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Review Article

Recent Trends in Phytosome Nanocarriers for Improved Bioavailability and Uptake of Herbal Drugs

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Abstract

Phytopharmaceuticals or herbal drugs have a significant therapeutic impact on healthcare systems. Though herbal extracts and their active constituents show excellent pharmacological *in vitro* effects, they still have indigent *in vivo* biological effects because of their considerable molecular weight and low lipid solubility, leading to low systemic availability. Phytosome is a novel approach for overcoming the drawbacks of conventional delivery methods of herbal actives. The phospholipids, mainly phosphatidylcholine, form a bond with herbal extracts or actives, forming a herb-lipid complex. The encapsulation of herbal actives with phospholipid allows an effective tool for the delivery to the affected area with enhanced pharmacological effect. Moreover, the amphiphilic nature of the phospholipid provides a good hydrophilic-lipophilic balance, thereby improving a better dissolution profile in the lipid-rich membranes of the gastrointestinal tract. This review focuses on the various phytosome nanocarriers to improve herbal medication bioavailability and uptake—recent trends in their industrial applicability, and applications in clinical management for various diseases, including other challenges.

Introduction

The capability of the dosage form to convey the medicament to its site of action at a rate and amount sufficient to achieve the desired curative effects is critical to the efficacy of any drug, whether it comes from a plant, animal, or synthetic drug. However, a wide range of pharmacokinetic difficulties emerges due to one or more biological barriers throughout the body. In addition, systemic bioavailability can also reduce due to factors preventing drug flux, for example, the destruction of medicine before crossing the biomembrane or ejection of the drug from the body after uptake by efflux transport systems. Therefore, it is evident that several parameters are needed to evaluate before designing the drug carriers.[1] Numerous innovative drug delivery systems and methods can be supplied via various channels to allow the medication to pass through the barrier at appropriate and safe dosages.[2] Hence, the essential characteristic regarding the design of a drug delivery system is the ease of use, which is not so simple in cases of oral delivery where an ideal concentration of drug in plasma is essential to elicit a therapeutic response, and if there are any variations in the concentrations, can lead to toxic effects or maybe of no benefit.[3]

Due to the rapid progress of allopathic drugs, they are more predominant in the healthcare system. Despite the significance of modern dosage forms, there are several issues with efficacy, bioavailability, biocompatibility, toxicity, and inactivity. Furthermore, there is an upsurge in natural remedies and herbal products as they are also gaining widespread attention for healing several body ailments with minimal side effects. Though herbal drugs are known for their significant pharmacological effects against many diseases, they produce minimal efficacy, safety, and batch-to-batch variation due to the lack of proper quality control because of the complexity of phytoconstituents.[4] Usually, it is known that the side effects associated with herbs are less, but numerous reports of serious reactions happened during their administration, indicating the need of proper safety profiles; proper validation and strict regulatory guidelines on the assessment of its quality, standardization of bioactive, therapeutic efficacy, safety monitoring is needed to be done for their future development.[5] In addition, phytotherapeutics requires a proper scientific approach to deliver the bioactive constantly to improve patient compliance, reducing repeated administration. Therefore, many herbal extracts have not been used clinically due to these obstacles. Drug carriers have developed to overwhelm these difficulties and improve the drug kinetics and dynamics.[6] The researchers have created various such systems to overcome the shortcomings of conventional medication drug carriers. Additionally, combining plant extracts into delivery channels will help improve and bulk dosing of these phytotherapeutics, as the activity of the herbal medicines depends on the overall

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synergism of all the constituents. Hence, integrating innovative delivery systems with traditional medicines is of utmost importance, especially for managing chronic diseases like asthma, diabetes, osteoporosis, and age-related diseases like dementia.[7]

Significant advancements in nanoparticles and nanomaterials have gained attention for the design of delivery methods because of their superior biological and chemical performance. Nanocarriers consist of safe and inert materials investigated for their potential role in medication delivery and unique characteristics. One such system for improved site-specific delivery of phytochemicals with enhanced absorption for the topical and oral route is a nanoliposomal delivery system, phytosome.[8]

This review will address the concept of phytosome as a carrier, its advances, clinical uses, challenges, industrial applicability, and translational obstacles to phytosomal drug delivery.

Phyto-phospholipid Complex: The Phytosome Concept

Phytosomes are a trademarked technology developed by Indena S.P.A., an Italian firm. They have described a 100% food-grade delivery system "proprietary" that optimizes the absorption and biodistribution of natural active ingredients by blending them with a healthy dietary component (lecithin). The term phytosome is made of two terms, 'Phyto,' which implies plant, and 'some,' represents cell-like, where the chemical constituents shield within the phospholipid bilayer. The lipid bilayer in the phytosome maintains the bioactive contact-facilitated drug delivery (CFDD). It is the process of transferring bonded nanoparticle lipid surfactant components into the outer leaflet of the specific cell membrane. The process is a lengthy second-order process due to the nanoparticle's continuous contact with specific membrane surfaces, resulting in the passage of herbal ingredients within a cell.[9] Phytosomes can make a better transition from a hydrophilic environment to the hydrophobic environment of the enterocyte cell membrane, then through the cell, and finally into the blood, as per their nature.

