to an integral part of the lipid membrane. Where the polar functionalities of the lipophilic guests interact via hydrogen bonds with the polar head of phospholipids (i.e. Phosphate and ammonium groups). Generating a specific pattern which can be qualified by spectroscopy.	In liposomes. The active part is dissolved in the fundamental domain of the cavity. With no possibility of molecular reaction between the surrounding lipid and hydrophilic substance.
Phytosome carry the phosphatidylcholine and plant component in the ratio of 1:1 or 2:1 depending on the substance (s) complexes. Involving chemical bonds. So, they better absorbed and shown improved bioavailability.	In a liposome, there is no formation of chemical bonds. The phosphatidylcholine molecules enwrap the water-soluble substance. There may be hundreds to thousands of phosphatidylcholine molecules enclosing the water-soluble compound.
Phytosomes react with the solvent having a low dielectric constant such as Acetone, Dioxane, Methylene chloride, Hexane and Ethyl acetate etc.	Liposomal drug complex is prepared in the occurrence of the water or buffer solution.

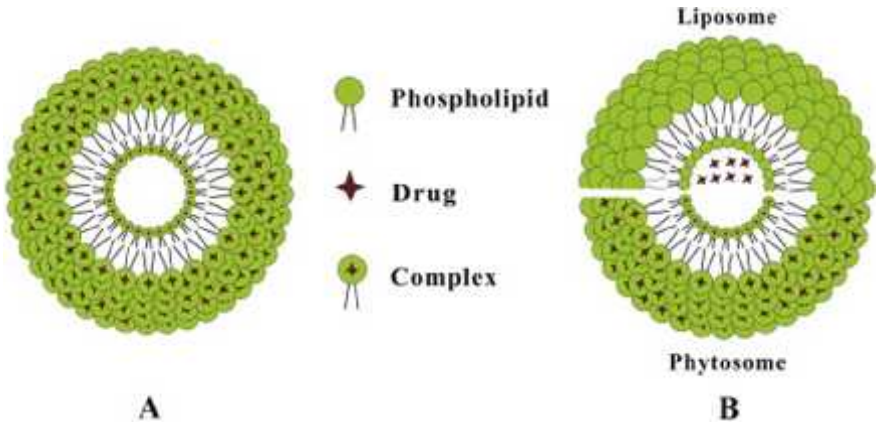


Fig. 3. The difference between phytosome and liposome. The molecular organization of phytosome (lower segment) and liposome (upper segment)

ADVANTAGES OF PHYTOSOME (Valenzuela et al. 1989; Saraf et al. 2010; Amin et al. 2012; Saha et al. 2013)

phytosomes have the following advantages:

a. There is an impressive improvement of the bioavailability of herbal essence because of their organization with phospholipid and enhanced assimilation in the intestinal tract.
b. They allow the non-lipophilic herbal essence to permit better absorption from the intestinal lumen.
c. The conceptualization of Phytosome is secure and the ingredients have all been permitted for pharmaceutical and cosmetic use.
d. They have been utilized to convey liver protecting flavonoids, as they are made of phytosomes which have high bioavailability. Apart from this, Phosphatidylcholine is hepatoprotective in nature.
e. This technology provides economical delivery of phytoconstituents and they also provide synergistic advantages in protecting the skin against exogenous or endogenous hazards in stressful environmental conditions when used in cosmetic.
f. They can be also utilized to improve the diffusion of drug through the skin in transdermal and dermal drug delivery.
g. These are foretop for the delivery of huge and an assorted group of drugs (peptides, protein).
h. The vesicular system is submissive, non-invading and is accessible for instant development.
i. Phosphatidylcholine, an indispensable portion of the cell membrane utilized in phytosome skill, perform as a transporter and also feeds the membrane.
j. There is no obstacle with drug frame-up during formulation manufacturing.
k. Also, the entrapment effectiveness is very large and furthermore programmed; because the drug himself forms vesicles after bonding with lipid.
l. They propose improved steadiness outline, because biochemical ties are generated among the phosphatidylcholine molecules and Phytoconstituents.
m. The quantity, necessity is abridged due to upgraded absorption of the active constituent.
n. Low hazard profile: This skill has no large-scale drug development risk since the toxicological outlines of the phytosomal components are well known in the systematic texts.
o. Comparatively easy to produce with no complex practical speculation essential for the manufacture of phytosomes.

the disadvantage of phytosome (Battacharya 2009) Phytoconstituent is quickly eradicated from phytosome.

MECHANISM OF PHYTOSOME FORMATION

The polyphenolic elements of herbal essence made themselves relatively healthy for straight conjugation with phosphatidylcholine. Phytosome ensues from the response of a stoichiometric quantity of the phospholipid-like phosphatidylcholine with the polyphenolic ingredients like simple flavonoids (some e.g. shown in the table. 4) in an aprotic solvent (Bombardelli et al. 1989). phosphatidylcholine is a multifunctional complex, the phosphatidyl part being lipophilic and the choline part being hydrophilic in behavior. The choline head of the phosphatidylcholine conjugate to these compounds while lipid-soluble phosphatidyl part containing the body and tail which then covers the choline bound material. Hence, the Phytomolecules yield a lipid soluble molecular compound with phospholipids named as phyto-phospholipid complex. Phytomolecules are anchored through chemical bonds to the polar choline head of phospholipids, as can be confirmed by precise spectroscopic methods (Bombardelli et al. 1991). Frequently, detailed chemical examination designates that, the unit phytosome is typically a flavonoid molecule connected with not less than one phosphatidylcholine molecule. The outcome is a tiny microsphere or cell is formed (Murray et al. 2008). In the blue phytosome spectrum, the spectrum from the polyphenol (red) is obscured by the orange spectrum from phosphatidylcholine. This is consistent with a physical entrapment of the polyphenol by the phosphatidylcholine molecule, as configured in Fig. 4.

