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Research Article

PHYTOSOME: A NOVEL REVOLUTION IN HERBAL DRUGS

Joseph A. Kareparamban*, Pravin H Nikam, Aruna P Jadhav and Vilasrao J Kadam

Department of Quality Assurance,Bharati Vidyapeeth's College of Pharmacy, Sector-8 C.B.D., Belapur, Navi Mumbai, Maharashtra, India.

ABSTRACT

Herbal drugs comprises of a vast array of active contents which furnishes us with a number of applications. But due to high polarity and poor lipophilicity the active contents are poorly absorbed resulting in poor bioavailability. These problem can be overcomed by formulating a suitable novel preparation of the herbal extract.Phytosomes are one of the novel drug delivery system containing hydrophilic bioactive phytoconstituents of herbs surround and bound by phospholipids.This phyto-phospholipid complex resembles a little cell which exhibit better pharmacokinetic and pharmacodynamic profile than the conventional herbal extract resulting in better bioavailability. This article highlights recent information, commercial preparation of phytosomes as well as the various other novel approaches for delivery of herbal constituents.

Keywords: Phytosomes, bioavailability, phospholipids, phytoconstituents

INTRODUCTION

Preparations of phytomedicine has been used for health maintenance since ancient times. The phytomedicine's posses a lot of therapeutic uses.Over the past century phytochemical and phyto-pharmacological sciences established the compositions,biological activities and health promoting benefits of herbal extracts and their derivatives.It was observed that most of the biologically active phytoconstituents such as the flavonoids and terpenoids are of highly polar nature or water soluble molecules.These highly water soluble constituents are poorly absorbed due to their poor lipid solubility,thus creating a hurdle to cross the highly lipid-rich biological membrane,which finally results in poor bioavailability.Many approaches have been developed for improving the bioavailability such as inclusion of solubility and bioavailability enhancers,structural modification and entrapement with lipophilic carriers[1-3].One such approach is the phytosome technology.The phytosome technology is a novel approach developed by Indena in an attempt to combat the issue of poor bioavailability.The term "phyto" means plant and "some" means cell like.This novel preparation comprises of incorporating a standardized plant extract into phospholipids to produce lipid compatible molecular complexes with enhanced absorption and bioavailability.The phytosome process produces a little cell whereby the valuable plant extracts are protected from degradation by digestive enzymes and gut bacteria.Phospholipids are complex molecules responsible for formation of cell membranes.Phospholipids are lipid molecules in which the glycerol is bonded to two fatty acids and the remaining portion occupied by the phosphate group[6].The phospholipid mostly employed is phosphatidylcholine derived from soybean(*Glycine max).*Phytosomes are obtained by reacting phosphatidylcholine with herbal extracts in an aprotic solvent.Phytosomes posses improved pharmacokinetic and pharmacological properties as compared to the conventional preparation.The flavonoid and the terpenoid component's of the herbal extract are able to

directly bind to the phosphatidylcholine moiety hence they are widely prepared.The phytosome process has been widely applied to herbal extract such as milk thistle (*Silybum marianum),*green tea *(Thea sinensis)*[4].

A NOVEL APPROACH: PHYTOSOME

Phytosome results from reaction of stoichiometric amount of phospholipid mostly phosphatidylcholine with a standardized herbal extract in an aprotic solvent.Phosphatidylcholine is a bifunctional compound, the phosphatidyl moiety being lipophilic in nature which is the head of the bifunctional compound and the choline moiety which is the tail of the bifunctional compound being hydrophilic in nature. The choline moiety of the phosphatidylcholine bounds to the hydrophilic phytoconstituents, whereas the lipid soluble phosphatidyl portion then envelopes the choline bound complex. As a result a phyto-phospholipid complex is formed with a better lipid solubility. The polar phytoconstituents binds to the choline head by means of a chemical bond. The term “phyto” means plant while “some” means cell like[8-10]. The phytosome technology produces a little micro sphere or little cell, which protects the plant extract or its active constituent from destruction by gastric secretion and gut bacteria due to the gastroprotective property of phosphatidylcholine [6].Fig .1

PREPARATION OF PHYTOSOME

Phytosomes are prepared by reacting 3-2 moles or preferably 1 mole of phosphatidylcholine with 1 mole of active phytoconstituents mostly the flavonoids and the terpenoids in an aprotic solvent such as dioxane or acetone from which complex can be isolated by precipitation with non solvent such as aliphatic hydrocarbons or by lyophilization or by spray drying[49]. In the phyto-phospholipid complex formation the ratio between these two components is in the range of 0.5-2 moles The most preferable ratio of phospholipid to phytoconstituents is 1:1[16-17] . The phospholipids mostly selected for phytosome preparations are selected from group consisting of soy lecithin (*Glycine max*)*;* phosphatidylcholine,
phosphatidylethanolamine,
phosphatidyiserine, in which acyl group may be same or different and mostly derived from palmitic, stearic, oleic, linoleic acid. The phospholipid mostly selected is phophatidylcholine[2-3]. Spectroscopic techniques reveal’s that the molecules of the phytoconstituents are bonded to phospholipid moiety by means of a chemical bond[29-30]. The common stages for the preparation of phytosomes are mentioned in fig 2.