Most of the bioactive constituents are hydrophilic, whose systemic availability is low. The poor absorption is either due to a multiple-ring molecule or the low mixing of phytoactive with oils and other lipids, which restricts their ability to pass through the small intestine in the case of oral drugs. Thus, the hydrophilic molecule can transform into a lipophilic molecular composite called phytosomes. The exceptionality of the phytosomal system is its ability to envelop both hydrophilic and lipophilic compounds, allowing a wide diversity of phytoactive to be enclosed using these vesicles. As a delivery channel, the benefits of phytosomes include their biocompatibility, capacity for self-assembly, the capability to transport large drug loads, and a variety of physicochemical and biological features that can alter to regulate their physiological features without losing nutrient safety. Due to the presence of natural phospholipids, the phytosomes are generally therapeutically inert with fewer side effects. The upgraded pharmacokinetic and therapeutic parameters of phytosome are becoming beneficial in medical and cosmetic industries.[10] There is supporting evidence indicating the increase in the bioavailability of herbal constituents by using phytosome technology. For example, to improve the permeability of phenolic compounds from *Diospyros kaki* extract through the biological membrane, a phytosome formulation was developed in nanometer size and high drug-loading efficiency. Moreover, compared to the phytosome, the free radical scavenging property was improved than the free extract, and further *in vivo* studies support its use as a food supplement as it did not affect lipidic or glycemic parameters and urinary parameters.[11]

The Drug-phospholipid Interaction and Its Preparation Methods

The phytoconstituent and the phospholipid have interconnected through their polar groups. Though the interaction exists between both phytoactive and phospholipids, the phospholipids tend to be a vital part in such a way; the phospholipid group has attached, then the two long fatty acid chains do not contribute to phytosome formation. In case of polyhenolic compounds, the complex was formed via chemical bonds. This has been identified by thermal analysis and Fourier Transform Infrared Spectroscopy (FT-IR) result of drug-phospholipid complexes with pure drug, and their physical mixture.[12] *Moringa oleifera* Leaf Polyphenol-Loaded Phytosome was prepared and investigated by Wanjiru and team in the year 2022 has recognized the presence of hydrogen bond in the complex. In the FT-IR of the complex, the peak at 1613 cm^{-1} showed the formation of hydrogen bond between the drug and the complex.[13] The differential scanning calorimetry data suggests that the interaction of catechin-phospholipid complex was due to hydrophobic interaction or hydrogen bonding. Semalty and team found that, the –O.H. groups of the phenol ring of catechin form hydrogen bonding and the hydrophobic interaction may be due to the aromatic ring.[14] While some investigators have suggested that Van der Waals forces could have a significant role, the connection is a -H bond. Long-chain fatty acids encapsulate the polar head of the complex as it moves to form a lipophilic surface.[15] These phyto-phospholipid give away aggregates dispersed in water, which look like tiny cells with liposomes. Even though structural similarities exist between liposomes and phytosomes, these two delivery systems have unique and differential properties. In liposomes, the highly polar compounds have spread in the interior space, where the hydrophilic or lipid-soluble compounds have disseminated inside the lipid bilayer. Hence there will be no H-bonding taking place. Pu and the coworker designed the molecular docking model to study the possible interaction between the protopanaxadiol and the phospholipid. It was found that the hydrophobic section of the phospholipid was entrapped in the two hydrophobic portions of PPD, and the formation of a hydrogen bond

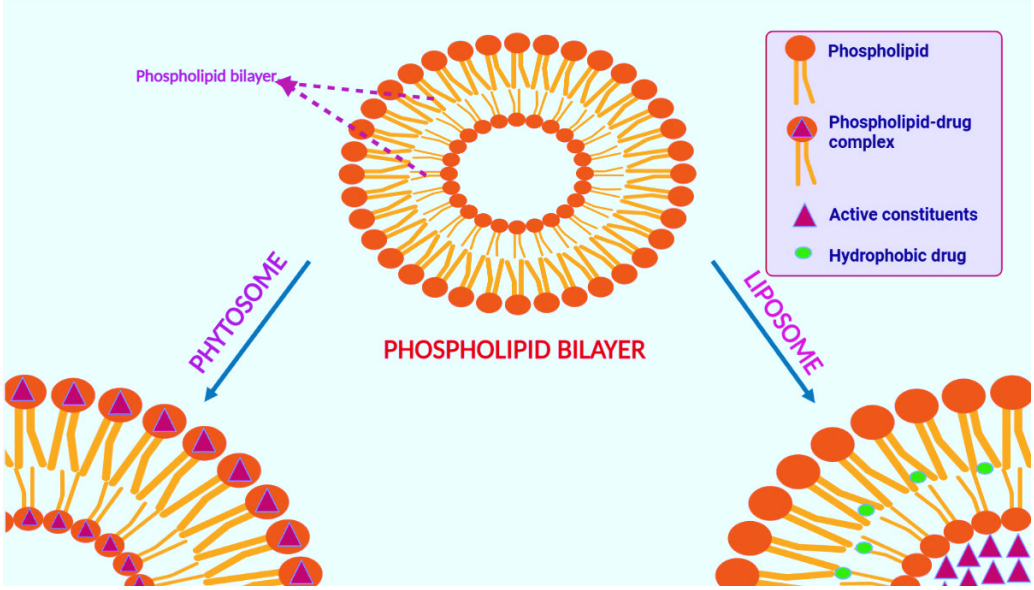

Figure 1. Difference in drug entrapment of phytosome and liposome.

between the –O.H. group and –P=O group, and the docking energy was -3.3 kcal/mol.[16] In phytosomes, the bioactive is loaded and chemically connected to the hydrophilic head of the phospholipid, which is a vital ingredient of the lipid bilayer.[17] Therefore, the main difference exists in the dispersion of the active components where, in phytosomes, it is a vital part that forms chemical bonds with the polar groups of the phospholipid. In the case of liposomes, they have closed vesicles in which the phytoconstituents are distributed evenly throughout the medium. Figure 1 describes the structural differences between a phytosome and a liposome.