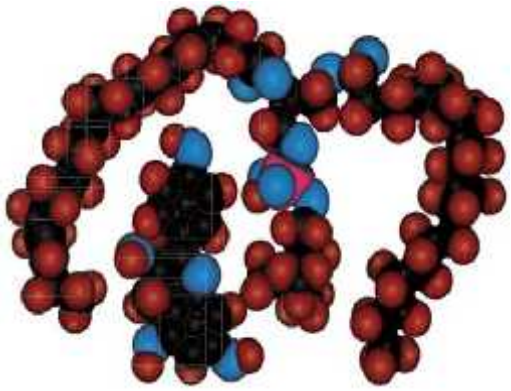


Fig. 4. Schematic of the phytosome Molecular Complex (Kidd 2009).

PREPARATION OF PHYTOSOME

1. Phytosomes are advance development which is made by interacting from 3-2 moles but if possible with one mole of a natural otherwise artificial phospholipid, such as phosphatidylcholine, phosphatidylethanolamine or phosphatidylserine with one mole of component for example- flavonolignans, either alone or in the natural combination of aprotic solvent such as dioxane or acetone from which complex can be extracted through precipitation with non-solvent for instance aliphatic hydrocarbons or lyophilization or else by spray drying. In the complex creation of phytosomes, the percentage between these two moieties is in the series from 0.5- 2.0 moles. The most desirable proportion of phospholipid to flavonoids is 1:1 (Bombardelli 1987). The different additives used in phytosome groundwork is given in Table.3.
2. Naringenin– phosphatidylcholine complex was formed by taking naringenin with an equimolar concentration of phosphatidylcholine. The equimolar concentration of phosphatidylcholine

and naringenin were placed in a 100 mL round bottom flask and refluxed in dichloromethane for 3 hours. After concentrating the solution to 5–10 mL, 30 mL of n-hexane was added to form the complex as a sludge afterward filtration. The sludge was collected and located in vacuum desiccators (Jain 2005).

3. The essential quantities of drugs and phospholipids were placed in a 100 ml round-bottom flask and dissolved in anhydrous ethanol. After ethanol was evaporated off under vacuum at 40 ◦C, the dehydrated deposits were collected and placed in desiccators overnight, then crushed in the mortar and separated by a 100 mesh. The resulting Silybin-phospholipid complex was moved into a glass bottle, flushed with nitrogen and stored in the room Temperature (Xiao et al.2006).
4. Preparation of silybin-phospholipids complex using ethanol as a reaction medium. Silybin and phospholipids were resolved into the medium after the organic solvent was evaporated under a vacuum state, and a silybin-phospholipids complex was prepared (Xiao et al.2006).
5. The mechanical Dispersion technique is utilized for the designing of marsupin-phospholipid complexes. A phospholipid is liquefied in a suitable solvent and the active ingredient is incorporated drop by drop while sonicating, the solution phospholipids complex is sometimes formed under reflux and stirring conditions to mark complete interaction (Sikarwar et al.2008)
6. Curcumin-phospholipid complexes are formed by incorporating the phospholipids to the ethanol solution of the hydroalcoholic extract of turmeric rhizomes, under reflux and with constant stirring. Set complex called phytosome can be secluded by precipitating with non-solvent, lyophilisation, and spray drying or else vacuum drying (Maiti et al. 2007). The step-by-step procedure of phytosome groundwork is represented in Fig. 5.

Table 3: DIFFERENT ADDITIVES EMPLOYED IN FORMULATION OF PHYTOSOMES

Class	Example	Uses
Phospholipid (Fuzzati et al. 1999; Zhang et al. 1994)	Soya phosphatidylcholine, Egg phosphatidylcholine, Dipalmitoylphosphatidylcholine, Distearyl phosphatidylcholine.	Vesicles creating the component.
Aprotic solvent (Gabetta et al. 2000)	dioxane, acetone, methylene chloride.	As a solvent.
Non-solvent (Gabetta et al. 2000)	n-hexane and non- solvent i.e. aliphatic hydrocarbon.	Complex precipitating solvent.
Alcohol (Basnet et al. 2011; Maghraby et al. 2008; Vanden et al. 1993)	Ethanol, Methanol.	As a solvent.
Dye (Aad et al. 2012)	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-Red 6 Carboxy fluorescence.	For CSLM study.
Buffering agent (Vanden et al. 1993; Maghraby et al. 2008)	Saline phosphate buffer (pH 6.5) 7 % v/v Ethanol Tris buffer ((pH 6.5).	As a hydrating medium.