PROPERTIES OF PHYTOSOME

Phytosomes are complex between a natural phytoconstituents and natural phospholipids, like soy phospholipids mostly phosphatidylcholine. These complex results from the reaction of stoichiometric amounts of phospholipids with the phytoconstituents in an aprotic solvent[13-16].

1. Phytosomes can accommodate the active principle that is anchored to the polar head of the phospholipids, which finally becomes an integral part of the membrane. The molecules are
2. Phytosomes are advanced form of herbal drugs which are better absorbed, utilized and which finally leads to better results than conventional dosage form. The increased bioavailability has been demonstrated by the pharmacokinetic studies as well as by pharmacokinetic tests in experimental animals and human subjects.
3. Phytosomes are lipophilic substances with definite melting point, freely soluble in non-polar solvents, and moderately soluble in fats.
4. Phytosomes when treated with water assume a micellar shape, forming structure that resemble liposomes exhibiting fundamental difference.

CHARACTERIZATION OF PHYTOSOME

There are various factors such as the physical size, membrane permeability, percentage of entrapped solutes, chemical composition of the preparing materials which play a vital role in determining the behavior of phytosomes in physical and biological system. The following are the characterization techniques used for phytosomes in characterizing its physical attributes

1. Transition temperature: The transition temperature of vesicular lipid system can be determinedby differential scanning calorimetry[25-26].
2. Entrapement efficiency: The entrapement efficiency of a phytosomal formulation can be determined by subjecting the formulation to ultracentrifugation technique[24].
3. Vesicle size and Zeta potential: The particle size and zeta potential of phytosomes can be determined by dynamic light scattering which uses a computerized inspection system and photon correlation spectroscopy[28-29].
4. Surface tension activity measurement: The surface tension activity of drug in aqueous solution can be measured by ring method Du Nouy ring tensiometer[27].

5. Spectroscopic evaluation: The spectroscopic evaluations are widely employed in order to confirm the formation of complex between phytoconstituents and the phospholipid moiety as well as to study the corresponding interaction between the two. The widely employed methods are

5.1 ^{1}HNMR

The NMR spectra are employed for estimating the complex formation between the active phytoconstituents and the phosphatidylcholine molecule. The NMR spectra of phytosome complex had been studied by Bombardelli. In nonpolar solvents there is amarked change in ^{1}H NMR signal originating from atoms involved in the formation of complex, without any summation of the signal peculiar to individual molecules. The signals from protons belonging to the phytoconstituents are broadened. In phospholipids there is broadening of signals while the singlet corresponding to the N-$(CH_3)_3$ of choline undergoes an upfield shift[29-30].

5.2 ^{13}CNMR

In the ^{13}C NMR of the phytoconstituents and the stoichiometric complex with the phosphatidylcholine when recorded in C_6D_6at room temperature all the phytoconstituents carbons where invisible. The signals corresponding to the glycerol and choline portion are broadened and some are shifted, while most of the resonance of the fatty acids chains retain their original sharp line shape[29-30].

5.3 FTIR

The spectroscopic evaluation of the formed complex can be confirmed by FTIR simply by comparing the spectrum of the complex and the individual components and that of the mechanical mixtures. FTIR can also be considered as a valuable tool in confirming the stability of the phytosomal complex. The stability can be confirmed by comparing the spectrum of the complex in solid form with that of the spectrum of micro-dispersion in water after lyophilization at different times[29-30].

PHYTOSOMES AND LIPOSOMES : A COMPARISON

Liposomes are also prepared by mixing suitable water soluble phytoconstituents in phosphatidylcholine in a definite ratio under suitable conditions. Here no chemical bond is formed, the phosphatidylcholine moiety just anchors the water soluble phytoconstituents as a result of which there may be hundreds or even thousands of phosphatidylcholine molecules surrounding the drug molecule. In case of phytosomes the phosphatidylcholine and the plant constituents form a complex in the ratio 1:1 or 2:1 and the process of phytosome formation involves chemical bond formation whereas the liposomes are completely devoid of the chemical bond formation between the phosphatidylcholine molecule and the phytoconstituents. Due to the lesser composition of the phospholipid content in case of phytosomes the phytosomes are more bioavailable and are absorbed to a better extent than the liposomes[16-18].Fig. 3

ADVANTAGES OF PHYTOSOMES

Phytosomes furnishes with the following advantages:[13-14]

1. Phytosomes produces a little cell where the valuable components of herbal extracts are protected from destruction by digestive secretions and gut bacteria.
2. It assures proper delivery of drug to the respective tissues.
3. The nutrient safety of the herbal extracts need not be compromised by conveying the herbal drug as means of phytosomes.
4. Dose requirement has been reduced due to the maximum absorption of chief constituents.
5. Marked enhancement in the bioavailabilty of drug occurs.
6. Entrapment efficiency is high and more over predetermined because drug itself is in conjugation with lipids in forming vesicles.
7. There is no problem in drug entrapment while formulating phytosomes.
8. Phytosomes shows better stability profile due to the formation of chemical bonds between phosphatidylcholine molecules and the phytoconstituents.
9. Phosphatidylcholine used in formulating phytosome process besides acting as a carrier also nourishes the skin as it is an essential part of a cell membrane.
10. Phytosomes are also superior to liposomes in skin care products.
11. Phytosomes proves to be of significantly greater clinical benefit.
12. Phosphatidylcholine used in preparation of phytosomes, besides acting as a carrier also acts as a hepatoprotective as a result it imparts a synergistic effect when hepatoprotective substances are employed.

