ABSTRACT

Phytosome is a scientific approach of delivering a pharmaceutical compound to achieve a significant therapeutic effect in an efficient manner by improving its performance in aspects of efficacy, safety and patient compliance. Phytosome exhibit better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts. This paper represents the complete overview of formulation methodology, chemical and biological properties, advantages and evaluation technologies, recent advances and applications of various standardized herbal extract phytosomes as a tool of drug delivery. Phytosomes have wide scope in cosmetics and many areas of them are to be revealed in future in the prospect of cardiovascular, anti-inflammatory, hepatoprotective and anticancer applications.

Keywords: Phytosome, Novel drug delivery system, Phosphatidylcholine, Bioavailability.

INTRODUCTION

Drug delivery refers to approaches, formulations, technologies and systems required to transport a pharmaceutical compound in the body safely and with its desired therapeutic effect. Novel drug delivery system is the process of delivering a pharmaceutical compound to achieve a significant therapeutic effect in an efficient manner by improving its performance in aspects of efficacy, safety and patient compliance. Some drugs possess a definite concentration range within where the maximum benefit is derived and the concentration levels above or below this range can lead to toxic effect or no therapeutic effect produced at all. The method of administrating a drug can also have significant effect in its efficacy. Therefore it is essential for the drug to “deliver at the exact place, at the definite concentration for the correct time period”. Therefore, there is a increased demand for new approaches to deliver the drugs to their target tissues, to increase the therapeutic effects of drugs, simultaneously to minimize their side effects. To enhance the bioavailability of selected phytomedicines, a new series of drug delivery system named “Phytosome” is designed. The meaning of the word “phyto” is plant and “some” refers to cell.

Most of the active phytoconstituents of plants are polar or water soluble molecules. But these phyto constituents (e.g. flavonoids, tannins, terpenoids etc.) are poorly absorbed either due to their large molecular size which cannot absorb by passive diffusion, or because of their insufficient lipid solubility; limiting their ability to pass across the lipid-rich biological membranes, causing poor bioavailability. Phytosomes have improved pharmacokinetic & pharmacological parameter helpful in the treatment of the acute and chronic liver disease of toxic metabolic or infective origin or of degenerative nature.

PHYTOSOME TECHNOLOGY

Phosphatidylcholine is a bifunctional compound comprising of the phosphatidyl moiety which is lipophilic and the choline moiety being hydrophilic in nature. Phosphatidylcholine binds with the flavonoid and terpenoid constituents of plant extract. Phytosomes are prepared by reaction of a stoichiometric amount of the phospholipid (phosphatidylcholine) with the standardized extract or polyphenolic constituents (like simple flavonoids) in a non polar solvent.

Specifically the choline head of the phosphatidylcholine molecule binds to phytoconstituents while the lipid soluble phosphatidyl portion comprising the body and tail which then envelopes the choline bound material. Therefore, the phospholipids and phytoconstituents produce a lipid compatible molecular complex known as phyto-phospholipid complex. The polar choline head
of the phospholipids are anchored with molecules through chemical bonds. Precise chemical analysis indicates the unit phytosome is usually a flavonoid molecule linked with at least one phosphatidylcholine molecule. The phytosome technology produces a little cell and the plant extract or its active constituent is protected from destruction by gastric secretions and gut bacteria owing to the gastro protective property of phosphatidylcholine.

**PREPARATION OF PHYTOSOME**

Phytosomes are formulated by patented processes consisting of standardized extract (having a standardized content of active principles) and/or active ingredients of herbs (like flavoliganans and terpenoids) bounded to the phospholipids like phosphatidylcholine (PC) by polar end. The phytosome technology produces small cells which protect the valuable components of the herbal extract from destruction by digestive secretions and gut bacteria. They improve transition of constituents from the polar phase to the circulation through enterocytes of the gut wall. PC is well absorbed orally and it is the principle molecular building block of cell membranes and is miscible with both polar and nonpolar mixtures. The choline head of the phosphatidylcholine molecule binds to phytoconstituents while the fat-soluble phosphatidyl portion envelopes the choline-bound material. This results in small microspheres or the production of cells known as phytosomes. Thus, phytosomes are also considered as a phytolipid delivery system.