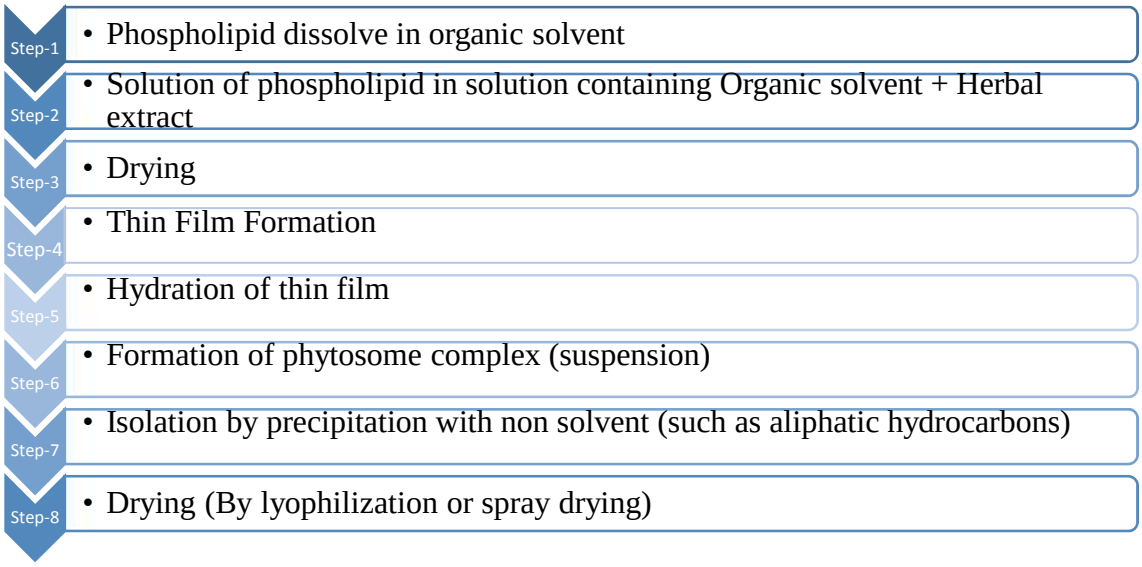
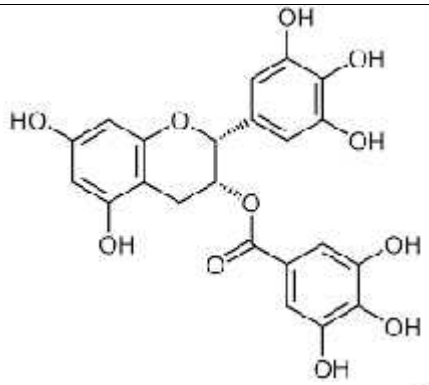
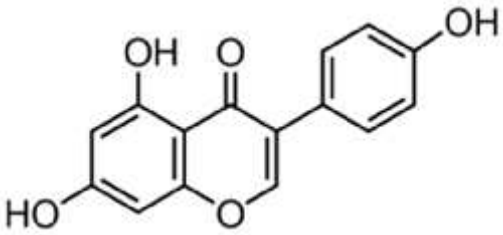
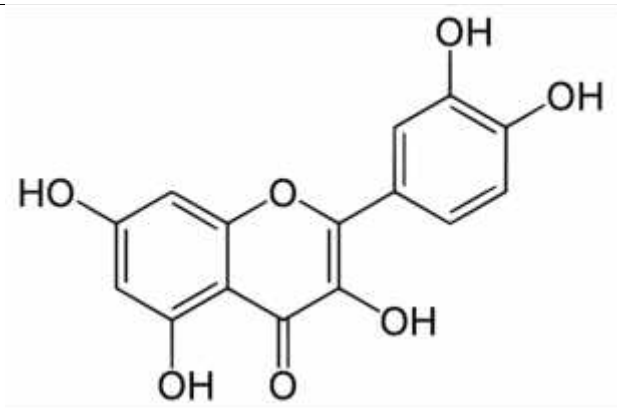
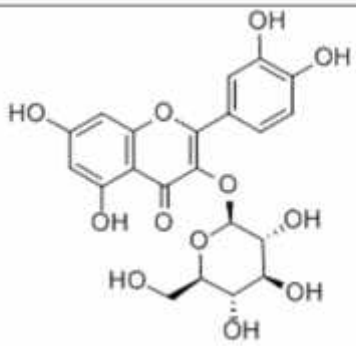
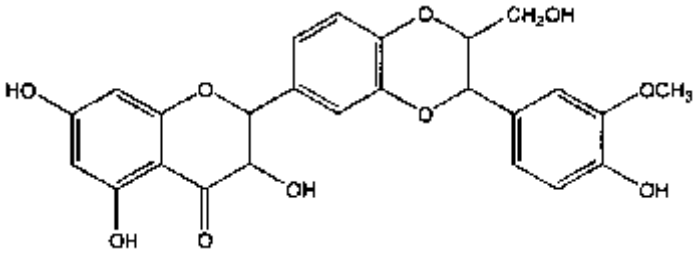
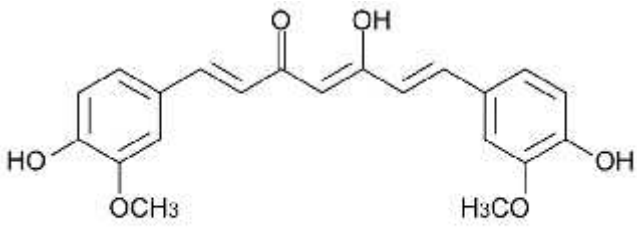
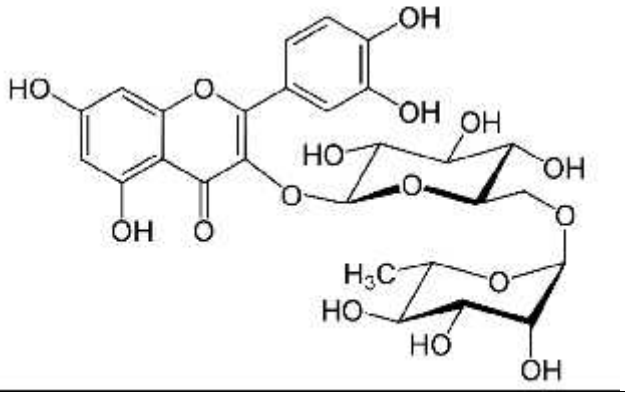
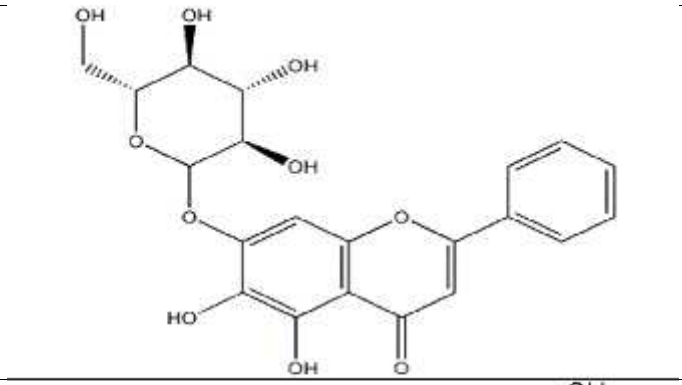
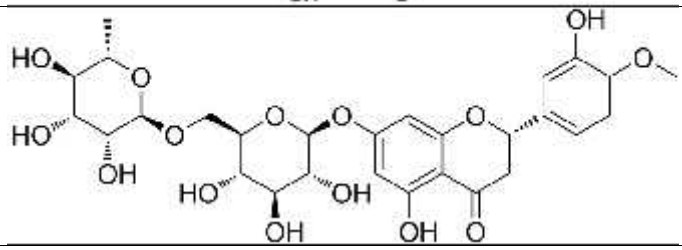
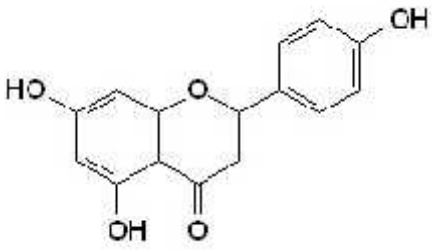


Fig.5 Common steps of preparation of Phytosomes (Jain 2005).

