HERBOSOMES AS A NOVEL DRUG DELIVERY SYSTEM FOR ABSORPTION ENHANCEMENT

Anuja P. Bhosale*, Akshay Patil and Mandeep Swami

Mahatma Gandhi Vidyamandir’s Pharmacy College, Panchavati, Nashik.

ABSTRACT
Herbosome technology enhances the bioavailability of herbal extracts. Herbosome act as bridge between novel delivery system and conventional delivery system. It is a complex of natural active ingredients and phospholipids (phosphatidylcholine, phosphatidylserine etc.) which increases absorption of herbal extract. Herbosome is the novel emerging technique applied to phyto-pharmaceuticals for the enhancement of bioavailability of herbal extract for medicinal applications. The goal of this review study is that herbosome have improved pharmacokinetics and pharmacological parameter, which in result can advantageously be used in treatment of various acute diseases as more amount of constituent present at the site of action (liver, brain, heart, kidney etc).

KEYWORD: Herbosomes, phospholipid, flavanoids, phytomedicine.

INTRODUCTION
The term “Herbo” means plant while “some” means cell-like. Most of the biologically active constituents of plants are polar or water soluble molecules. However, water soluble phytoconstituents (flavonoids, tannins, glycosides) are poorly absorbed either due to their large molecular size which cannot absorb by passive diffusion or due to their poor lipid solubility, severely limiting their ability to pass across the lipid rich biological membrane, resulting poor bioavailability when taken orally or applied topically. Also isolation and purification of individual components from whole herbal extract lead to partial or total loss of therapeutic activity, the natural synergy become lost which is due to chemically related constituents in herbal extract. The effectiveness of any herbal product (or medication) is dependent upon delivering an effective level of the active compounds.[1]
The herbosomes structures contain the active ingredients of the standardized plant extract or its constituents bound to phospholipids, mainly phosphatidylcholine phosphatidylerine producing a lipid compatible molecular complex. Phosphatidylcholine is not only a passive "carrier" for the bioactive flavonoids of the herbosomes, but is itself a bioactive nutrient for liver disease. Flavonoids (e.g., anthocyanidins from bilberry, catechins from green tea, silymarin from milk thistle) are the most bioactive constituents of phytomedicines. The phospholipid molecular structure includes a water-soluble head and two fatsoluble tails. Because of this dual solubility, the phospholipid acts as an effective emulsifier. Herbosomes have improved pharmacokinetic and pharmacological parameter which in result can advantageously be used in the treatment of the acute and chronic liver disease of toxic metabolic or infective origin or of degenerative nature. It can also be used in anti-inflammatory activity as well as in pharmaceutical and cosmetic compositions.\(^2\)

**OBJECTIVE**

i. To enhance permeability of phytoconstituents across the biological membranes.

ii. To increase absorption of lipid insoluble polar phytoconstituents through different routes.

iii. To solve the problem of drug entrapment.

**PROPERTIES**

*Physical properties*

- Herbosomes are lipid substances with clear melting point.
- Average size range from 50nm to a few hundred micrometers.
- They are easily soluble in non-polar solvent, insoluble in water and moderately soluble in fats.
- Micellar shape Herbosomes are formed when herbosome treated with water.

*Chemical properties:* In Herbosomes the active principal is anchored to polar head of phospholipids, becoming an integral part of membrane.

**METHOD OF PREPARATION**

Herbosomes novel complexes which are prepared by reacting from 3-2 moles but preferably with one mole of natural or synthetic phospholipids, like phosphatidylcholine, phosphatidylethanolamine or phosphatidylerine with one mole of component like flavolignans, either alone or in the natural mixture in aprotic solvent such as dioxane or acetone. The herbosome complex can be then isolated by precipitation with non solvent such
as aliphatic hydrocarbons or lyophilization or by spray drying. In the complex formation of herbosomes the ratio between these two moieties is in the range from 0.5-2.0 moles. The most preferable ratio of phospholipids to flavonoids is 1:1.\[6\]

Naringenin-PC complex was prepared by taking naringenin with an equimolar concentration of phosphatidylcholine (PC). The equimolar concentration of PC and naringenin were placed in a 100 ml round bottom flask and refluxed in dichloromethane for 3 hrs on concentrating the solution to 5-10 ml, 30 ml of n-hexane was added to get the complex as a precipitate followed by filtration. The precipitate was collected and placed in vacuum desiccators.\[7\]

