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Phyto-vesicles: conduit between conventional and novel drug delivery system

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ABSTRACT

Objective: To discuss the preparation, characterization, targeting and formulation aspect of phospholipids based drug delivery system i.e. Phyto-vesicles. **Methods:** The methods of phyto-vesicles preparation on R & D scale and different analytical techniques to characterize them have been discussed. **Result:** Phyto-vesicles are the advanced form of herbal drug delivery systems as its structure includes water soluble head and two fat soluble tails which act as an effective emulsifier. **Conclusion:** It is concluded that phytovesicular delivery system has improved pharmacokinetic and pharmacodynamic parameter as compared to conventional system Therefore, phyto-vesicles are called as conduit between conventional and novel drug delivery system.

1. Introduction

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs like Ginseng and Rosemary have an optimum concentration range within which maximum benefit is derived and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, very slow progress in the efficacy of the treatment of severe diseases has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. Keeping in view the above facts new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS) are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry and molecular biology. An ideal drug delivery system possesses two elements (i) ability to target (ii) to control the drug release. Targeting will ensure high efficiency of the drug and reduce the side effects especially when dealing with drugs that are presumed to kill cancer cells but can also kill healthy cells when delivered to them. The prevention of side effects is achieved by controlled release of drug[1]. Therefore, different types of delivery system are used for variety of synthetic drugs, phytomolecules and herbal extracts to ensure better bioavailability and targeted delivery. Some of these delivery system are Cubosomes, Colloidosomes, Ethosomes, Aquasomes, Niosomes, Liposomes and Nanoparticles. Cubosomes are bicontinuous cubic phases consisting of two separate, continuous but nonintersecting hydrophilic regions divided by a lipid layer that is contorted into a periodic minimal surface with zero average curvature. Colloidosomes are solid microcapsules formed by the self assembly of colloidal particles at the interface of emulsion droplets. They are hollow, elastic shells whose permeability and elasticity can be precisely controlled. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. It contains phospholipids, alcohol in relatively high concentration and water. Aquasomes are spherical particles used for drug and antigen delivery. The particle core is composed of non crystalline calcium phosphate or ceramic diamond and is covered by a polyhydroxyl oligomeric film. Colloidal dispersion of drugs covalently bound to a lipid and may exists as ultrafine

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vesicular, micellar or hexagonal aggregates, depending on the chemical structure of the drug-lipid. Liposomes (Table 1) are small artificial vesicles of spherical shape that can be produced from natural non toxic phospholipids and cholesterol. Because of their size, hydrophobic and hydrophilic character as well as biocompatibility, liposomes are promising systems for drug delivery. A niosome is non-ionic surfactant formed mostly by cholesterol incorporation as an excipient. They are structurally similar to liposome in having a bilayer, however, the materials used to prepare niosomes make them more stable and thus niosomes offer many more advantages over liposomes[2-3]. Nanoparticles having diameter of 10-1 000 nm are drug loaded particles which can be embedded or dissolved in nanoparticles prepared by taking natural polymer or synthetic chemicals as the carriers. Comparison of these delivery systems is given in Table 2.

Over the past century, phytochemical and phytopharmalogical sciences established the composition, biological activities and health promoting benefits of numerous plant products. But many phytomedicines like epigallocatechin obtained from green tea leaves, grape procynadins, silybin obtained from silymarin are limited in their effectiveness because they are poorly absorbed when taken by mouth either due to their large molecular size, which cannot absorb by passive diffusion or due to their poor lipid solubility limiting their ability to pass across the lipid rich biological membranes resulting to poor bioavailability. To overcome this complexation with certain other clinically useful nutrients substantially improves the bioavailability. The nutrients so helpful for enhancing the absorption are the phospholipids. Phospholipids are complex molecules employed as natural digestive aids and as carrier for both fat miscible and water miscible nutrients[4-8].