Table 4: SOME BIOFLAVONOIDS USED IN PHYTOSOME (Mukherjee 2001)

No.	Flavonoids	Structure
1	Epigallocatechin-3-gallate (EGCG)	
2	Genistein	

3	Quercetin	
4	Isoquercetin	
5	Silibinin	
6	Curcumin	
7	Rutin	

8	Baicalein	
9	Hesperidin	
10	Naringenin	

CHARACTERIZATION OF PHYTOSOME

There are numerous issues, for instance, the physical size, membrane permeability, percentage of entrapped solutes, chemical composition of the preparing materials which perform a dynamic character in influencing the performance of phytosomes in physical in addition to biological systems. The following are the characterization methods utilized for phytosomes in characterizing the aforementioned physical attributes.

- **transition temperature:** The transition temperature of the vesicular lipid system can be settled via differential scanning calorimetry (Ceve et al. 1995).
- **entrapment efficiency:** The entrapment efficiency of a phytosomal preparation can be determined by exposing the preparation to ultracentrifugation method (Varde et al. 2012).
- **vesicle size and zeta potential:** The particle size and zeta potential of phytosomes can be confirmed by dynamic light scattering, which usages a computerized examination system and photon correlation spectroscopy (Saha et al. 2013).
- **surface tension activity measurement:** The surface tension activity of the drug in aqueous solution can be determined by the Du Nouy ring tensiometer (Saha et al. 2013).
- **visualization:** Visualization of phytosomes can be accomplished using Scanning Electron Microscopy (SEM) shown in fig. 6 and by Transmission Electron Microscopy (TEM) shown in fig.7 (Kumari et al. 2011).
- **vesicle stability**: The steadiness of vesicles can be measured by calculating the size and structure of the vesicles over time. The mean size is calculated by DLS and structural changes are monitored by TEM (Tripathy et al. 2013).

- **spectroscopic evaluation:** The spectroscopic estimations are broadly hired in order to authorize the establishment of a complex between phytoconstituents and the phospholipid moiety in addition to studying the equivalent reaction among the two. The extensively working means are
    - **H^1 NMR -** The NMR spectra are hired for assessing the complex development among the active phytoconstituents and the phosphatidylcholine molecule. The NMR spectra of the phytosome complex had been studied by Bombardelli. In nonpolar solvents, there is a noticeable change in ^1H NMR signal initiating from atoms tangled in the construction of complex, without any outline of the signal peculiar to individual molecules. The signs from protons have its place into the phytoconstituents are magnified. In phospholipids, there is a magnification of signals while the singlet corresponding to the N-$(CH_3)_3$ of choline undergoes an upfield shift (Gabrtta et al. 1989).
    - **C^{13} NMR-** In the ^{13}C NMR of the phytoconstituents and the stoichiometric complex with the phosphatidylcholine when documented in C6D6 at room temperature, all the phytoconstituents carbons were invisible. The signal equivalent to the glycerol and choline portion are magnified and some are shifted, while a maximum of the resonance of the fatty acid chains holds their original sharp line shape (Amin et al. 2012).
    - **FTIR-** The spectroscopic assessment of the designed complex can be verified by FTIR only by relating the spectrum of the complex and the individual components and that of the mechanical mixtures. FTIR can also be measured as appreciated means in checking the firmness of the phytosomal complex. The stability can be established by comparing the spectrum of the complex in solid form with that of the spectrum of micro-dispersion in water after lyophilization at dissimilar periods (Amit et al. 2013).
- **in vitro and in vivo evaluations:** Models of in-vitro and in-vivo assessments are designated on the basis of the predictable therapeutic action of biologically energetic phytoconstituents existing in the phytosomes [54]. For instance, in vitro antihepatotoxic activity can be evaluated by the antioxidant and free radical scavenging action of the phytosomes. For calculating antihepatotoxic activity in-vivo, the result of organized phytosomes on animals in contrast to thioacetamide, paracetamol or alcohol- induced hepatoxicity can be examined (Kidd 1996; Wellington et al. 2001). Skin sensitization as well as tolerability studies of glycyrrhizic acid-Phytosome® ointment, a commercial product, describes the in vivo safety evaluation methodology (Delgi 2004).

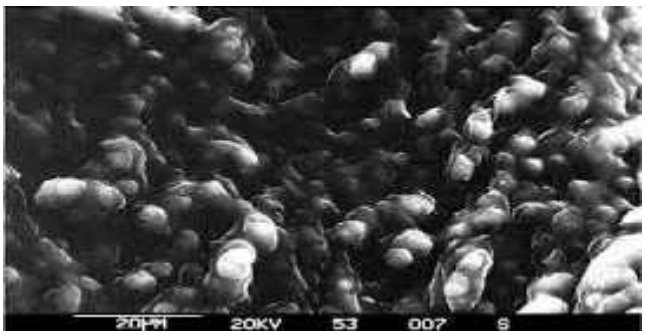


Fig. 6: SEM photomicrograph of phytosomes (Semalty et al. 2010)

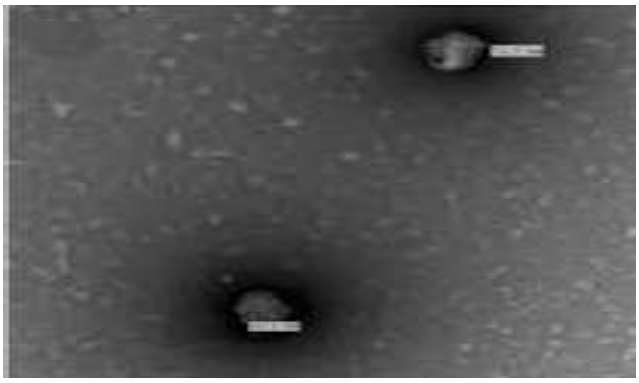


Fig. 7: TEM photograph of phytosomes (Gupta et al. 2011)