Another significant difference is in the molar ratios of phospholipid to phytoactive ingredients present. In liposomes, the ratio is 5:1, but in phytosomes, it is generally 1:1 to 3:1. Therefore, the phytosomes are generally extra stable and have more drug entrapment efficacy because of these properties. Vu and the team compared the liposome and phytosome of rutin to check its antioxidant efficacy. Here, the authors tried to make rutin liposomes and rutin phytosomes via the thin-film hydration method with different stoichiometric ratios of rutin to 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (1:1, 1:2, and 1:4). It was found that the ratio of 1:1 or 1:2, there was no change in FT-IR spectra for the formation of liposome or phytosome. As the ratio was increased from 1:4, 1:6, and 1:8; It was clearly observed the formation of liposomes or phytosome, and there was an enhancement in drug-loading with the smallest mean particle size. A ratio of 1:6 was chosen to make the phytosome and liposome. The entrapment efficiency of rutin phytosomes was higher than its liposome formulation (24% for phytosome and 17% for liposome), which denotes that the phytoconstituents have linked to both interiors as well as outer membrane of the lipid vesicle. Likewise, the phytoactive can be entrapped within the lipid bilayer in the case of liposomes, showing its low entrapment efficiency.[18]

According to Bombardelli, phytosome production involves the reaction of phospholipids with phyto actives at a stoichiometric ratio. Three components must be present for the phytosome delivery system: the carrier (phospholipid), phytoconstituents, and the solvents with the stoichiometric ratio needed for its formation.[19]

Phospholipids

The sources of phospholipids can be synthetic or natural, from soy, sunflower, eggs, chickens, bovine, milk, and rapeseed.[19] Also, industrially produced phospholipids are available, but when obtained from different sources, they have different properties, and consequently, their applications also differ for use in the pharmaceuticals, cosmetic, and food industries. Due to their amphiphilic nature, they are soluble in both aqueous and lipophilic mediums. Phospholipids comprise a glycerol backbone coupled to two fatty acids, with a phosphate group occupying the third remaining site in glycerol.[20] Phosphatidylcholine (P.C.), phosphatidylethanolamine, and phosphatidylserine are the most commonly employed phospholipids in phytosome formulation. P.C. is the most extensively used of the phospholipids mentioned. It has two neutral tail groups, fatty acids with a polar head group holding an oxygen atom that tends to gain electrons and a nitrogen atom that prefers to lose electrons, making it amphiphilic. In addition to their amphiphilic nature, they also possess significant advantages for drug delivery systems. Figure 2 represents the basic structure of phospholipids.

Phyto active constituents

The pharmacological effect of an herbal formulation depends on the multi-component mixture present in the extract, as some of the herbal extracts lose their efficacy after being purified or fractionated. This step can be practical in some cases but also devoid of the required pharmacological or toxic effect. Therefore, encapsulation of the extracts

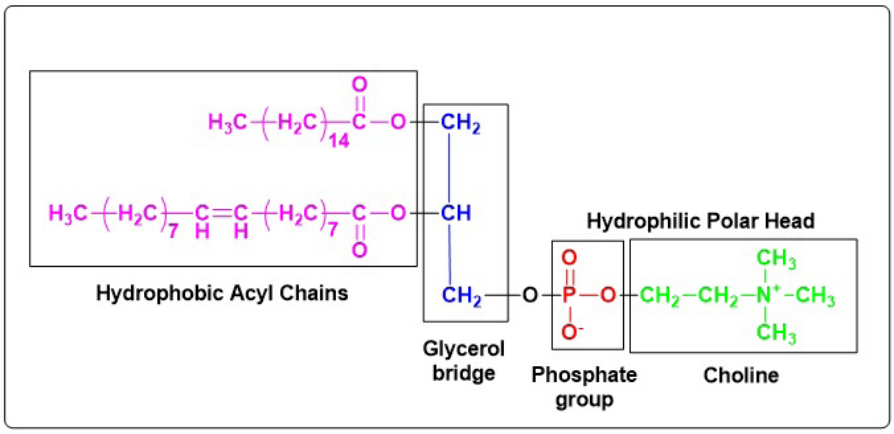

Figure 2. The basic structure of phospholipids.

or bioactive is essential in such cases. In addition, in the case of phytoconstituents, most of them are polyphenols that contain one or more –O.H. groups attached to the phenolic ring, making them more polar. Polyphenols are classified into flavonoids and phenolic compounds, which have abundant subtypes. These subtypes have lipophilic properties and can pass the biological membrane but cannot dissolve in the aqueous gastrointestinal fluids and vice versa. For example, the polyphenol in herbal extracts such as hesperidin limits its penetration through the membrane, as the membrane is rich in lipids.