Advantages of phospholipids based carrier systems in comparison to other delivery systems are (i) these systems show enhanced permeation of drug through skin for transdermal and dermal delivery, (ii) these are platform for the delivery of large and diverse group of drugs (peptides, protein molecules), (iii) their composition is safe and the components are approved for pharmaceutical and cosmetic use, (iv) Low risk profile- the toxicological profiles of the phospholipids are well documented in the scientific literature, (v) high market attractiveness for products with proprietary technology, (vi) Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes & (vii) the vesicular system is passive, non-invasive and is available for immediate commercialization.

Phyto-vesicles often known as herbosomes developed to incorporate standardized plant extracts or water soluble constituents into phospholipids (such as Phosphotidylcholine (PC) derived from soy bean, Phosphotidylserine) to produce lipid compatible molecular complexes, called as phyto-vesicles and significantly improve their absorption and bioavailability. PC is not merely a passive "carrier" for the bioactive flavonoids of the Phyto-vesicle but is itself a bioactive nutrient with documented clinical efficacy for liver diseases such as alcoholic hepatic steatosis; drug induced liver damage and hepatitis. To appreciate the uniqueness of Phyto-vesicles it is necessary to differentiate them from liposomes. The main difference between Phyto-vesicles and liposomes is that in Liposomes the active principle is dissolved in the medium contained in the cavity or in the layers of the membranes whereas in the Phyto-vesicles it is an integral part of the membrane, being the molecule anchored through chemical bonds to the polar head of the phospholipids. The unit phyto-vesicle is a molecular-level association involving as few as two molecules (one PC plus one polyphenol). The unit liposome is an aggregate of hundreds of phospholipids molecules into a spherule, within which other molecules are compartmentalized but not specifically bonded. Whereas, the liposome concept remains unproven as an oral delivery vehicle, the phyto-vesicle is known to dramatically enhance oral delivery[12,13]. Various Phyto-vesicles herbal formulations are given in Table 3. Phyto-vesicles of selected plant drugs would make the drugs better bioavailable, dramatically enhance bioavailability due to their complex with phospholipids, ensure faster drug delivery and improve absorption in intestinal tract. Phyto-vesicles permeates the non-lipophillic membrane and absorbed better in intestinal lumen. Phyto-vesicles would be given in small quantity and desired result can be achieved. Therefore it has been proved that the Phyto-vesicles technology is a breakthrough model for (i) marked enhancement of bioavailability (ii) significantly greater clinical benefit (iii) assured delivery to the tissues (particularly liver tissues)[9-11]

2. Material and method

2.1. Chemicals

All the chemicals used were of analytical grade and were obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai

2.2. Method of preparation

Most of the bioactive constituents of herbal drugs are water soluble molecules. However, water soluble phytoconstituent like many flavonoids are poorly absorbed e.g. *Ginkgo biloba* (*G. biloba*) and silymarin (i) either due to their multiple-ring large size molecules which can not be absorbed by simple diffusion, or (ii) due to their poor miscibility with oils and other lipids, severely limiting their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine. Water-soluble phytoconstituent molecules (mainly polyphenol) can be converted into lipid-compatible molecular complexes, which are called Phyto-vesicles. Mareno and Lampertico (1991), Jiang et al (2001), reported the methods of Phyto-vesicle preparation[14-27] on R & D scale as discussed below:

2.2.1.Method 1

PC + Drug (1:1)
⇓
Place in 100 mL RBF containing aprotic solvent (Dichloromethane)
⇓
Reflux for 3 h
⇓
Concentrate the solvent up to 5–10 mL
⇓
Add 30 mL of polar solvent
⇓
Precipitation
⇓
Filter and store in vacuum desiccators

2.2.2. Method 2

PC + Drug (1:1)
⇓
Place in 100 mL RBF containing anhydrous Alcohol
⇓
Evaporate ethanol under vacuum at 40 ℃
⇓
Place dried residues in desiccators for overnight
⇓
Crush in mortar and sieve with 100 mesh sieve
⇓
Phyto-vesicles
⇓
Transfer into a glass bottle and store at room temperature