APPLICATION OF PHYTOSOME

Silymarin Phytosome

Yanyu et al. prepared the silymarin phytosome and studied the pharmacokinetics in rats. In the study the bioavailability of silybin in rats was increased remarkably after oral administration of prepared silybin-phospholipid complex due to the impressive improvement of

the lipophilic property of silybin-phospholipid complex and which led to the improved biological effect of silybin[4]. Tedesco et al. reported that silymarin phytosome exhibits better anti-hepatotoxic activity than silymarin alone and can play a vital role in protection against the toxic effects of aflatoxin B1 on performance of broiler chicks[21].

Mascarella et al. investigated that in one study of 232 patients with chronic hepatitis 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 compared to a group of control[7]. Bombardelli et al. reported silymarin phytosome in which silymarin was complexed with phospholipids. Phytosomes showed higher specific activity and a longer lasting action than single constituents with respect to the reduction of oedema, inhibition of myeloperoxidase activity, antioxidant and free radical scavenging activity[17]. Barzaghi et al. conducted a human study designed to assess the absorption of silybin when directly bounded to phosphatidylcholine. Plasma silybin levels were determined after administration of single oral dose of silybin phytosome and a similar amount of silybin from milk thistle in healthy volunteers. The results indicated that the absorption of silybin from silybin phytosome is approximately seven times greater as compared to the absorption of silybin from regular milk thistle[22-23].Grange et al. conducted a series of studies on silymarin phytosome, which contained a standardized extract from the seeds of *S.marianum,* administered orally and found out that it could protect the fetus from maternally ingested ethanol[23][42].

Phytosome of green tea

Green tea leaves *(Theasinensis)*is characterized by prescence of a polyphenolic compound epigallocatechin 3-O-gallate as the key component. These compounds are potent modulators of several biochemical process linked to the breakdown of homeostasis in major chronic-degenerative diseases such as cancer and atherosclerosis. Green tea also furnishes us with a number of beneficial activities such as antioxidant, anticarcinogenic, antimutagenic, hypocholesterolemic, cardioprotective effects. Inspite of such beneficial activities furnished by polyphenols from green tea extract the polyphenols suffer from the problem of poor bioavailability. The complexation of polyphenols derived from green tea with phospholipids strongly improves the oral bioavailability[5]. A study on absorption of phytosomal preparation was performed in healthy human volunteers along with non complexed green tea extract following oral administration. Over the study period of 6 hours the plasma concentration of total flavonoids was more than doubled when comparison was done between the phytosomal and the non-phytosomal preparation was done. Antioxidant capacity was measured as TRAP (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[9].

Quercetin-phospholipid phytosomal complex

Maiti et al. developed the quercetin-phospholipid phytosomal complex by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy as compared to the non-phytosomal conventional preparation in rat liver injury induced by carbon tetrachloride[51].

Phytosomes of grape seed

Grape seedphytosome is composed of oligomeric polyphenols (grape proanthocyanidins or procyanidins from grape seed extract, *Vitis vinifera*) of varying molecular size complexed with phospholipids. The main properties of procyanidin flavonoids of grape seed are an increase in total antioxidant capacity and stimulation of physiological defenses of plasma, protection against ischemia/reperfusion induced damages in the heart,protective effects against atherosclerosis thereby offering marked protection against the cardiovascular system and other organs through a network of mechanism that extend beyond their antioxidant effect.

In another study, rabbits were fed with a high cholesterol diet for 6 weeks, to markedly elevate their blood cholesterol level and to induce atherosclerotic lesions in their aortas and carotid arteries. One group of rabbit received grape seed phytosome in their feed for the first 6 weeks, then 4 weeks of high cholesterol diet. These developed significantly less aortic plaque than did the control group which received conventional standardized grape seed extract in similar regimen. In randomized human trial, young healthy volunteers received grape seed phytosome once daily for 5 days. The blood TRAP (Total Radical-trapping Antioxidant Parameter) was measured at several time intervals during 1st day, then also on 5th day. Already by 30

minutes after administration on 1st day, blood TRAP levels were significantly elevated over the control which received conventional standardized grape seed extract[31].