Conversely, phytoactive such as curcumin or rutin cannot dissolve in the gastric fluid due to their lipophilic character.[15] Bioactive phytochemicals such as andrographolide, gymnemic acid, emodin, berberine, embelin, and oxymatrine would utilize to produce phytosomes. The novel approach focuses on developing a multi-layered nanocarrier system for the delivery of phytoactive. Moreover, the synergistic combination or the improved efficacy of antitumor effects will produce via different mechanisms, or the exact mechanism would obtain by co-administration of naturally occurring antitumor phytoactive from both fungal and herbal origins. However, a nanosized vesicle composed of Monascin (MNS) and ankaflavin (ANK)-loaded casein micelles (CAS MCs) which will entrap in the phytosomal resveratrol (RSV), was formulated.[21]

Solvents used to prepare phytosome

The choice of solvents significantly impacts the phospholipid complex formation, including the solubility of phospholipid and active compounds. Hydrophobic solvents such as dioxane, dichloromethane, methylene chloride, ethyl acetate, n-hexane, and acetone would produce phytosomes. Solvents such as ethanol and methanol can be used to prepare phytosomes, for food applications, and for obtaining the complex in high yield, as it leaves fewer residues and causes minimal environmental damage,[22] but the use of such solvents is limited. The combination of protic and aprotic solvents for the production of phytosome improves the solubility, as suggested by previous studies.[23] Supercritical fluids (SFC) formulate phytosomes as an excellent alternative solvent.

Using the same bioactive, different solvents to formulate phytosomes will add corresponding characteristics to the developed phytosome. For example, in the preparation and optimization of rutin nanophytosomes using ratios of 1:1 (I), 1:2 (II), and 1:3 (III), rutin to phospholipid using a blend of chloroform and methanol 4:1. The formulation was kept under storage to identify the best ratio by assessing the mean particle size for seven days. On the first day, formulation I had the lowermost particle size of 99 ± 6 compared to formulations II and III, 119 ± 7 and 123 ± 10 nm. However, after seven days of storage, formulation II only had an acceptable particle size of 403 ± 30 nm, whereas other formulations had a much higher comparison. The same approach was applied to assess the incorporation of cholesterol to determine the best rutin-to-phospholipid ratio. Therefore, three ratios of rutin to phospholipid to cholesterol were prepared 1:2:0.2, 1:2:0.5, and 1:2:1 and kept at storage for 21 days to evaluate the optimum ratio by measuring the particle size. On the first day, a particle size of 164.5 ± 11 nm will observe with a ratio of 1:2:0.2, and 582.5 ± 43 nm was the observed particle size after 21 days. This composition will accept compared to others and has shown a zeta potential of –45.2 mV, representing high physical stability and entrapment efficiency. From the results, it was shown that the concentration of cholesterol has an influence on drug-loading. As the concentration of cholesterol increases, the drug-loading capacity decreases, but the change was insignificant (P> 0.05). The drug-loading of different ratios, i.e., 1:2:0.2, 1:2:0.5, and 1:2:1, were found to be 80.4 ± 1.3, 72.5 ± 1.4, and 72.3 ± 1.1, respectively. Similarly, three rutin nanophytosome formulations (1:1, 1:2, and 1:3) rutin to phospholipid will prepare by dissolving in absolute ethanol, where all the nanophytosomes have particle sizes below 100 nm. Formulation 1:3 will identify the best particle size, highest entrapment efficiency of 99 %, and highest stability.[24]

Stoichiometric ratio of bioactive compound to phospholipid

The phytosome complex production involves collaborating between natural or synthetic phospholipids and

phytoactive, usually with molar ratios extending from 0.5 to 2.0.[25] Previously, a stoichiometric ratio of 1:1 produced the best results. For example, andrographolide-phospholipid complexes formulation involves mixing andrographolide and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) at a molar ratio of 1:1.[26] However, it does identify that this ratio could not be the same for preparing phytosomes with different phytoconstituents. In the instance of hesperidin phytosome, a molar ratio of 1:1 yielded the most significant solubility in distilled water and PBS at pH 2.5 and 7.4, partition coefficient n-octanol/distilled water and n-octanol/PBS pH 7.4, and drug content (95.54 ± 4.01%). However, for the preparation of silymarin phytosome, a molar ratio of 1:5 showed the best physical parameters with a mean diameter of 133.534 ± 8.76 nm, having a polydispersity index of 0.399 ± 0.078, entrapment efficiency of 97.169 ± 2.412% w/w, loading capacity of 12.18 ± 0.3%, and had good stability after freeze-thaw stability test.[27] In case of *Centella asiatica* (L.) The urban extract's quality by design (QbD) approach optimizes the drug-to-phospholipid ratio, reaction temperature, and time. The influence of both formulation and process variables like phospholipid-to-drug-ratio, reaction temperature, and time can be easily studied using the QbD. All these parameters would influence drug-loading efficiency and the efficiency of drg-loading increases with increasing these variables. Different ratios of 0.5:1, 1.01:1, 1.75:12.49:1, or 3:1 Phospholipon® 90H and standardized *Centella* extract (SCE) were prepared by the modified solvent evaporation technique at 40 °C, 44 °C, 50 °C, 56 °C or 60 °C, respectively. Upon further studies, maximum drug loading of 95% w/w with a stoichiometric ratio of 3:1 at 60 °C and for 3 hours.[28] Similarly, an entrapment of 93.26 ± 0.82 %w/w during apigenin phytosomes formation used a 1:2 ratio of apigenin to phospholipid at 60 °C.[29]

The different methods of phytosome preparation include:

Solvent evaporation technique using a rotary evaporator

The popular and commonly used method of preparation is the solvent evaporation technique. In this process, the phospholipid was dissolved in an organic solvent, and the drug or the extract was also solubilized and evaporated using the rotory evaporator. Liu and team prepared the evodiamine-phospholipid complex for enhancing the systemic availability using the solvent evaporation method.[30] In a recent study by X. Chen, Glycyrrhizinate phytosome was prepared for nasal vaccination using the solvent evaporation technique. Tetrahydrofuran was used as the solvent for the complex formation, and the complex formed shows good drug-loading capacity, and increased solubility in *n*-octanol.[31] Similarly, Alotaibi and team investigated the effect of silymarin phytosome and sylymarin alone for its hepatoprotective activity. The phytosomes were prepared by solvent evaporation, and the oral bioavailability was increased by ~6 fold compared to its pure silymarin. All these results unitedly emphasize that phytosome helps in enhancing the solubility, oral availability, ultimately leads to beneficial therapeutic effects.[32] Figure 3 shows the steps involved in phytosome preparation. The nanosized soy phytosome-based thermogel does formulate using the soy extract and phosphatidylcholine, which is dissolved in ethanol and refluxed for 2 hours under vacuum using rotavapor at 30 °C, 120 rpm. The drug release (77.16% within 2 hours), as well as the zeta potential values (–51 ± 7.06 mV), suggest the release characteristics and stability profile of the phytosomes as compared to the crude extract. The chrysin-loaded phytosomes employ either soy phosphatidylcholine (SPC) or egg phospholipid (EPL) as the phospholipid source. The average size and zeta potential of the phytosome produced using EPL be 117 nm and –31 mV, respectively, with a uniform size distribution (polydispersity index: 0.30). The phytosome produced via EPL was more stable, with a ratio of 1:3. The average particle

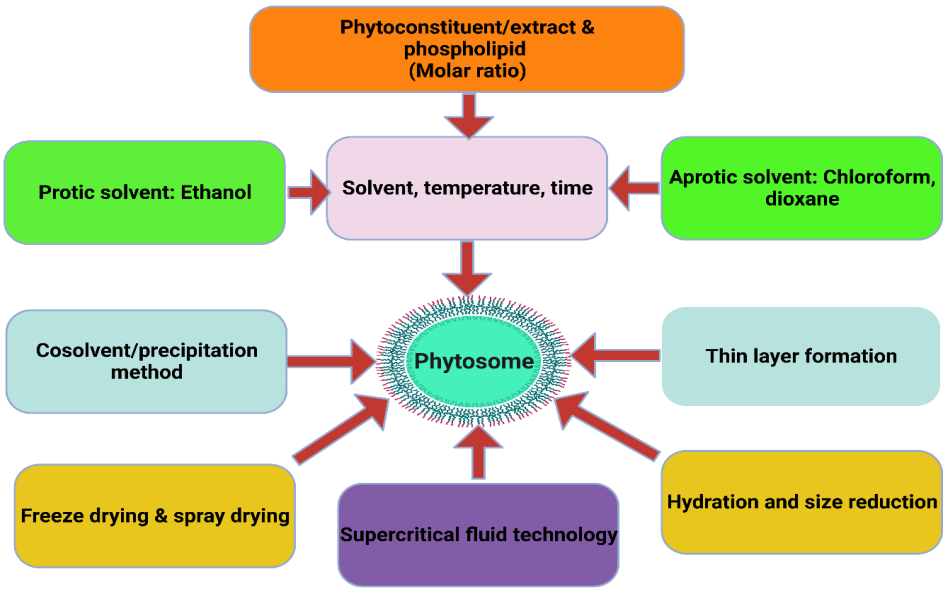

Figure 3. Phytosome preparation.

size and zeta potential of phytosomes (1:3) prepared using SPC show 134 ± 0.1 and –33 ± 4.1 mV, respectively.[33]

Salting out method or anti-solvent precipitation process

The antisolvent process is a rapid and easy method for obtaining ultrafine drug particles—the ultrafine drug particle for the absorption and dissolution of oral dosage forms. The anti-solvent precipitation is a suitable technique for preparing drugs with ultrafine actives. The drug is dissolved in the solvent and then combined with the solvent in which the drug is not soluble (antisolvent). The drug precipitates while combining the solution and the antisolvent. However, the disadvantage associated with this method is that the phytoconstituent must be soluble in the solvent. DMSO or acetone was the solvent for the antisolvent precipitation process.[34] Here, a specific quantity of herbal extract and phospholipids is allowed to reflux with 20 ml of common organic solvents at a temperature not above 50°C for 2 to 3 hrs. Then it is concentrated to a minimum volume, followed by the addition of an anti-solvent like n-hexane so that the complex forms as precipitates, and after vacuum filtration, keeping it in air-tight amber-colored glass bottles.[23] Protopanaxadiol-phospholipid complex (PPD-PLC) was prepared by a slightly modified anti-solvent method. Pu and team observed that, the solubility in water and *n*-octanol of the complex was increased 6.53- and 1.53 times. Moreover, the suspension was formulated using the complex, and its dissolution rate was increased. In another study, Habbu and coworkers prepared Bacopa-phospholipid complex through anti-solvent method, and the studied the cognitive effects in aged mice. The results shows improved cognitive function because of better absorption.[35]