Selection of preparation methods depends upon the following factors: (i) particle size requirement, (ii) Drug-carrier compatibility. (iii) Reproducibility of the release profile and the method of preparation, (iv) stability issues & (v) no toxic product associated with the final products

3. Properties of Phyto-vesicles

3.1. Physicochemical

On the basis of spectroscopic data it has been shown that the main phospholipids substrate interaction is due to the formation of hydrogen bonds between polar heads of phospholipids (Phosphate and ammonium group) and the polar functionalities of the substrate. When treated with water the Phyto-vesicles assumes a micellar shape. The structure of Phyto-vesicles depicts that the active principle is anchored to the polar head of phospholipids which becomes an integral part of the membrane. The formation of hydrogen bonds can be deduced from the comparison of ^{1}H-NMR and ^{13}C-NMR spectra of the complex with those of the spectra of pure component[28].

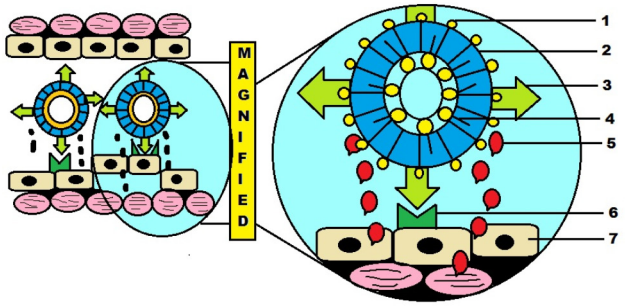

Figure 1. Targeting of drug to parenchymal cells (hepatocytes) of liver using ligand – 1 PC head – 2–Phyto-vesicles 3– Ligand molecule, 4– PC tail, 5– Water soluble drug, 6– Receptor, 7–parenchymal cell.

3.2. Biological

Phyto-vesicles are the advanced form of delivery system

Table 1.
Liposomal herbal formulation[3].

Formulations	Active ingredients	Applications of liposome formulations	Biological activity
Quercetin liposomes	Quercetin	Reduced dose, enhance penetration in blood brain barrier	Antioxidant; Anticancer
Liposomes encapsulated silymarin	Silymarin	Improve bioavailability	Hepatoprotective
Liposoma artemisia Arborescens	ArtemisiaArborescens Essential oil	Targeting of essential oils to cells, enhance penetration into, cytoplasmatic barrier	Antiviral
Ampelopsin liposome	Ampelopsin	Increase efficiency	Anticancer
Paclitaxel liposome	Paclitaxel	High entrapment efficiency and ph sensitive	Anticancer
Curcumin liposome	Curcumin	Long-circulating with high entrapment efficiency	Anticancer
Garlicin liposome	Garlicin	Increase efficiency	Lungs
Flavonoids liposomes	Quercetin and rutin	Binding of flavonoids with Hb is enhanced	Hemoglobin
Usnic acid liposome with β-CD	Usnic acid	Increase solubility and localization with prolonged release profile	Antimycobacterial
Wogonin liposome	Wogonin	Sustained release effect	Anticancer
Colchicine	Colchicine	Liposome Enhance skin accumulation, prolong drug release and improve site specificity	Antigout
Catechins liposomes	Catechins	Increased permeation through skin	Antioxidant and Chemo preventive
Breviscapine liposomes	Breviscapin	Sustained delivery of breviscapine	Cardiovascular Diseases

Table 2.
Comparison of physical parameters of different drug delivery systems.