Phytosomes are prepared by reacting 3-2 moles (preferably with one mole) of a natural or synthetic phospholipid, like phosphatidylcholine, phosphatidyl ethanolamine or phosphatidyserine, either alone with one mole of phytoconstituents or in the natural mixture in an aprotic solvent, like dioxane or acetone, in a 1:2 ratio. The optimum ratio of phospholipid to phytoconstituent is 1:1. The complex thus formed can be isolated by precipitation with an aliphatic hydrocarbon or lyophilization or spray drying.

**CHARACTERIZATION & EVALUATION OF PHYTOSOMES**

The behavior of phytosomes is governed by factors such as the physical size, membrane permeability, percentage of entrapped solutes, chemical constituents and the amount and purity of the starting materials. Hence, phytosomes can be characterized in terms of their physical characters like shape, size, distribution, amount of drug captured, entrapped volume, amount of drug released and chemical composition.

A) Characterization techniques used for phytosomes:

1. **Visualization:**
   Visualization of phytosomes can be achieved using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).

2. **Vesicle size and Zeta potential:**
   The particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy.

3. **Entrapment Efficiency:**
   The entrapment efficiency of a drug by phytosomes can be measured by the ultracentrifugation technique.

4. **Transition Temperature:**
   The transition temperature of the vesicular lipid systems can be determined by differential scanning calorimetry.

5. **Surface Tension Activity Measurement:**
   The surface tension activity of the drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

6. **Vesicle Stability:**
   The stability of vesicles can be determined by assessing the size and structure of the vesicles with respect to time. The average size is measured by DLS and structural changes are monitored by TEM.

7. **Drug Content:**
   The amount of drug can be quantified by a modified high performance liquid chromatographic method or by a suitable spectroscopic method.

B) Spectroscopic Evaluations:

To confirm the formation of a complex or to study the reciprocal interaction between the phytoconstituents and the phospholipids, the given spectroscopic methods are used:

1. **1H-NMR:**
   The NMR spectra of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine have been studied. In nonpolar solvents, there is a marked change of the 1H-NMR signal originating from the atoms involved in complex formation, without any summation of the signal corresponding to the individual molecules. The signals from the protons of the flavonoids are to be broadened that the proton cannot be resolved. In phospholipids, all the signals broaden while the singlet corresponding to the N-(CH$_3$)$_3$ of choline undergo an uplift shift. The sample is heated to 60°C results in the appearance of some new broad bands, corresponding to the resonance of the flavonoid moiety.

2. **13C-NMR:**
   In the 13C-NMR spectrum of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine, all the flavonoid carbons are clearly invisible, when recorded in CD$_3$Cl at room temperature. The signals from the glycerol and choline portion of the lipid (between 60-80 ppm) are shifted and broadened, whereas resonances of the fatty acid chains retain their original shape of sharp line. When heated to 60°C, all the signals of the flavonoid moieties reappear, which are still very broad and partially overlapping.

3. **FTIR:**
   The formation of the complex can be also be confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and spectrum of mechanical mixtures.
FTIR spectroscopy is important for the control of the stability of phytosomes when micro-dispersed in water or when incorporated in cosmetic gels. The stability of phytosomes can be confirmed by comparing the spectrum of the complex in solid form (phytosomes) with the spectrum of its micro-dispersion in water after lyophilization at different time interval. For simple formulations, it is essential to subtract the spectrum of the excipients (blank) from the spectrum of the cosmetic at different time interval, comparing the remaining spectrum of the complex itself.