Mechanical dispersion process

Here, the phospholipid is dissolved in an organic solvent and keeps in contact with the drug's aqueous phase. The phosphatidylcholine is dissolved in an organic solvent such as diethyl ether and then subjected to slow injection into the already prepared aqueous solution of the phytoconstituents to form the encapsulated product. After that, does remove the organic layer generates the formation of the phytosome complex.[36] The phosphatidylcholine complex of gymnemic acid preparation involves the mechanical dispersion process. For the preparation of the phytosome, 100g of phosphatidylcholine does dissolve in 1 lt of ethanol, and it was then refluxed at 60 °C in a mechanical stirrer and added drop by drop the gymnemic acid prepared in ethanol in water (1:1) mixture. Then the combination was concentrated under vacuum and dried under vacuum at 40 °C for 68 hours. The prepared complex was soluble in n-octanol and chloroform, as its pure gymnemic acid was not soluble in the low polar solvents such as n-octanol and chloroform. The complex produced shows an increased *in vitro* release rate. The amorphous nature of the complex formed indicates the phyto-phospholipid complex, as evidenced by the X-ray diffraction study.[37]

Lyophilization method

The phytoactive constituents and the phosphatidylcholine are dissolved in a suitable solvent (organic/water), subsequently stirring till the phytosome production, then it is separated with the help of lyophilisation.[23] If the solvent is organic such as acetone, quench freezing method is employed for lyophilization in a dry ice bath without affecting the collapse of the structure of the formulation. However, the maintaining the margin of safety is important between the temperature of the sample and collapse.[38] Perrie and colleagues used a simple technique for lyophilization of different phospholipid and cholesterol complexes of ovalbumin. The dynamic mechanical analysis method (DMA), freeze drying microscopy (FDM), modulated differential scanning calorimetry (MDSC) were employed for the determination of collapse temperature of the complex-cryoprotectant mixtures. The collapse temperature found to be between -39 and -33 °C. The ramp freezing and snap freezing approaches were studied, and found that ramp frozen method caused fewer changes in physicochemical parameters with better drug-loading.[39] Freitag and the team prepared a diosmin-loaded phytosome using the lyophilization method. For that, diosmin does wholly dissolve in DMSO, and the solution obtained was added to the solution of soybean phospholipid dissolved in t-butyl alcohol, after that stirring for 3 hours on a magnetic stirrer till the complex formation. Finally, the complex separation by lyophilization at a pressure of 40 mbar. The diosmin-loaded herbal nanocarrier prepared using lyophilization improves the dissolution rate (related to its crude form and marketed products) and the permeation through non-everted rat sacs.[40]

Gas anti-solvent process (GAS), compressed anti-solvent technique (PCA), and supercritical anti-solvent methods are novel techniques for the preparation of phyto-phospholipid complex.[41]

The preparation of phytosome recently, the method used, the solvent used for the preparation, and the characterization of the phytosome are shown in Table 1.

Experimental Biomedical Applications of Phytosomes

Phytosomes, the unconventional form of delivery of herbal drugs, aid the absorption through the biomembrane quickly and produce better results than the conventional dosage forms; further confirmed by pharmacokinetic and pharmacodynamic tests in animals and humans trials.

Phytosome for liver protection

Targeting the drug to the desired site at the desired time is a challenging task. However, the phytosome complex will enhance the solubility in the bile salts, thereby aiding in liver targeting of active moiety directly into the hepatic cell. The phosphatidylcholine liquefies the fat and thus helps cure fatty liver disease besides its hepatoprotective effect. The phosphatidylcholine obtained from soy phospholipids is hepatoprotective and cures alcohol-induced liver damage and drug abuse.[49] *Andrographis paniculata* is a

Table 1. The preparation methods of phytosome prepared recently, the method used, the solvent used for the production, and their characterization of the phytosome.

Marketed phytosome	Method of preparation	Solvents employed	Drug: phospholipid ratio	Temp.	Chemical analysis	Characterization	Ref.
Thymoquinone-loaded Soy-Phospholipid	Anti-Solvent precipitation method	Dichloromethane	1:1,1:3, 1:5		FT-IR, vesicle size measurement, TEM, *in vitro* release	-As per the TEM data, the complex assumes the shape of the sphere. The decreased peak strength indicates the formation of the complex (FT-IR) -Vesicle sizes range from 43.7 to 129.9 nm.	42
Terminalia arjuna phytosome	Solvent evaporation process	Dichloromethane and n-hexane at 40 °C for 1.5 hrs.	-	40 °C	Entrapment efficiency, DSC. SEM and FT-IR	-DSC results show two peaks at 140.72 °C and 212.45 °C, indicating melting lipid components and interactions. The highest entrapment efficiency of 97.9 ± 0.4%w/w.	43
Curcumin phytosome	Rotary Evaporation method	Dichloromethane, n-hexane, phosphate buffer 6.8	1:1, 1:2. 1:3, 1:4 and 1:5	40 °C	SEM, Entrapment efficiency, *in vitro* release study.	-Particle size: 181.6nm, -Zeta potential: 33.2 mV. -Entrapment efficiency: 76 to 87 %.	44
Cocoa pod phytosome	Solvent evaporation	Ethanol	1:3 for 24 hrs	40 °C	Particle size distribution	-The average globule size was 627 nm	45
Ursolic acid-phospholipid complex	Solvent-assisted grinding method	Methanol, ethanol. Acetone, ethyl acetate	0.5:1, 1:1. 1.5:1 and 1:2	40 °C	DSC, XRD, TEM, *in vivo* pharmacokinetic study	-SEM results show that the complex produced was found to be smooth surfaced, which was due to the molecular dispersion of ursolic acid in phospholipid. TEM analysis indicates the formation of the nanometer-scale vesicle. -The improved ADME profile as compared to the ursolic acid alone with enhancement in bioavailability	46
Myricetin nanophytosome	Thin layer film hydration-sonication technique	Dichloromethane, acetone, ethanol	Myricetin:phosphatidylcholine: cholesterol (1:1:0.4, 1:2:0.4, 1:3:0.4)	35 °C	Particle size and entrapment efficiency	-Particle size (nm)-233.6 to 250	47
Novel protamine-decorated tripterine phytosomes	Solvent evaporation process	Anhydrous ethanol	1:3	40 °C	Particle size, SEM, and TEM	-Particle size -250 nm -Zeta potential (+21.6 mV) -TEM data suggest the formation of a dark outer layer over phytosomes.	48