Phytosomes of curcumin

Maiti et al. developed the phytosomes of curcumin (flavonoid from turmeric, *Curcuma longa linn*) and naringenin (flavonoid from grape, *Vitis vinifera*) in two different studies. The antioxidant activity of complex was significantly higher than pure curcumin in all dose levels tested. In the other study the developed phytosome of naringenin produced better antioxidant activity than the free compound with a prolonged duration of action, which may be due to decrease in the rapid elimination of the molecule from the body[47-48].

Phytosomes of *Gingko biloba* leaves

Studies have shown that ginkgo phytosome (prepared from standardized extract of *Ginkgo biloba* leaves) produced better results compared to the conventional standardized extract from plant (GBE, 24% ginkgo flavones glycoside and 6% terpene lactones). In a bioavailability study conducted with healthy human volunteers the level of GBE constituents (flavonoids and terpenes) from the phytosomal form peaked after 3 hours and persisted longer for atleast 5 hours after oral administration. It was found that the phytosomal GBE produced a 2-4 times greater plasma concentration of terpenes than did the non-phytosomal GBE. Its major indication are cerebral insufficiency and peripheral vascular disorders and it can also ameliorate reduced cerebral circulations. Its improved oral bioavailability and good tolerability makes it the ideal ginkgo product even for long term treatment. Studies with ginkgo phytosomes in patients with peripheral vascular disorders have shown to produce 30-60% greater improvement compared to regular standardized GBE[33].Studies were also conducted on gingko phytosome which yielded better result as compared to the conventional form. For conducting the aforesaid studies ginkgo phytosome was administered for 5 days in guinea pigs, in whom the bronchoconstriction was induced by three different agonists (histamine, PAF and Acetylcholine). The bronchospastic inhibition was measured at the maximum peak, expressed as variations versus the basal values. The result indicated that ginkgo phytosome can not only counteract direct bronchoconstriction but also it posses the tendency to reduce the TXA2 mediated bronchoconstriction of histamine and PAF as compared to the conventional forms, thus indicating the improved efficacy of ginkgo phytosome in combating the allergen induced bronchospasm. Studies have also proved the improved efficacy of ginkgo phytosome over the conventional standardized extract in protecting rat isolated hearts against ischemia. The above mentioned results clearly gives an indication about the upper hand that phytosome posses over the conventional preparations, thus proving it's utility for herbal phytoconstituents[54].

PATENTED TECHNOLOGIES RELATED TO PHYTOSOME

A number of innovative process has been carried out in the field of phytosomes. The academic scientists are conducting a number of formulation research studies on phytosome, the studies are also conducted by industrial laboratories. These studies encompasses the current areas of research and the recent innovations that can be made possible in phytosomes. Some of the patented technologies of phytosome and other related technologies along with their applications and innovations are listed in table. 1

COMMERCIALLY MARKETED FORMULATION

Phytosome are the advanced form of herbal extract which are better absorbed as compared to the conventional standardized herbal extract. These are the patented technologies developed by INDENA SPA Milan, Italy. A number of herbal standardized extract especially the polyphenolic and the terpenoids fraction are widely formulated as phytosome. Some of the marketed formulations are listed in the table. 2

CONCLUSION

A wide number of phytoconstituents are present in herbal drugs especially the flavonoidal and the terpenoidal fraction furnishes with a number of application. The poor absorption and the poor bioavailability associated with the polar phytoconstituents limits its use. These hindrances can be tackled by formulating an appropriate drug delivery system. Phospholipid based drug delivery system have been found promising for better and effective delivery of drug and can enhance the rate and extent of drug absorption across the lipoidal biomembrane. Phytosome are one of the phospholipid based drug delivery system with a better absorption and stability profile as compared to other phospholipid based drug delivery system. Phytosome can play a vital role in efficient delivery of

phytoconstituents such as the flavones and the xanthones. Apart from the aforesaid use phytosome also has a wide scope in cosmetics as well. Many areas of phytosome will be revealed in the future as part of their pharmaceutical use.

NOVEL APPROACHES FOR DELIVERY OF HERBAL CONSTITUENTS

Liposomes

Liposomes are artificial microscopic vesicles consistiong of an aqueous core enclosed in one or more phospholipid layers, used to convey vaccines, drugs, enzymesor other substances to target cells or organs[46].

Nanoparticles

Nanoparticles are particles of less than 100nm in diameter that exhibit new or enhanced size-dependent properties compared with larger particles of same material[46].

Microemulsion

A thermodynamically stable dispersion of two immiscible liquids, stabilized by surfactants. A microemulsion is an emulsion whose particles are less than 1 micron in size[46].

Phytosome

It is a newly introduced patented technology developed to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes they are also known as herbosome[46].

Transfersomes

A transfersomes carrier is an artificially designed to be like cell vesicle or a cell engaged in exocytosis and thus suitable for controlled and potentially targeted drug delivery. Transfersome consist of phosphatidylcholine and cholate and are ultra deformable vesicles with enhanced skin penetrating properties[46].