C) In vitro and in vivo evaluations:
Models of in-vitro and in-vivo evaluations are selected on the basis of the expected therapeutic activity of the biologically active phytoconstituents present in the phytosomes. For example; in-vitro antihepatotoxic activity can be assessed by the antioxidant and free radical scavenging activity of the phytosomes. For evaluating antihepatotoxic activity in-vivo, the activity of prepared phytosomes on animals against thioacetamide, paracetamol or alcohol-induced hepatotoxicity can be examined. Skin sensitization and tolerability studies of commercial phytosome product glycyrrhetinic acid ointment, explain the in vivo safety evaluation methodology. Filburn et al. studied the bioavailability of a silybinphosphatidylcholyl complex in dog models to examine the pharmacokinetic parameters of this new complex form.

ADVANTAGES OF PHYTOSOMES
1. There is an enhancement of the bioavailability of botanical extracts due to their complexation with phospholipids and improved absorption in the intestinal tract.
2. They permeate the non-lipophilic botanical extract to allow better absorption from intestinal lumen.
3. The formulation of phytosomes is safe and its components have all been approved for pharmaceutical and cosmetic use.
4. The liver-protecting flavonoids can be made easily bioavailable by phytosomes.
5. Phosphatidylcholine is also hepatoprotective and provides a synergistic effect for liver protection.
6. This technology offers cost-effective delivery of phytoconstituents and synergistic benefits when used as functional cosmetics to protect the skin against exogenous or endogenous hazards.
7. They enhanced permeation of drug through skin for transdermal and dermal delivery.
8. Due to their improved skin penetration and high lipid profile they can be widely used in cosmetics.
9. Phosphatidylcholine, an essential part of the cell membrane used in phytosome technology, nourishes the skin and also acts as a carrier.
10. The drug entrapment efficiency is high and predetermined, because after conjugation with lipid the drug itself forms vesicles.
11. The chemical bonds formed between the phosphatidylcholine molecules and phyto constituents provide better stability profile.
12. The phytosomal system is passive and non-invasive.
13. The improved absorption of the main constituent minimizes the dose requirement.
14. This technology has no large-scale drug development risk since the toxicological profiles of the phytosomal components are well documented in the scientific literature.
15. Relatively simple to manufacture with no complicated technical investment required for the production of phytosomes.
16. They also have many applications in the cosmetic, veterinary and pharmaceutical fields.
17. Phytosomes and liposomes are very different structurally. Unlike phytosomes, liposomes are formed by mixing a water-soluble substance with phosphatidylcholine. There may be hundreds or even thousands of phosphatidylcholine molecules surrounding the water-soluble compound without forming any chemical bond. Whereas in the phytosome process, the phosphatidylcholine and the individual plant components actually form a 1:1 or a 2:1 molecular complex depending on the substance(s) complexes and chemical bonds are also involved.

DISADVANTAGES OF PHYTOSOMES
Phytoconstituent is rapidly eliminated from phytosomes. The duration of action is short.

APPLICATIONS OF PHYTOSOMES
Ginkgo Phytosome:
The major therapeutic indications for the standardized Ginkgo biloba leaves extract concern cerebral insufficiency and peripheral vascular disorders. These tremendous medicinal properties of Ginkgo biloba are due to the presence of flavonoids and terpene lactones such as ginkgolides and bilobalides. To further improve its absorption and bioavailability, GBE (Ginkgo biloba extract) has been complexed with soy phospholipids (1:3 w/w). Studies have shown GINKGO PHYTOSOME produced better results when compared to the conventional standardized extract from the plant. It was found that the phytosomal Ginkgo biloba extract produced a 2-4 times greater plasma concentration of terpenes than nonphytosomal Ginkgo biloba extract.