Delivery system	Size and shape	Stability	Geometry	Applications
Cubosomes	Discrete, sub-micron, nanostructure particles	Thermodynamically stable	Bicontinous cubic phases consisting of two separate, continous but nonintersecting hydrophilic regions divided by lipid layer with zero average of curvature.	Exotic delivery vehicles in personal care and consumer products
Colloidosomes	Micrometers to millimeters and generally non-spherical in shape	Mechanically stable	Multiple compartments are generated using water-in-oil-in-water double emulsions with controlled morphology as templates	Promising vehicles for macromolecular Delivery in pharmaceutical, cosmetics, and food industries
Ethosomes	Tens of nanometer to microns and spherical	Stable at 4 ℃	–	Promising carrier for transdermal delivery of drug
Aquasomes	Range from 60-120 nm and spherical in shape	Maximum stable for 30 days the brushite is unstable and converts to hydroxyapatite upon prolong storage	Comprised of a solid phase nanocrystalline core coated with oligomeric film to which the drug moieties or biologically active molecule are adsorbed with or without modification	Successful carrier system For bioactive molecules like peptide, protein, hormones, antigens and genes to specific sites. Enzyme activity and sensitivity towards molecular conformation made aquasome as a novel carrier for enzymes like dnases and pigment/ dyes
Niosomes	Round in shape and size range was found to be 1.54 – 2.64 μm	Optimum storage condition for niosomes was found to be 4°	The vesicle holds hydrophilic drugs within the space enclosed in the vesicle, while hydrophobic drugs are embedded within the bilayer itself	Potentially applicable to many pharmacological agents for their action against various diseases including cancer and leishmaniasis.
Liposomes	Range from 20 nm to 1 000 nm and generally spherical in shape	An increase in physical stability of liposomes can be achieved by increasing amount of charge on liposomes	Vesicles having concentric bilayers of lipids filled with water and typically carrier for hydrophilic drugs	Topical applications of drugs, such as corticosteroids, antifungal, local anesthetics and retinoid, encapsulated in liposomes result in increased concentrations of the agents in the epidermis and dermis compared to conventional formulations. On the other hand, the systemic concentrations of these drugs (plasma, liver and spleen) are reduced compared to the controls. These results prove that liposomes are suitable vehicles for a selective drug delivery in the skin
Nanoparticles	Range from 20 nm to 1000 nm and shape varies according to nanospheres, nanocapsules etc	Very stable dispersions of oil in water, these emulsions are stabilized by a negative zeta potential which prevents droplet coalescence upon random collisions of particles	Single layered cell and filled with oil and typically carrier for lipophillic substance	Tumor targeting, for oral delivery of peptides and proteins, targeting of nanoparticles to epithelial cells in the GI tract using ligands, for gene delivery, drug delivery into the brain
Phyto-vesicles	Small in size and spherical in shape	–	Vesicles comprises of choline head of the phosphatidylcholine molecule binds to the drug while the lipid soluble phosphatidyl portion comprising the body and tail which then envelopes the choline bound material.	Cardio-protective, Hepatoprotective,Immunomodulator, Antioxidant,Anticancer etc

for herbal products which have better bioavailability than the conventional herbal extracts. The increased bioavailability has been demonstrated by pharmacokinetic studies or by pharmacodynamic tests in experimental animals and human subjects. Some of the examples of increased bioavailability are given below: (i) Leucoselect® Phyto-vesicles when complexed with soy phospholipids results in markedly improved bioavailability of procyanidins which have cardiovascular activity as well as antioxidant activity, (ii) Gingkoselect® Phyto-vesicles shows improved bioavailability of reduce cerebral performance, (iii) SILIPHOS® prevents the liver damage[4,8] & (iv) Greenselect® Phyto-vesicles improved their low and erratic oral bioavailability. Therefore we can say that Phyto-vesicles are conduit between conventional and novel delivery system[29].

3.3. Pharmacokinetic profile of Phyto-vesicles

The Phyto-vesicles formulation increases the absorption of active ingredients when topically applied on the skin, and improves systemic bioavailability when administered orally. In water medium, it will assume a micellar shape, forming a liposome-like structure. Pharmacokinetic studies in rats, dogs and in humans have shown increased

Table 3.
Phyto-vesicles herbal formulations [3, 37-42].