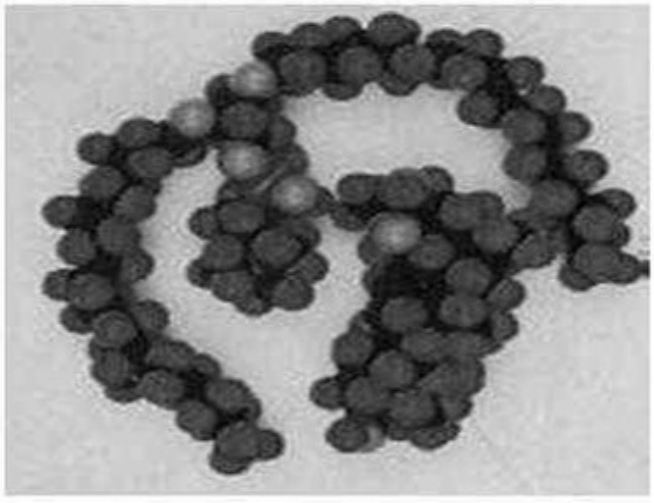

Fig .1: Organisation of phytosome complex

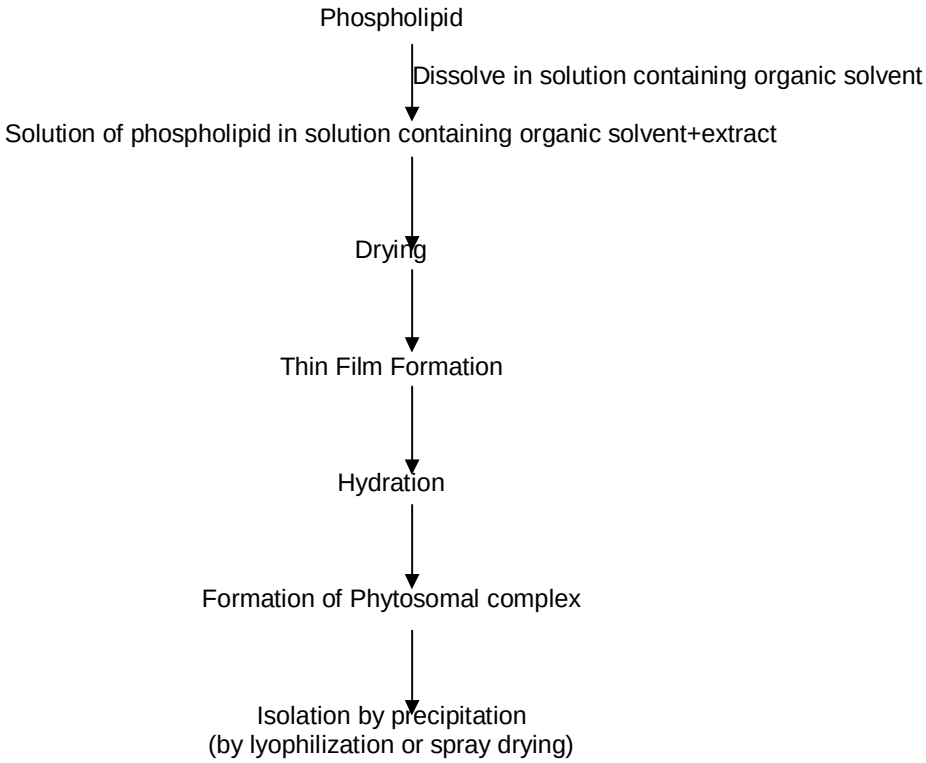

Fig. 2: Stages of Phytosome preparation[46]

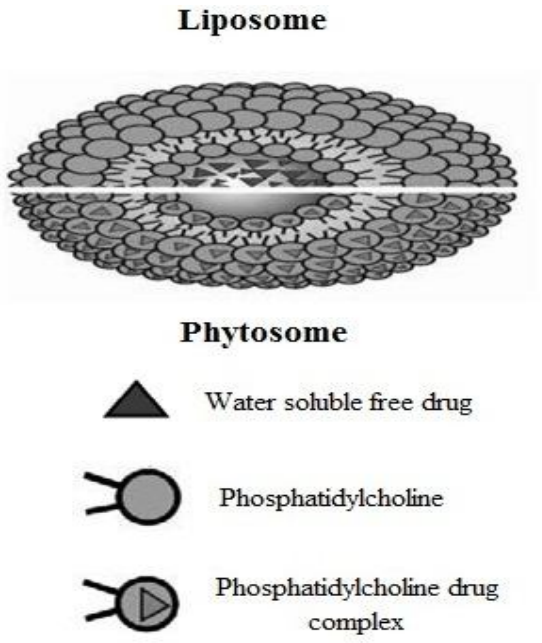

Fig. 3: Difference between phytosome and liposome
The molecular organization of liposome upper segment
The molecular organization of phytosome lower segment.