Preparation of silybin-phospholipids complex using ethanol as a reaction medium. Silybin and phospholipids were resolved into the medium, after the organic solvent was removed under vacuum condition, and a silybin-phospholipids complex was formed.\[8\]

The required amount of the drug 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 dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh. The resultant silybin-phospholipids complex was transferred into a glass bottle, flushed with nitrogen and stored in the room temperature.\[8\]

Mechanical Dispersion method is used for the preparation of marsupin-phospholipid complexes. Phospholipid is dissolved in suitable solvent and active ingredient is added drop by drop while sonicating the solution phospholipids complex is sometimes prepared under reflux and stirring conditions to affect complete interaction.\[9\]

Curcumin phospholipid complexes are prepared by adding the phospholipids to the ethanol solution of the hydro alcoholic extract of turmeric rhizomes, under reflux and with stirring. Prepared complex called Herbosome can be isolated by precipitation with non-solvent, lyophilisation, and spray drying or vacuum drying.\[10\]

**PHOSPHATIDYLCHOLINE AND HERBAL EXTRACT**

Phospholipids are a class of lipids and are a major component of all cell membrane. In humans and other higher animals the phospholipids are also employed as natural digestive aids and as carriers for both fat-miscible and water miscible nutrients. They are a major component of biological membrane and can be isolated from either egg yolk or soy beans from which they are mechanically extracted or chemically extracted using hexane.
Bhosale et al. World Journal of Pharmacy and Pharmaceutical Sciences

Phosphatidylcholine is a bifunctional compound, the phosphatidyl moiety being lipophilic and the choline moiety being hydrophilic in nature. Specifically the choline head of the phosphatidylcholine molecule binds to the components of herbal extract while the lipid soluble phosphatidyl portion then envelopes the choline bound material that results in a little micro sphere or cell is produced. The term "phyto" means plant while "some" means cell-like. What the Phytosome process produces is a microsphere cell that protects valuable components of the herbal extract from destruction by digestive secretions and gut bacteria.\cite{24, 25}

Many popular standardized herbal extracts comprising of flavanoids, polyphenolics, terpenes, alkaloids, volatile oils are employed for the preparation of phytosomes. The hypothesis of an interaction with phospholipids originated from a histochemical finding indicating that anthocyanosides from \textit{Vaccinium myrtillus} L. showed a strong affinity for specific cellular structures rich in phospholipids. Evidence that flavonoids as well as saponins and triterpenic acids, do form real complexes with phospholipids was obtained about years ago when these complexes could be prepared and chemically standardized. Mainly flavanoids and polyphenolics are complexed with the phospholipids molecules and forms phytosomes. More than 4,000 naturally occurring flavonoids have been identified, each with its own distinctive molecular structure and 3-D shape. Flavonoids are part of a broader class of dietary antioxidants called polyphenols and are distinctive for their triple ring structures.\cite{5, 24}

The poor absorption of polyphenolics is likely due to two main factors. First, these are multiple-ring molecules not quite small enough to be absorbed from the intestine into the blood by simple diffusion nor does the intestinal lining actively absorb them, as occurs with some vitamins and minerals. Second, they typically have poor miscibility with oils and other lipids. This severely limits their ability to pass across the lipid-rich outer membranes of the enterocytes, the cells that line the small intestine. PC is miscible both in the water phase and in oil/lipid phases, and is excellently absorbed when taken by mouth. The molecular properties of PC and precise chemical analysis indicate the unit phytosome is usually a herbal extract molecule linked with at least one PC molecule. A bond is formed between the two molecules to create a hybrid molecule. This hybrid is highly lipid - miscible, better suited to merge into the lipid phase of the enterocyte’s outer cell membrane. Once there, it can cross the enterocyte and reach the circulating blood.
Phosphotidylcholine orientation

DIFFERENCE BETWEEN HERBOSOMES AND LIPOSOMES-

**Herbosomes**
1. In herbosome active chemical constituents molecules are anchored through chemical bonds to the polar head of phospholipids.
2. In herbosome, phosphatidylcholine and the individual plant compound form a 1:1 or 2:1 complex depending on the substance.
3. It has much better bioavailability and absorption.