Formulations	ActiveIngredients	Applications of nanostructure formulations	Biological activity
Ginkgo biloba Phyto-vesicle	Flavonoids	Flavonoids of GBP stabilize the ROS	Cardio-protective, antioxidant activity
Ginkgo select Phyto-vesicle	Flavonoids	Inhibits lipid peroxidation (LPO), stabilize the ROS	Hepatoprotective, antioxidant
Silybin phyto-vesicle	Flavonoids	Absorption of silybin phyto-vesicle from silybin is approximately seven times greater	Hepatoprotective, antioxidant for liver and skin
Ginseng phyto-vesicle	Ginsenosides	Increase absorption	Immunomodulator green tea phyto-vesicle
Epigallocatechin	Increase absorption Nutraceutical,	Antioxidant, anticancer	
Grape seed phyto-vesicle	Procyanidins	The blood TRAP n Total Radical-trapping Antioxidant Parameter) were significantly elevated over the control	Systemic antioxidant
Cardio-protective HawthornPhyto-vesicle	Flavonoids	Increase therapeutic efficacy and absorption	Cardio-protective and antihypertensive
Quercetin phyto-vesicle	Quercetin	Exerted better therapeutic efficacy	Antioxidant, anticancer
Curcumin Phyto-vesicles	Curcumin	Increase antioxidant activity and Increase bioavailability	Antioxidant, anticancer Naringenin
Phyto-vesicles	Naringenin	Prolonged duration of action	Antioxidant activity
18 β -glycyrrhetinic acid Phyto-vesicle	18 β -glycyrrhetinic acid from licorice rhizome	Prolonged duration of action and provide soothing effect	Soothing
Centella Phyto-vesicle®	Triterpenes	Healing by forming scar tissue	Cicatrizing, trophodermic
Crataegus Phyto-vesicle®	Vitexin-2 X -O-rhamnoside from Hawthorn flower	Prolonged duration of action	Antioxidant
PA2 Phyto-vesicle®	Proanthocyanidin A2 from horse chestnut bark	Prolonged duration of action	Anti-wrinkles, UV protectant
Sericoside Phyto-vesicle®	Sericoside from Terminalia sericea bark root	Prolonged duration of action	Anti-wrinkles
Visnadex®	Visnadin from Amni visnaga umbel	Increased coronary blood flow	Vasokinetic
Oleaselecttm phyto-vesicle	Olive oil polyphenols.	Prolonged duration of action	Anti oxidant, anti inflammatory
Echinacea Phyto-vesicle	Echinacosides	Adjusting the immune response to the desired level	Immunomodulant
Bilberry Phyto-vesicles	Extract of bilberry which provides anthocyanosides	Reduce abnormal blood vessel permeability,	Potent antioxidants
Palmetto Phyto-vesicles	Fatty acids, alcohols and sterols	Prolonged duration of action	Prostate enlargement

bioavailability of Siliphos than Silybin extract[30,31]. In a comparative study in humans, analyzing the absorption of curcumin Phyto-vesicle (Meriva) and curcumin the overall curcuminoid absorption was about 29-fold higher for Meriva compared to the unformulated curcuminoid mixture. The anti-inflammatory and anti-oedemigenous effects of the Glycyrrhetinic acid were assessed in the experimental model of Croton oil-induced oedema reduction. At the same dose (0.16 μM), the action of the 18 β -Glycyrrhetinic Acid Phyto-vesicles was found to be greater and to last longer than that of 18 β -glycyrrhetic acid alone. Similar is the case with Ginkgoselect® Phyto-vesicle and Greenselect® Phyto-vesicle. This means that the complex with the Phyto-vesicle not only increases the active ingredient tolerability and absorption, but also improves its efficacy[32].