Table 1: Patented Technologies of Phytosome

Title of patent	Innovation	Patent no.	Reference
Phospholipids complexes of olive fruits or leaves extracts having improved bioavailabilty	Phospholipids complexes of olive fruits or leaves extracts or their compositions containing it which imparts improved bioavailability	EP/1844785	32
Compositions comprising *Ginkgo biloba* derivatives	Compositions containing fractions derived from *Ginkgo biloba* useful for treating asthma	EP/1813280	33
Fatty acids monoesters of sorbityl furfural and compositions for cosmetic and dermatological use	Fatty acid monoesters of sorbityl furfural selected from two different series of compounds in which side chain is a linear or branched C3-C19 alkyl radical optionally containing at least one ethylenic unsaturation	EP1690862	34
Treatment of skin and wound repair with thymosin β4	Complexation of thymosin β4 along phospholipids for treatment of skin disorder	US/2007 0015698	35
Soluble isoflavone compositions	Isoflavone compositions exhibiting improved solubility, taste, colour and texture characteristics	WO/2004/ 045541	36
An antioxidant preparation based on plant extracts for the treatment of circulation and adiposity prodlems varicose veins, arteriosclerosis, high blood pressure and haemorrhoids	Preparations based on plant extracts which has an antioxidant effect and is particularly useful in treatment of circulation problems such as phlebitis,	EP/12114084 37	
Complexes of saponins with phospholipids and orsynthetic phospholipids posses pharmaceutical and high lipophilia and improved cosmetic compositions bioavailability and are suitable for containing them use as active principle in pharmaceutical,dermatologic and cosmetic composition		Complexes of saponins with natural EP0283713	38

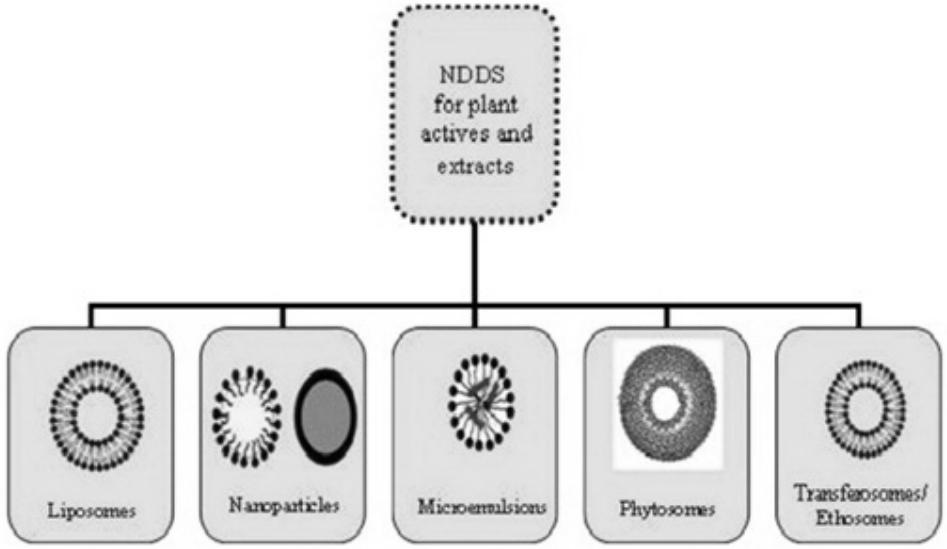

Fig. 4: NDDS for delivery of herbal constituents

Table 2: Commercially Marketed Formulations

Centella phytosome	Triterpenes	*Centella asiatica*	Leaf	Cicatrizing agent
Crataegus phytosome	Vitexin-2-O-rhamnoside	Hawthorn Flower	Flower	Antioxidant
Escin β-sitosterol phytosome	Escin β- sitosterol	Horse chestnut	Fruit	Antioedema
Gingkoselect phytosome	Ginkgolides,bilobalides	*Gingko biloba*	Leaf	Vasokinetic
Ginselect phytosome	Ginsenosides	*Panax ginseng*	Rhizome	Adaptogenic
Ginkgo biloba terpenes phytosome	Ginkgolides,bilobalide	*Ginkgo biloba*	Leaf	Soothing agent
Ginkgo biloba dimeric phytosome	Dimeric flavonoids	*Ginkgo biloba*	Leaf	Lipolytic
Greenselect phytosome	Polyphenols	Green tea	Leaf	Obesity
Leucoselect phytosome	Polyphenols	*Vitis vinifera*	Seed	Antioxidant
Meriva	Curcuminiods	Turmeric	Rhizome	Osteoarthritis
PA_2 phytosome	Proanthocyanidin A_2	Horse chestnut	Bark	U.V.protectant
Sericoside phytosome	Sericoside	*Terminalia Sericea*	Bark	Anti-Wrinkles
Siliphos	Silybin	Milk thistle	Seed	Hepatoprotective
Silymarin phytosome	Silymarin	Milk thistle	Seed	Hepatoprotective
Virtiva	Ginkgoflavonglucosides	*Ginkgo Biloba*	Leaf	Vasokinetic
Visnadex	Visnadin	*Ammi Visnaga*	Seed	Vasokinetic