Table 5: PATENTED TECHNOLOGIES OF PHYTOSOME

Title of patent	Patent No.
Phospholipid complexes of olive fruits or leaves essence having enhanced bioavailability (Doering et al. 2006).	EP/1844785
Compositions containing Ginkgo biloba derivatives for the treatment of asthma and allergic conditions (Comoglio et al. 1995).	EP1813280
Fatty acid monoesters of sorbitol furfural and compositions intended for cosmetic and dermatological practice (Pandey et al. 2012).	EP1690862
Cosmetic as well as dermatological preparation for the bioremediation of aging or photo injured skin (Xiao et al. 2006).	EP1640041
Bioremediation of skin, and wound repair, with thymosin β4 (Battacharya 2009).	US/2007/0015698
Soluble isoflavone compositions (Chauhan et al. 2009).	WO/2004/045541
An anti-oxidant formulation founded on herb essence for the treatment of circulation and adiposity complications (Maghraby et al. 2000).	EP1214084
Complexes of saponins with phospholipid and pharmaceutical and cosmetic compositions comprising them (Fry et al. 1978).	EP0283713

RECENT RESEARCH WORK ON PHYTOSOMES

Abundant study works are being conducted by the scientists and the current research discloses that the phytosome expertise is a novel process for enhancing the absorption and bioavailability of herb essence pointedly dropping the amount level. The appropriateness of this technique and enlarged request of herbal remedies for countless ailment management in the present situation has covered the technique of novel studies (More et al. 2012).

- Giorgio et al. examined the clinical utility of oral supplementation with a combination product comprising an alpha-lipoic acid, curcumin phytosome, and B group vitamins in 180 patients with carpal tunnel syndrome (CTS). The treatment was connected with high fulfillment levels and good compliance, signifying the potential clinical helpfulness of this supplementation before and after surgery in CTS patients programmed for the surgical decompression of the median nerve (Giorgio et al. 2014).
- Gianni et al. assessed the advantageous possessions of Green select phytosome, a proprietary lecithin formulation of a caffeine-free green tea catechin essence in a controlled registry study on 50 asymptomatic subject borderlines of metabolic syndrome factors and with amplified plasma oxidative stress. Compared to the control (lifestyle and dietary changes alone), Green select Phytosome was particularly operative for weight/waist changes. The outcomes

emphasized the significance of addressing multiple factors tangled in the expansion of metabolic disease with apheliotropic agent accomplished by enhancing the valuable effects of lifestyle and dietary changes and foster the attainment of a globally improved health profile (Gianni et al. 2013).

- Zaveri et al. have designed the curcumin-phospholipid complex in a molar ratio of (1:2) of curcumin and phospholipid. They varied the formation of the complex by FT-IR Spectroscopy and DSC analysis. They likened the skin permeation of curcumin with the complexed curcumin and originate that the complexed curcumin disclosed a 60% greater permeation of curcumin through rat skin. They stated that the phospholipid complex has supplementary transdermal penetration than pure curcumin (Zaveri et al. 2011).
- Cuomo et al. examined the comparative absorption of a standardized curcuminoid mixture and its equivalent lecithin formulation (Meriva) in a randomized, double-blind crossover design human study. They informed the enhanced absorption and improved plasma curcuminoid profile of the Meriva at a dose comparatively lower than unformulated curcuminoid mixture (Cuomo et al. 2011).
- Gupta, Dixit described that combination of the high amount of curcumin in a topical preparation cannot offer improved bioavailability. They formulated complex of curcumin with phosphatidylcholine and evaluated them on the basis of TLC, DSC, Melting point and FTIR. They likened the action of vesicular systems like liposome, niosome, phyto-vesicle. In consequence, they got that the phyto-vesicle are having outstanding antioxidant and anti-aging properties compared with other vesicular systems that may be due to the amphiphilic nature of the complex, which greatly improves the water and lipid miscibility of the curcumin (Gupta et al. 2011).
- Cao et al. prepared Oxymatrine-phospholipid complex (OMT-PLC) to enhance the lipid solubility and efficiency of OMT. The aim of their study was to discover the efficacy of the mixture of a microemulsion and an OMT-PLC as a topical delivery vehicle for improving the absorption and effectiveness of OMT. They characterized numerous physicochemical properties and in vitro and in vitro permeability through the skin. They resolved that the combination of a microemulsion and phospholipid complex represents an effective vehicle for topical delivery of OMT (Cao et al. 2011).
- Forster et al**.** described in their review that the topical delivery of plant derived formulation can be successfully done in cosmetic groundwork by phospholipid complexation (Forster et al. 2009).
- Kid stated the hydration of the superficial corneous layer is related to the liposomal like properties of the phospholipid of the complex. Gingkoselect phytosomes enjoy a transdermal action which helps the ginseng saponin present in the phospholipid complex to pass through the skin (Kid 2009).
- Yanyu et al. formulated the silymarin phytosome and examined its pharmacokinetics in rats. In this study, the bioavailability of silybin in rats was enhanced noticeably after oral administration of formulated silybin-phospholipid complex due to an inspiring enhancement of the lipophilic property of silybin-phospholipid complex and enhancement of the biological effect of silybin (Yanyu et al. 2006).

- ❖ Maiti et al. Established the Quercetin-phospholipid complex by easy and reproducible technique and also presented that the preparation expressed improved therapeutic efficiency than the molecule in rat liver injury made by carbon tetrachloride (Maiti et al. 2005).
- ❖ Busby et al. described that the practice of a silymarin phytosome displayed a better photo-protectant action from ethanol-induced behavioral deficits than uncomplexed silymarin (Busby et al. 2002).
- ❖ Grange et al. conducted a series of an experiment on silymarin phytosome, comprising a standardized extract from the seeds of S. marianum, administered orally and found that it could protect the fetus from maternally ingested ethanol (Grange et al. 1999).
- ❖ Moscarella et al. investigated in one study of 232 patients with chronic hepatitis (viral, alcohol or drug induced) treated with silybin phytosome at a dose of 120 mg either twice daily or thrice daily for up to 120 days, liver function returned to normal faster in patients taking silybin phytosome related to a group of controls (49 treated with commercially available silymarin, 117 untreated or given placebo) (Moscarella et al. 2006).
- ❖ Bombardelli et al**.** described Silymarin phytosomes, in which Silymarin (A standardized mixture of flavanolignans extracted from the fruits of S. marianum) was complexed with phospholipid. Phytosomes displayed much higher specific activity and a longer lasting action than the single components, with respect to percent reduction of edema, inhibition of myeloperoxidase activity, antioxidant, and free radical scavenging properties (Bombardelli et al. 1991).