4. Targeting of Phyto-vesicles

Targeting of drug to certain site or to certain organ is a very challenging job as it not only involves the targeting of drug to a certain site but also necessary to retain it for the desired duration so as to elicit the desired pharmacological response. However, Phyto-vesicles facilitates the liver targeting (Figure 1) by increasing the solubility in the bile salts. Also targeting can be achieved by attaching ligand in such a manner that it should be presented in its right orientation for binding to the target receptors e.g. Monoclonal antibodies must bind to the drug carrier with their Fc part, so that their antigen binding site (Fab) is free to interact with the antigenic targets on cells [33-35].

5. Possible formulations of Phyto-vesicles

Four types of formulations are possible for Phyto-vesicles namely (i) Soft gelatin capsules-Phyto-vesicles complex can be dispersed in oily vehicles to obtain suspension to be filled in soft gelatin capsule, e.g. Hawthorn Phyto-vesicle which helps to strengthen the heart and cardiovascular system and may be beneficial for angina, irregular heartbeat, hypertension, coronary heart disease, congestive heart failure, (ii) Hard gelatin capsules involve direct volumetric filling process, e.g. Panax ginseng Phyto-vesicle™ which promotes adaptogenic function & resistance to stress, Grape Seed Phyto-vesicle™ provide

natural Antioxidant Protection, Green Tea Phyto-vesicles have powerful antioxidant effects, preserving cell health to improved longevity, health and well-being and Ginkgoselect Phyto-vesicle improves memory, brain function, cerebral and peripheral circulation, oxygenation, and blood flow (iii) Tablet-Phyto-vesicles complex should be diluted with 60%-70% of excipients to optimize its technological properties and to obtain tablet with appropriate technological and biopharmaceutical charactertics, e.g. *Centella asiatica* leaf Phyto-vesicles involves in treatment of vein and skin disorders (iv) Topical dosage form-the ideal process to incorporate the Phyto-vesicles complex in emulsion is to disperse the phospholipids complex in a small amount of the lipidic phase and add it to the already created emulsion at low temperature (<40 ℃)[28,29] e.g. Glycyrrhetinic acid Phyto-vesicle have anti-inflammatory, anti-irritant, anti-puffiness, especially effective for eye area, soothing to irritated skin.

6. Characterization of Phyto-vesicles

6.1. Shape

The vesicle shape can be easily visualized by scanning electron microscopy (SEM) micrographs for surface analysis and/or transmission electron microscopy (TEM) for sections analysis.

6.2. Size

The vesicle size and zeta potential of the formulation can be measured with the Zeta meter. The size of the Phyto-vesicles is influenced by the composition of the formulation. The size of the vesicles increase with increasing the phospholipids concentration.

6.3. Entrapment efficiency

After preparing Phyto-vesicles, entrapped drug is separated by dialysis, centrifugation, or gel filtration and resultant solution is analyzed by appropriate assay method for the entrapped drug.

[Where; Entrapment efficiency (EF) = (Amount entrapped/total amount)×100]

6.4. Drug-carrier interaction

Any physical interaction such as change in melting point is analyzed by DSC (Differential scanning calorimeter) whereas any chemical interaction such as formation of hydrogen bond is analyzed by various spectroscopic technique such as Fourier transform Infra-red (FT-IR), ^{1}H-NMR and ^{13}C-NMR.

6.5. Transition temperature

Determined by using Differential scanning calorimeter (DSC) which gives sharp peak for pure extract and broader peak for Phyto-vesicles which further confirms the interaction between extract and carrier molecule.

6.6. In-vitro release

A method of *in-vitro* drug release rate study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pipette into a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 200 mL of buffer solution in a 250 mL beaker with constant shaking at 25 ℃ or 37 ℃. At various time intervals, the buffer is analyzed for the drug content by an appropriate assay method that can also be studied with Franz diffusion cell[36-42].

7. Conclusion

From the above discussion it may be concluded that Phytovesicular delivery system has improved pharmacokinetic and pharmacodynamic parameter as compared to conventional system which is advantageous not only in cosmetics preparations but also in various acute and chronic diseases related to liver, heart, brain and kidney.

Conflict of interest statement

We declare that we have no conflict of interest statement.

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