medicinal plant having liver protectant effects because of andrographolide (AN). However, the systemic availability was the limiting factor for its targeted delivery. For instance, Jain and companion formulated an andrographolide-loaded phytosome with soya-phosphatidylcholine for enhancing the systemic absorption. The AN-loaded phytosome has better bioavailability as compared to its plain AN . *In vivo* study results reveal the increased serum SGOT and SGPT levels accompanied by its enhanced protection percentage on liver tissue as compared with the plain AN.[50] In a study by Giudice and team, investigated the importance of herbal therapy for the liver disease, and the study was conducted in dogs. The nutritional supplement was the combination of silymarin phytosome, choline chloride, l-cystine, artichoke, and vitamin E (Epacare pet)+pasta for 30 days. Here, sylymarin phytosome is the active constituent present, allowing to increase the bioavailability of silymarin for engancing therapeutic efficacy as a liver protectant. Finally the feed supplementation produces therapeutic benefit on the free radical scavenging effect and liver enzymes in normal dogs.[51] A study found that phytosomal curcumin has shown chemopreventive effects on hepatocellular carcinoma development *in vivo* (transgenic mouse model).[52] Milk thistle (*Silybum marianum*) is a liver-protectant prescribed mainly for treating hepatitis C, hepatocarcinoma, nonalcoholic fatty liver disease, and gall bladder disorders. El-Gazayerly and coworkers performed a comparative study of the free radical scavenging and liver-protective benefits of silymarin phytosomes to extract in rats suffering from carbon tetrachloride-induced hepatotoxicity. The phytosomes produced from silymarin have shown the decreased concentration of SGPT induced by carbon tetrachloride induction, as related to its milk thistle extract and the phosphatidylcholine being a surfactant enhances the solubility of many constituents in the milk thistle extract. Besides its use as hepatoprotection, silymarin is frequently a renal protectant, as in the case of acetaminophen, cisplatin, cyclosporine, and vincristine-induced renal toxicity. Correspondingly, researchers compared the silybin-phosphatidylcholine complex in dogs with commercially available standardized extract of silymarin and found that the phytosome complex administration markedly improved the bioavailability in dogs for the treatment of liver dysfunction.[53] *Ginkgo biloba*, a vital herb in the conventional Chinese system of medicine, is often prescribed for cardioprotection, anti-asthmatic, antidiabetic, and has potent central nervous system activities such as improvement in memory, mental alertness, and reduction in mental fatigue. Meanwhile, Naik and Panda 2007 found the antioxidant effects and the hepatoprotection effects of *Ginkgo biloba* phytosomes (GBP) in carbon tetrachloride-induced liver injury in rodents. The administration of GBP to carbon tetrachloride-induced liver-damaged rats outcomes in elevated levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (G.R.) levels; finally observed that the phytosome helps in the restoration of the enzyme levels as well.[54]

Phytosome as antioxidant

Telange and companions prepared the mangiferin phytosomal soft nanoparticles to study solubility, dissolution study, *ex vivo* penetration, oral availability, and antioxidant effects.[55] The *Ginkgo biloba* has various health benefits. The results obtained were promising when studying the influence of its phytosome on antioxidant effects on the rat model. The effects of phytosome were due to free-radical scavenging activity and providing liver protection.[56] The umbelliferone is present in many medicinal plants among the Apiaceae family, particularly effective after U.V. exposure because of its free radical scavenging properties. However, the issues such as solubility, permeation, and antioxidant effects, including their photoprotective effects, bring down their efficacy. In this research work, umbelliferone-loaded phytosomes were prepared and studied the *in vitro* antioxidant effects and the rat skin to measure the antioxidant enzymes present. The finding demonstrates that the phytosome complex shows an excellent antioxidant profile and better *ex vivo* permeation than its drug alone.[57]

Phytosome as a cardiac protectant

Ginkgo biloba phytosome has a protective effect on isoproterenol (ISO) induced cardiotoxicity, and the antioxidant effects investigate in rats. The dose of ISO used was 85 mg/kg for the genesis of myocardial infarction. 100 mg and 200 mg/kg body weight of the phytosome is administered twice a day (oral). The phytosome showed significant cardioprotection by decreasing the serum marker enzymes and lipid peroxidation, and there is an increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase.[58]

Phytosome action on the nervous system

Mancini *et al.*[59] compared the liposomes and phytosomes of *Annona muricata* for major depressive disorder (MDD). To enhance the permeation through the blood-brain barrier (BBB) and minimize gastric biotransformation, liposome, and phytosome prepare. Further analysis of the formulation, the phytosome-loaded product shows better encapsulation efficiency and less toxicity (did hCMEC/D3 cell line). Moreover, the presence of cholesterol in the complex helped improve the permeation, as evidenced by the study results. Wattanathorn and the company studied the combination of phytosome-loaded mulberry fruit and ginger for its effect on dementia. They observed that the cholinergic function improved with the administration of the phytosome (*in vitro*).[60] *Geophila repens* shows good anticholinesterase activity used to improve memory and intelligence. Rajamma *et al.*[61] studied the phytosome of *Geophila repens* and made an intranasal gel for the improved permeation for the therapy of Alzheimer's disease. Moreover, transcutol was incorporated to enhance

permeability. The results show that the complex produced a significant acetylcholinesterase inhibition without causing skin irritation; preclinical studies (*in vivo* imaging) confirmed this.