Table 6: COMMERCIAL PRODUCTS ON PHYTOSOMES (www.indena.com.; http://www.vitamedics.com.)

To inspect the several benefits of phytosomes, especially their aptitude to improve the bioavailability of polar phytoconstituents, various therapeutic applications of phytosomes have been discovered. The commercial products available in market and some patents on phytosome are listed below

Sr.No.	Trade Name	Indications
1.	Silybin phytosome	Hepatoprotective, Antioxidant.
2.	Grape seed (Leucoselect) phytosome	Antioxidant, Anticancer.
3.	Ginseng phytosome	Immunomodulator
4.	Hawthorn phytosome	Antihypertensive, Cardioprotective.
5.	Sericoside phytosome	Skin improver, Anti-Wrinkles
6.	Ginko select phytosome	Anti-aging, Protects Brain
7.	Olea select phytosome	Anti-hyperlipidemic, Anti-inflammatory
8.	Green select phytosome	Anti-cancer, Antioxidant
9.	Echinacea phytosome	Immunomodulatory, Nutraceuticals
10.	Bilberry (Mertoselect) phytosome	Antioxidant, Improvement of Capillary Tone.
11.	Palmetto (Sabalselect) phytosome	Anti-oxidant, Benign Prostatic hyperplasia
12.	Anti-oxidant, Benign Prostatic hyperplasia	Circulation Improver, Vasokinetic
13.	Centella phytosome	Brain tonic, Vein and Skin Disorder
14.	Glycyrrhiza phytosome	Anti-inflammatory, Soothing
15.	Melilotus (Lymphaselect) phytosome	Hypotensive, Indicated in Insomnia
16.	Curcumin (Merivaselect) phytosomes	Cancer Chemopreventive Agent

17.	Mertoselect phytosome	Antioxidant
18.	PA2 phytosome	Anti-Wrinkles, UV protectant.
19.	Escin β sitosterol phytosome	Anti-oedema.
20.	Ximilene and Ximenia oil phytosome	Skin Smoother, Micro Circulation Improver
21.	Ruscogenin phytosome	Anti-inflammatory, Improve Skin circulation
22.	Zanthalene phytosome	Soothing, Anti-Irritant, Anti-Itching
23.	Curbilene phytosome	Skin care, Matting Agent
24.	Esculoside phytosome	Vasoactive, Anti-cellulite, Microcirculation improver

CONCLUSION

In current eras, the evolving skill of drug delivery and drug targeting is also being functional to phytopharmaceuticals. Suitable drug delivery systems are required for the ideal delivery of active ingredients. Phytosomes an innovative drug delivery system intended for the safe, effective and proper delivery of active drug constituents, particularly phytoconstituents that are hydrophilic in nature and are poorly absorbed. The article thus analyses the welfares, physical characteristics, chemical properties, and method of preparation of phytosomes. The preparation procedure for phytosome is easy and can be effortlessly progressed to a marketable scale. Phytosomes have enhanced pharmacokinetic and pharmacological parameter, which in outcome can favorably be utilized in the treatment of diseases. Various zones of phytosome will be exposed in the upcoming days as part of their pharmaceutical use.

REFERENCES

1. Aad G, Abajyan T, Abbott B, Abdallah J, Khalek SA, Abdelalim AA, Abdinov O, Aben R, Abi B, Abolins M, AbouZeid OS. Observation of a new particle in the search for the Standard Model Higgs boson with the ATLAS detector at the LHC. Physics Letters B. 2012 Sep 17;716(1):1-29.
2. Amin T, Bhat S (2012) A Review on Phytosome Technology as a Novel Approach to Improve the Bioavailability of Neutraceuticals. International Journal of Advancements in Research and Technology 1: 1-15.
3. Amit P, Tanwar YS, Rakesh S, Poojan P. Phytosome: Phyto lipid drug delivery system for improving the bioavailability of the herbal drug. J Pharm Sci Biosci Res. 2013;3(2):51-57.
4. Angeloni C, Maraldi T, Ghelli A, Rugolo M, Leoncini E, Hakim G, Hrelia S. Green tea modulates alpha1adrenergic stimulated glucose transport in cultured rat cardiomyocytes. J Agric Food Chem. 2007; 55: 7553-7558.
5. Basnet P, Skalko-Basnet N. Curcumin: an anti-inflammatory molecule from a curry spice on the path to cancer treatment. Molecules. 2011 Jun 3;16(6):4567-98.
6. Battacharya S (2009) Phytosome: Emerging strategy in the delivery of herbal drugs and nutraceuticals. PharmTimes 41: 3.
7. Bombardelli E, Curtis B, and Della LR: Complexes between phospholipids and vegetal derivatives of biological interest, Fitoterapia 1989;90(Suppl.1): 1-9.
8. Bombardelli E, Mustich G (1991) Bilobalide-phospholipid complex, their uses and formulation containing them. U. S. Patent No. EPO-275005.
9. Bombardelli E, Spelta M. Phospholipid-Polyphenol Complexes: A New Concept in Skin Care Ingredients. Cosm & Toil.1991; 106:69-76.