REFERENCES

1. Bombardelli E, Curris B and Della LR: Complexes between phospholipids and vegetal derivatives of biological interest, *Fitoterapia* 1989;90(suppl.1): 1-9.
2. Gupta A, Ashawat MS, Saraf S: Phytosome: A novel approach towards functional cosmetics,*J Plant Sci* 2007; 2(6): 644-649.
3. Cott J: Natural Product Formulation, Available in Europe for Psychotropic Indications; Psychotropic Indications, *Psychopharmacol Bull* 1995 ; 31: 745.
4. Parris K, Kathleen H: A review of bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex, *Altern Med Rev* 2005; 10(3): 193-203.
5. Manach C, Scalbert A, Morand C: Polyphenols: Food source and bioavailability, *Am J Clin Nutr* 2004; 79: 727-747.
6. Bhattacharya S: Phytosomes: Emerging strategy in delivery of herbal drugs and nutraceuticals, *Pharma Times* 2009; 41(3): 8-12.
7. Mascarella S: Therapeutic and antilipoperoxidant effects of silybin-phosphatidylcholine complex in chronic liver disease, Preliminary results, *Curr Ther. Res* 1993; 53(1): 98-102.
8. Franco PG, Bombardelli E: Complex coppouns of bioflavonoids with phospholipids, their preparation and use and pharmaceutical and cosmetic composition containing them 1998, U.S.Patent No. EPO 275005.
9. Bombardelli E, Spelta M: Phospholipid-polyphenol complexes: A new concept in skin care ingredients, *Cosm and Toil* 1991;106: 69-76.
10. Bombardellli E, Giuseppe M, bilobalide phospholipid complex, their uses and formulation containing them,1991, U.S.Patent No. EPO 275005.
11. Dang Yi New product concept UPC code 0300540111783, 2000.
12. Bombardelli E, Critoni A, Morazzoni P: Phytosomes in Functional Cosmetics, *Fitoterapia* 1994; 95: 387-401.
13. Sharma S, Sikarwar M: Phytosome: A review, *Plant indica* 2005; 1(2): 1-3.
14. Bombardelli E: Phytosome: New cosmetic delivery, *Boll chim farm* 1991; 130(11): 431-438.
15. Semalty A, Semalty M, Singh R: Phytosome in herbal drug delivery: A review, *Indian Drugs* 2006; 43(12): 937-946.
16. Phytosomes: A Technical Revolution in Phytomedicine [online] 2010 [cited 2010 Mar 22] Available from: URL: http//www.indena.com.
17. Jose MM, Bombardelli E: Pharmaceutical Composition Containing Flavanolignans and Phospholipida Active Principles. U.S.Patent EPO 209037 1987.
18. Bombardelli E, Mustich G. Bilobalide Phospholipid Complex, Their Uses and Formulation Containing Them .U.S.Patent EPO-275005. 1991.
19. Valenzuela A, Aspillaga M, Vial S, Guerra R: Selectivity of Silymarin on the Increase of the Glutathione Content in Different Tissues of Rat, *Planta Med* 1989; 55: 420-422.
20. Francesco DP, Anna BM, Angela B, Maurizio L, Andrea C: Green Select Phytosome as an Adjunct to Low Calorie Diet For Tretment of Obesity: A Clinical Trial, *Altern Med Rev* 2009; 14: 154-160.
21. Ravarotto L: Efficacy of Silymarin-Phospholipid Complex in Reducing the Toxicity of