**Liposomes**
1. In liposomes, the active principle is dissolved in the medium of activity or in the layers of the membrane. No chemical bonds are formed.
2. In liposomes, hundreds and thousands of phosphatidylcholine molecules surround the water soluble molecule.
3. Its bioavailability and absorption is lesser than herbosomes.
ADVANTAGES OVER THE CONVENTIONAL DOSAGE FORM\textsuperscript{[3,4,5]}

1. Herbosomes show better stability as chemical bond is formed between phospholipids molecule and phytoconstituents(s).
2. Dose of phytoconstituents is reduced due to more bioavailability of phytoconstituents in the complex form.
3. Duration of action is increased.
4. Herbosomes are simple to manufacture.
5. Phytoconstituents complex with phospholipids are more stable in gastric secretion and resist the action of gut bacteria.
6. Enhanced permeability of phytoconstituents across the biological membranes.
7. Absorption of lipid insoluble polar phytoconstituents through different routes shows better absorption, hence shows significantly higher therapeutic effects.
8. Phosphatidylcholine used in the formation of herbosome, besides acting as a carrier also possesses several therapeutic properties and gives the synergistic effect.
9. Drug entrapment is not a problem with Herbosome as the complex is biodegradable.
10. Herbosomes are widely used in cosmetics due to their more skin penetration and high lipid profile.

APPLICATION OF PHYTOSOME

Herbosomes are used in treatment of various diseases like liver disease and heart disease. It is also used as antiinflamatory, lipolytic, vasokinetic, anti-oedema, cicatrizing, trophodermic, neutraceutical immunomo-dulator, antioxidant for skin and liver, cardioprotective, anti-wrinkles and UV protectant.

The fruit of milk thistle plant contain flavonoid known for hepatoprotective effect. Silymarin has been shown to have positive effect in treating liver diseases of various kinds, including hepatitis, cirrhosis, fatty filtration of liver and inflammation of bile duct.\textsuperscript{[20]} Silybin protect the liver by conserving glutathione into the Parenchyma Cells.\textsuperscript{[21]} While Parenchyma cell (PC) helps repair and replace cell membrane. These constituents likely offer the synergistic benefit of sparing liver cell from destruction. Yanyu et al. (2006) prepared the silymarin phytosome and studied its pharmacokinetics in rats. In the study the bioavailability of silybin in rat was increased remarkably after oral administration of prepared silybin-phospholipid complex. Tedesco et al. (2004), reported silymarin phytosome show better antihepatotoxic
effect than silymarin alone and can prove protection against the toxic effect of aflatoxin B1 on performance of broiler chicks.

Mukerjee et al. (2008) developed a novel hesperetin phytosome by complexing hesperetin with hydrogenated phosphatidylcholine. This complex was then evaluated for antioxidant activity in CCL4 intoxicated rats along with pharmacokinetic studies. It was found that the phytosome had a sustained release property for over 24 h and enhanced antioxidant activity. Bombardelli and Mustichl (1991) reported silymarin phytosomes in which silymarin was complexed with phospholipids. It shows much higher specific activity and a longer lasting action than the single components, with respect to percent reduction of oedema, inhibition of myeloperoxidase activity, antioxidant and free radical scavenging properties.

Maiti et al. (2005) developed the quercetin-phospholipids complex by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride.

Barzaghi et al. (1990) conducted human study designed to assess the absorption of silybin when directly bound to phosphatidylcholine. Plasma silybin levels were determined after administration of single oral doses of silybin phytosome and a similar amount of silybin from milk thistle in healthy volunteers. The result indicated that the absorption of silybin from silybin phytosome is approximately seven times greater compared to the absorption of silybin from regular milk thistle extract.

Grange et al. (1999) conducted a series of studies on silymarin phytosome, containing a standardized extract from the seeds of *Silybum marinum*, administered orally and found that it could protect the fetus from maternally egested ethanol.