10. Bombardelli E, Zio US. Patent No-EPO209037, Pharmaceutical compositions containing flavanolignans and phospholipida active principles, 1987.
11. Bordoni A, Hrelia S, Angeloni C, Giordano E, Guarnieri C, Caldarera CM, Biagi PL. Green tea protection of hypoxia/reoxygenation injury in cultured cardiac cells. J Nutr Biochem. 2002; 13:103-111.
12. Busby A, La Grange L, Edwards J, King J (2002) The use of a silymarin/ phospholipid compound as a fetoprotectant from ethanol-induced behavioral deficits. J Herb Pharmacother 2: 39-47.
13. Cao FH, OuYang WQ, Wang YP, Yue PF, Li SP (2011) A combination of a microemulsion and a phospholipid complex for topical delivery of oxymatrine. Arch Pharm Res 34: 551-562.
14. Cevc G, Schatzlein A, Blume G (1995) Transdermal Drug Carriers: Basic Properties, Optimization and Transfer Efficiency in Case of Epicutaneously Applied Peptides. J. Control Release. 36: 3-16.
15. Chauhan NS, Gowtham R, Gopalkrishna B (2009) Phytosome: A potential phyto-phospholipid carriers for herbal drug delivery. J. Pharm Res 2: 1267-1270.
16. Comoglio A, Tomasi A, Malandrino S, Poli G, Albano E (1995) Scavenging effect of the silipide-A new silybin-phospholipid complex on ethanol derived free radicals, Biochem. Pharmacol 50: 1313-1316.
17. Cott J. Natural Product Formulations Available in Europe for Psychotropic Indications. Psychopharmacol Bull. 1995; 31:745.
18. Cuomo J, Appendino G, Dern AS, Schneider E, McKinnon TP, et al. (2011) Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. J Nat Prod 74: 664-669.
19. Dang Yi. New product concept. UPC code 0300540111783. 2000.
20. Dayan N, Touitou E (2000) Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials 21: 1879-1885.
21. Delgi U, Urbino SD. Tolerability and cutaneous sensitization study in healthy volunteers after topical application of the product glycyrrhetinic acid-Phytosome® ointment, Unpublished data submitted by CTFA, 2004; 36: 2.
22. Doering T, Traeger A, Waldmann-Laue M (2006) Cosmetic and dermatological composition for the treatment of aging or photodamaged skin. EP1640041.
23. El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: from drug delivery to model membranes. European journal of pharmaceutical sciences. 2008 Aug 7;34(4):203-22.
24. Forster M, Bolzinger MA, Fessi H, Briançon S (2009) Topical delivery of cosmetics and drugs. Molecular aspects of percutaneous absorption and delivery. Eur J Dermatol 19: 309-323.
25. Fry DW, White JC, Goldman ID (1978) Rapid Secretion of Low Molecular Weight Solute from Liposomes without Dilution. Anal. Biochem. 90: 809-815.
26. Fuzzati N, Gabetta B, Jayakar K, Pace R, Peterlongo F. Liquid chromatography–electrospray mass spectrometric identification of ginsenosides in Panax ginseng roots. Journal of Chromatography A. 1999 Aug 27;854(1):69-79.
27. Gabetta B, Fuzzati N, Griffini A, Lolla E, Pace R, Ruffilli T, Peterlongo F. Characterization of proanthocyanidins from grape seeds. Fitoterapia. 2000 Apr 1;71(2):162-75.
28. Gabetta B, Zini GF, Pifferi G: Spectroscopic Studies on Idb-1016 A New Flavanolignan Complex, Plant Med 1989;55: 615.
29. Gianni B, Andrea L, Shu H, Maria RC, Beatrice F, et al. (2013) Greenselect Phytosome for Borderline Metabolic Syndrome. Evidence-Based Complementary and Alternative Medicine 1-7.

30. Gold JL, Laxer DA, Rochon PA, 2000. Herbal remedies; a critical perspective. Ann R Coll Physician Surg Can; 33:497-498.
31. Gupta A, Ashawat MS, Saraf S: Phytosome: A novel approach towards functional cosmetics, J Plant Sci 2007; 2(6): 644-649.
32. Gupta N K, Dixit VK, Development and evaluation of a vesicular system for curcumin delivery, Archives of Dermatological Research, 2011, 303, 89-101.
33. Gupta S, Singh RP, Lokwani P, Yadav S, Gupta SK (2011) Vesicular system as targeted drug delivery system: an overview. Int. J Pharm Tech 3: 987-1021.
34. Hikino H, Kiso Y, Wagner H, Fiebig M. Antihepatotoxic actions of flavonolignans from Silybum marinum fruits. Planta Med. 1984; 50: 248-50.
35. Jain NK (2005) Liposomes as drug carriers, controlled and novel drug delivery, 1st edition, CBS publisher 321-326.
36. Kareparamban J, Nikam P, Jadhav A, Kadam V (2012) Phytosome: a novel revolution in herbal drugs. International journal of research in pharmacy and chemistry 2: 299-310.
37. Kidd PM. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. Altern Med Rev. 2009 Sep 1;14(3):226-46.
38. Kidd PM. Phosphatidylcholine, A superior protectant against liver disease. Altern Med Rev.1996; 1:258-74.
39. Kumari P, Singh N, Cheriyan P, Neelam (2011) Phytosome: a novel approach for phytomedicine. International Journal of Institutional Pharmacy and Life Sciences. 1: 89-100.
40. La Grange L, Wang M, Watkins R, Ortiz D, Sanchez ME, et al. (1999) Protective effects of the flavonoid mixture, silymarin, on fetal rat brain and liver. J Ethnopharmacol 65: 53-61.
41. Maffei Facino R, Carini M, Aldini G, Bombardelli E, Morazzoni P, et al. (1994) Free radicals scavenging action and anti-enzyme activities of procyanidins from Vitis vinifera. A mechanism for their capillary protective action. Arzneimittelforschung 44: 592-601.
42. Maghraby GM, Williams EC, Barry BW (2000) Oestradiol skin delivery from ultra deformable liposomes: refinement of surfactant concentration. Int. J.Pharm. 196: 63-74.
43. Maiti K, Mukherjee K, Gantait A, Ahamed HN, Saha BP, et al. (2005) Enhanced therapeutic benefit of the quercetin-phospholipid complex in carbon tetrachloride-induced acute liver injury in rats: a comparative study. Iranian Journal of Pharmacology and Therapeutics 4: 84-90.
44. Maiti K, Mukherjee K, Gantait A, Curcumin–phospholipid complex, Preparation, therapeutic evaluation and pharmacokinetic study in rats. International Journal of Pharmaceutics. 2007; 330(1-2): 155–163.
45. Malandrino S, Pifferi G: Idb-1016 Silybin Phosphatidylcholine Complex, Drugs Future 1990; 15: 226-227.
46. Manach C, Scalbert A, Morand C. Polyphenols: Food Sources and Bioavailability. Am J Clin Nutr. 2004; 79:727-47.
47. Mascarella S. Therapeutic and Antilipoperoxidant Effects of Silybin-Phosphatidylcholine Complex in Chronic Liver Disease, Preliminary Results. Curr Ther Res. 1993; 53:98-102.
48. More M, shende M, kolhe D, Jaiswal N (2012) Herbosomes: herbo-phospholipid complex an approach for absorption enhancement. International journal of biological and pharmaceutical research 3: 946-955.