Aflatoxin B1 in Broiler Chicks, *Poult Sci* 2004; 83: 1839-43.
22. Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E: Pharmacokinetic Studies on Idb 1016, A Silybin Phosphatidylcholine Complex In Healthy Human Subjects, *Eur J Drug Metab Pharmacokinetics* 1990; 15: 333-38.
23. La Grange L, Wang M, Watkins R, Ortiz D, Sanchez ME, Konst J, Lee C, Reyes E: Protective Effects of The Flavonoids Mixture, Silymarin, On Fetal Rat Brain And Liver, *J Ethnopharmacol* 1999; 65: 53-61.
24. GMM Maghraby E, Williams AC, Barry BW: Oestrodiol Skin Delivery From Ultra deformable Liposomes: Refinement of Surfactant Concentration, *Int J.Pharm* 2000;196: 63-74.
25. Fry DW, White JC, Goldman ID: Rapid Sectretion of Low Molecular Weight Solute From Liposomes Without Dilution, *Anal.Biochem* 1978;90: 809-815.
26. Cevc G, Schatzlein A: TransdermalDrug Carriers: Basic Properties, Optimization and Transfer Efficiency in Case of Epicutaneously Applied Peptides, *J. Control Release* 1995;36: 3-16.
27. BAIV Berge, VAB Wartzendruber, Geest J: Development of an Optimal Protocol For Ultrastructural Examination of Skin By TEM, *J.Micros* 1997; 187: 125-133.
28. Dayan N, Touitou E: Carrier For Skin Delivery of Trihexyphenidyl HCl: Ethosomes v/s Liposomes , *Biomaterials* 2002; 21: 1879-1885.
29. Gabetta B, Zini GF, Pifferi G: Spectroscopic Studies on Idb-1016 A New Flavanolignan Complex, *Plant Med* 1989;55: 615.
30. Malandrino S, Pifferi G: Idb-1016 Silybin Phosphatidylcholine Complex, *Drugs Future* 1990; 15: 226-227.
31. Facino RA, Carini M, Aldini G: Free Radicals Reaction and Anti-Enzymic Activities of Vitis Vinifera: A Mechanism For Capillary Protection, *Arzeniem Forsch* 1994;44: 592-601.
32. Franceshi F, Giori A: A Phospholipid Complex of Olive Fruits or Leaves Extracts Having Improved Bioavailability Patent No. EP1844785, 2007.
33. Dipierro F: Composition Comprising *Ginkgo Biloba* Derivatives For Treatment of Asthmatic and Allergic Conditions Patent No. EP1813280, 2007.
34. Bertelli V: Fatty acid Monoesters of Sorbityl Furfural and Composition For Cosmetic and Dermatological use Patent No. EP1690862,2006.
35. Kleinman HK, Goldstein AL: Treatment of Skin and Wound Repair With Thymosin Beta 4 Patent No. 20070015698, 2007.
36. Khare AB: Soluble Isoflavone Composition, WO/2004/045541, 2004.
37. Merizzi G: An Antioxidant Preparation Based on Plant Extracts For Treatment of Circulation and Adiposity Problem, EP1214084, 2002.
38. BomBardelli E, Petri GF, Pozzi R: Complexes of Saponins With Phospholipids and Pharmaceuticals and Cosmetic Composition Containing Them, EP0283713, 1988.
39. Hikino H, Kiso Y, Wagner H, Fiebig M: Antihepatotoxic Action s of Flavanolignans from *Silybum Marianum* fruits, *Planta Med* 1984;50: 248-250.
40. Mukherjee K, Maiti K, Venkatesh M, Mukherjee PK: Phytosome of Hesperetin, A Value Added Formulation With Phytomolecules.

60th Indian Pharmaceutical Congress; New Delhi , 2008: 287.
41. Yanyu X, Yunmei S, Zhipeng C, Quineng P: The Preparation of Silybin-Phospholipid Complex and The Study on Its Pharmacokinetics in Rats, *Int J Pharm* 2006;307: 77-82.
42. Busby A, La Grange L, Edwards J, King J: The use of A Silymarin Phospholipid Compound As A Fetoprotectant From Ethanol-Induced Behavioral Defeits, *J Herb Pharmacother* 2002;2: 39-47.
43. Jiang YN, Yu ZP, Yan ZM, Chen JM: Studies on Preparation of Herbal Epimedii Flavonoid Phytosomes and Their Pharmaceutics, *Zhongguo Zhong Yao Za Zhi 2001*; 26(2): 105-108.
44. Marena C, Lampertico M: Preliminary Clinical Development of Silipide: A Complex of Silybin in Toxic Liver Disorders, *Planta Med* 1991;57: A 124-125.
45. Magistretti MJ, Bombardelli E: Pharmaceutical Compositions Containing Flavanolignans and Phospholipids Active Principles U.S. Patent No. EP0209037 1987.
46. Jain NK, Controlled and Novel Drug Delivery, 1stEdition, CBS Publisher, 2005, 321-326.
47. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK: Enhanced Therapeutic Potential of Naringenin-Phospholipid Complex in Rats, *J pharm Pharmacol*, 2006;58(9): 1227-1233.
48. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK: Curcumin-Phospholipid Complex: Preparation, Therapeutic Evaluation and Pharmacokinetics Study in Rats, *Int J Pharm*, 2007;330(1-2): 155-163.
49. Vitamedics, Phytosome Products (online). 2008 (cited 2008 Sep 19). Available from : URL: http://www.vitamedics.com
50. Schwitters B, Masquelier J: OPC in Practice: Biflavanals and Their Application. Alfa Omega Rome. Italy 1993.
51. Maiti K, Mukherjee K, Gantait A, Ahamed HN, Saha BP, Mukherjee PK: Enhanced Therapeutic Benefit of Quercitin-Phospholipid Complex in Carbon Tetrachloride Induced Acute Liver Injury in Rats: A Comparative Study, *Iran J Pharmacol Ther* 2005;4: 84-90.
52. Venkatesan S, Babu BS, Vyas SP: Protected Particulate Drug Carriers For Prolonged Systemic Circulation- A Review, *Indian J.Pharm.Sci* 2000;62 : 327-333.
53. Abrol S, Trehan A, Katare OP: Comparative Study of Different Silymarin Formulations: Formulation, Characterization and in vitro/in vivo Evaluation, *Current Drug Delivery* 2005;2: 45-51.
54. Naik SR, Pilgaonkar VW: Evaluation of Antioxidant Activity of *Ginkgo Biloba* Phyosomes in Rat Brain, *Phytotherapy Research* 2006;20: 1013-1016.
55. Joshi A, Chaturvedi S and Kumar V. Phytosomes: A Revolution in Herbal Drugs *Pharma Review*, Kongposh Publications 2007.