Grape seed phytosome composed of oligomeric polyphenols of varying molecular size, complexed with phospholipids. The main properties of procyanidin flavonoids of grape seed are, increase in total antioxidant capacity and stimulation of physiological antioxidant defenses of plasma, protection against ischemia/refusion induced damages in the heart, and protective effects against atherosclerosis thereby offering marked protection for the cardiovascular system and other organs through a network of mechanisms that extend beyond their great antioxidant potency.\[23\] Green tea has got several long term beneficial activities such as antioxidant, anticarcinogenic, antimutagenic, antiatherosclerotic,
hypocholesterolemic, cardioprotective and antibacterial effect. Despite such potential action green tea polyphenols have very poor oral bioavailability from conventional extracts. The complexion of green tea polyphenols with phospholipids strongly improves their poor oral bioavailability. A study on absorption of phytosomal preparations was performed in healthy volunteers along with non complexed green tea extract following oral administration. During the study period of 6 h the plasma concentration of total flavonoids was more than doubled when coming from the phytosomal versus the nonphytosomal extract. Antioxidant capacity was measured as total radical-trapping antioxidant parameter. The peak antioxidant effect was a 20% enhancement and it showed that the phytosome formulation had about double the total antioxidant effect (www.phospholipids online.com) protectant. After screening and selection of potential extracts or constituents from plant, phytosomes can be developed for different therapeutic effect like antidiabetic, immunomodulator, anticancer, antinflammatory, heart diseases and for various therapeutic purposes in future.

EVALUATION OF HERBOSOMES

**Microscopic and Other Techniques**

1) **Visualization:** Visualization of herbosomes can be achieved using Transmission Electron Microscopy (TEM) and by Scanning Electron Microscopy (SEM) electron microscopic techniques.[11]

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 (PCS).[12]

3) **Entrapment efficiency:** The entrapment efficiency of a drug in herbosomes can be measured by the ultracentrifugation technique.[13]

4) **Transition temperature:** The transition temperature of the vesicular lipid systems can be determined by differential scanning calorimeter.[14]

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.[15]

6) **Vesicle stability:** The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. The mean size is measured by DLS and structural changes are monitored by TEM.[16]
7) **Drug content:** The amount of drug can be quantified by a modified high performance liquid chromatographic (HPLC) method or by a suitable spectroscopic method.\textsuperscript{[17]}

To confirm the formation of a complex or to study the reciprocal interaction between the phytoconstituent and the phospholipids, the following spectroscopic methods are used.\textsuperscript{[18]}

1) **1H-NMR:** The NMR spectra of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine have been studied by \textsuperscript{[19]} in polar solvents, there is a marked change of the 1H-NMR signal originating from the atoms involved in the formation of the complex, without any summation of the signal peculiar to the individual molecules. The signals from the protons belonging to the flavonoid are to be broadened that the proton cannot be relieved. In the phospholipids, there is broadening of all the signals while the singlet corresponding to the N-(CH3)3 of choline undergo an uplift shift. Heating the sample to 60°C results in the appearance of some new broad bands, which correspond mainly to the resonance of the flavonoid moiety.

2) **13C-NMR:** In the 13C-NMR spectrum of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine, particularly when recorded in C6D6 at room temperature, all the flavonoid carbons are clearly invisible. The signals corresponding to the glycerol and choline portion of the lipid (between 60-80ppm) are broadened and some are shifted, while most of the resonance of the fatty acid chain retains their original sharp line shape. After heating to 60°C, all the signal belonging to the flavonoid moieties reappear, although they 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 their mechanical mixtures. FTIR spectroscopy is also a useful tool for the control of the stability of herbosomes when micro-dispersed in water or when incorporated in very simple cosmetic gels. From a practical point of view, the stability can be confirmed by comparing the spectrum of the complex in the solid form (herbosomes) with the spectrum of its micro-dispersion in water after lyophilization, at different times.

**CONCLUSION**

Herbosomes results from the reaction of stoichiometric amount of phospholipid with standardized herbal extract or polyphenolic constituents like (flavonoids, terpenoids, tannins,
xanthones etc) in nonpolar solvents. Phytosomal complexes were first investigated for cosmetic applications, but mounting evidence of potential for drug delivery has been cumulated over the past few years, with beneficial activity in the realms of cardiovascular, anti-inflammatory, hepatoprotective and anticancer applications. Herbosomes shows much better absorption profile following oral administration owing to improve lipid solubility which enables them to cross the biological membrane resulting enhanced bioavailability.

REFERENCES