49. Moscarella S, Giusti A, Marra F, Marena C, Lampertico M, Relli P, Gentilini P, Buzzelli G. 1993. Therapeutic and antilipoperoxidant effects of silybin phosphatidylcholine complex in chronic liver disease: preliminary results, Curr Ther Res 53:98-102.2006.
50. Mukherjee PK 2001. Evaluation of Indian Traditional Medicine. Drug Information J.; 35(2):623-631.
51. Mukherjee P.K, While A. 2006. Integrated Approaches towards drug development from Ayurveda and other Indian System of Medicine.J. Ethnopharmacol 103:25-35.
52. Murray D. Phytosomes-Increase the absorption of herbal extract, Available at:www.doctormurray.com/articles/silybin.htm Accessed-Sept.28, 2008.
53. Pandey Shivanand, Patel Kinjal, Phytosomes: Technical Revolution in Phytomedicine, International Journal of PharmTech Research, 2010, 2, (1), 627-631.
54. Phytosomes: A technical revolution in phytomedicine. Available at: HTTP:// www.indena.com Accessed- may-20, 2010.
55. Rathore P (2012) Planterosomes: potential phyto-phospholipid carriers for the bioavailability enhancement of herbal extracts. International journal of pharmaceutical science and research 3: 737-755.
56. Saha S, Sarma A, Saikia P, Chakrabarty T (2013) Phytosome: A Brief Overview. Scholars Academic Journal of Pharmacy 2: 12-20.
57. Saraf S, Kaur CD (2010) Phytoconstituents as photoprotective novel cosmetic formulations. Pharmacogn Rev 4: 1-11.
58. Semalty A, Semalty M, Rawat, MSM & Franceschi F, Supramolecular phospholipid-polyphenolics interaction: The PHYTOSOME® strategy to improve the bioavailability of Phytochemicals, Fitoterapia, 2010, 81, 306 – 314.
59. Sikarwar MS, Sharma S, Jain AK. Preparation, characterization and evaluation of marsupsin phospholipid complex. AAPS Pharm SciTech, 2008; 9(1): 129-137.
60. Singh A, Singh AP, Verma N. Phytosome: A Revolution in Herbal Drug Delivery System. Asian Journal of Chemistry. 2011 Dec 1;23(12):5189.
61. Singha A, Saharanb V, Singha M, Bhandaria A. Phytosome: Drug Delivery System for Polyphenolic Phytoconstituents. Iranian Journal of Pharmaceutical Sciences Autumn. 2011; 7(4): 209-219.
62. Tedeschi E, Menegazzi M, Yao Y, Suzuki H, Förstermann U, Kleinert H. Green tea inhibits human inducible NO Synthase expression by downregulating signal transducer and activator of transcription-1a activation. Mol Pharmacol. 2004; 65: 111-120.
63. Tripathy S, Patel D, Baro L, Nair S (2013) A review on phytosomes, their characterization, advancement and potential for transdermal application, Journal of Drug Delivery and Therapeutics 3:147-152.
64. Townsend PA, Scarabelli TM, Pasini E, Gitti G, Menegazzi M, Suzuki H, Knight RA, Latchman DS, Stephanou A. Epigallocatechin 3-O- gallate inhibits STAT-1 activation and protects cardiac myocytes from ischemia/reperfusion-induced apoptosis. FASEB J. 2004; 18:1621-1623.
65. Valenzuela A, Aspillaga M, Vial S, Guerra R (1989) Selectivity of silymarin on the increase of the glutathione content in different tissues of the rat. Planta Med 55: 420-422.
66. Vanden Berge JC, Zweers GA. Myologia. Handbook of avian anatomy: Nomina anatomica avium. 1993:189-247.
67. Varde N, Mehta N, Thakor N, Shah V, Upadhyay U (2012) Phytosomes a potential phospholipid nanoparticulate carrier for the bioavailability enhancement of herbal extracts. International journal of comprehensive pharmacy 10: 1-7.

68. Venkatesan N, Babu BS, Vyas SP. Protected particulate drug carriers for prolonged systemic circulation, Indian J. Pharm. Science, 2000; 62: 327-333.
69. Verma H, Prasad SB, Yashwant SH. Herbal drug delivery system: A modern era prospective. Int J Current Pharma Rev Res. 2013; 4: 88-101.
70. Vitamedics, Phytosome Products [online]. 2008 [cited 2008 Sep 19]. Available from: URL: http://www.vitamedics.com.
71. Wellington K, Jarvis B. Silymarin. A review of its clinical properties in the management of hepatic disorders. Bio Drugs. 2001; 15: 465-89.
72. Xiao Yanyu, Song Yunmei, Chen Zhipeng, Ping Qineng. The preparation of silybin–phospholipid complex and the study on its pharmacokinetics in rats. International Journal of Pharmaceutics. 2006; 37: 77–82.
73. Zaveri M, Gajjar H, Kanaki N, Patel S (2011) Preparation and evaluation of drug-phospholipid complex for increasing transdermal penetration of phytoconstituents. International Journal of Institutional Pharmacy and Life sciences 1: 80-93.
74. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994 Dec 1;372(6505):425-32.