Silybin Phytosome:
A standardized extract from Silybum marianum (Milk thistle) is an excellent liver protectant but very poorly absorbed orally. So in order to enhance its bioavailability, silybin was complexed with phospholipids to form SILIYBIN PHYTOSOME which is better absorbed due to an impressive improvement in lipophilic property when compared to an equal amount of silybin in conventional Milk thistle extracts. Silybin improves the bile solubility. Because of SILIYBIN PHYTOSOME, more silybin is being delivered to the...
liver and gallbladder, this form is the ideal form for individuals with gallstones or fatty-infiltration of the liver - two conditions characterized by decreased bile solubility.

Phytosomes showed much higher specific activity and a longer lasting action than the single constituents, with respect to percent of edema reduction, myeloperoxidase activity inhibition, antioxidant and free radical scavenging properties and also better fetoprotectant activity from ethanol-induced behavioral deficits than uncomplexed silymarin\textsuperscript{40,41,42}.

**Grape Seed Phytosome:**
Grape seed extract is a rich source of one of the beneficial group of plant flavonoids- the proanthocyanidins (PCO). The primary uses of PCO extracts are in the treatment of venous and capillary disorders including venous inadequacy, varicose veins, capillary fragility and retina disorders like diabetic retinopathy. It also inhibits destruction of collagen by enhancing vitamin C levels. GRAPE SEED PHYTOSOME offers the most beneficial source of procyanidolic oligomers\textsuperscript{43}.

**Greenselect Phytosome:**
A standardized Green tea extract generally contains a polyphenolic fraction (\textit{Thea sinensis}) characterized by the presence of the key compound, epigallocatechin 3-O-gallate. These compounds are effective modulators of several biochemical processes linked to breakdown of homeostasis in major chronic-degenerative diseases such as cancer and atherosclerosis and also got several long term beneficial activities such as antioxidant, antimutagenic, hypocholesterolemic, cardioprotective, antiatherosclerotic anticarcinogenic and antibacterial effects. Despite such potential actions, to improve their poor oral bioavailability polyphenols are complexes with phospholipids\textsuperscript{44}.

**Curcumin Phytosome:**
Antioxidant activity of CURCUMIN PHYTOSOME (containing turmeric, \textit{Curcuma longa}) was prolonged when compared to that of free compound which may be due to decrease in the rapid elimination of the molecule from body\textsuperscript{45}.

**Leucoselect Phytosome:**
It is composed of oligomeric polyphenols (Grape procyanidins) of varying molecular size, complexed with phospholipids\textsuperscript{46,47}.

**Mirtoselect Phytosome:**
It contains an extract of Bilberry which provides anthocyanosides\textsuperscript{47}.

**Sabalselect Phytosome:**
It includes an extract prepared from Saw palmetto berries through supercritical carbon dioxide extraction\textsuperscript{47}.

**Lymphaselect Phytosome:**
It includes a standardized extract from \textit{Melilotus officinalis}. This preparation is particularly indicated for venous disorders such as chronic venous insufficiency of the lower limbs\textsuperscript{46,47}.

**Oleaselect Phytosome:**
It is a newer preparation from Olive oil polyphenols. These are potent antioxidants (free radical scavengers), inhibit oxidation of LDL cholesterol and have anti-inflammatory activity\textsuperscript{46,47}.

**Polinacea Phytosome:**
It is an immunomodulating preparation made from \textit{Echinacea angustifolia}. It includes echinacosides and a unique high-molecular weight polysaccharide. This preparation especially enhances immune function in response to a toxic challenge\textsuperscript{47,48}.

**CONCLUSION**
Phytosomes are novel formulations of herbal extracts having improved pharmacokinetic and pharmacological parameter as compared to conventional herbal extract. This technology offers cost-effective delivery of phytoconstituents and synergistic benefits when used as functional cosmetics to protect the skin against exogenous or endogenous hazards. This new drug delivery system when applied to botanicals open new avenues to explore maximum therapeutic potential of plant substances of polar nature. Many patents are already approved for innovative formulations and techniques. Phytosomes have bright future in cosmetics and many areas of them are to be revealed in future in the prospect of anti-inflammatory, cardiovascular, hepatoprotective and anticancer applications.

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