Researchers have investigated various phytosome-loaded formulations *in vivo* to find the tissue distribution of the active ingredients in the brain. Bacopa-phospholipid (BPC) does formulate for finding its antiamnesic activity in natural aging-induced amnesic mice. BPC administration improves aged mice's cognitive function by enhancing acetylcholinesterase inhibitory activity. There is an enhanced serum concentration of bacoposide-I and bacoposide-II for BPC compared to its bacosides extract.[35] *Centella asiatica* has various clinical applications, including anti-inflammatory and brain tonic. The administration of the phytosome produces an enhancement of Brain-derived neurotrophic factor (*Bdnf*) in the prefrontal cortex to enhance cognitive performance.[62] Sbrini and their team in 2020 have investigated the effect of phytosomes containing *Centella asiatica* and *Curcuma longa* on the concentration of *Bdnf* level in the prefrontal cortex of adult rats. The enhancement of mTOR-S6- regulated transcription thus suggests the possible therapeutic benefits, especially for memory and cognitive abilities.[63]

Phytosome as anti-asthmatic

The management of asthma attacks results in the systematic destruction of airways. Chronic treatment often results in side effects and high costs—usually, corticosteroids (inhaler therapy) and long-acting beta-agonists for mild-to-severe persistent asthma. A total of 32 asthma subjects have been enrolled in the multicentered study, using Boswellia phytosome (Casperome 500 mg/day) as the complementary intervention and the standardized treatment regimen (inhaled corticosteroids) beta-agonists) for four week period. Vincentiis and Pierro conducted the study. The treatment reduces the number of desired inhalations associated with the standard drug alone.[64] Yu and the team studied the naringenin-dipalmitoylphosphatidylcholine phytosome for the inhalation treatment of acute lung injury in the male Sprague Dawley rat model. The phytosome dry powder inhalations were developed for the sustained effects and enhanced pulmonary bioavailability and for protecting the acid-induced lesions in the rat model and found that the formulation can eliminate the acute lung injury.[65]

Phytosome for wound healing

Metallic nanoparticles such as gold nanoparticles (AuNPs) usually have wound healing and antioxidant effects on living cells. Demir and colleagues prepared and characterized AuNP and *Calendula officinalis*-loaded phytosome using a ratio of 3:1:1 (phosphatidylcholine: cholesterol: calendula extract). The prepared complex exhibited good wound healing activity as obtained from *in vitro* scratch assay for wound healing effects.[66] It was known from ancient times that sinigrin is among the main glucosinolates present in the plants of the Brassicaceae family (mustard family). Even though sinigrin has numerous biological actions such as anticancer, antimicrobial, and anti-inflammatory effects, its wound-healing effects are unknown. In one report, the wound-healing effect of the sinigrin phytosome using HaCaT cells does found. After 42 h, the sinigrin- complex appeared at 79 %, whereas the sinigrin alone arrived at only 50 % wound healing activity.[67]

Phytosome for cancer treatment

Alhakamy and a coworker studied the scorpion venom-functionalized quercetin phytosome to manage breast cancer. The results show increased caspase-9, Bax, Bcl-2, and p53 mRNA expression for the phytosome.[68] Taxifolin is a pharmacologically active moiety known for its anti-inflammatory, antimicrobial, antiangiogenic liver protective, and anticancer properties. However, because of its hydrophilic character, its use became limited. In this study, taxifolin-rich ethyl acetate was combined with the phospholipids so that the resultant phytosomes had subjected to an *ex vivo* study against MCF-7 cell lines. The phytosomes show enhanced cytotoxic activity with decreased IC_{50} value.[69] Genistein (Gen) is especially suited for treating hepatocellular carcinoma (HCC). However, due to low aqueous solubility and first-pass metabolism, Komeil and the team have investigated genistein's phytosome to improve pharmacokinetics. The mouse model's *in vivo* study finds its important use as an antitumor agent for HCC and helps the accumulation of Gen aglycone in hepatic cells.[70] Aloe vera extract loaded phytosome has antitumor activity against the MCF-7 cell line.[71] Phosphatidylcholine is the main component present in lecithin (98 % w/w). However, various sources of lecithin include egg lecithin, soy lecithin, or purified phospholipids employed for the pharmaceutical industry's production as dispersing, emulsifying, and stabilizing agents. The mitogenic effects of phosphatidylcholine nanoparticles on MCF-7 Breast cancer cells have been done and showed that the nanoparticles dispersed in buffer with pH 7.0 have some changes in the cancer cells' plasma membrane, thereby affecting epidermal growth factor receptor (EGFR) and cell proliferation.

Interestingly, no impacts observe when the nanoparticles dissolve in pH 5.0 buffer.[72] Icariin, a flavonol glycoside, has pleiotropic pharmacological actions and has cytotoxic effects against ovarian cancer cells, and Alkamy and coworkers studied the significance of Icarrin phytosomes against ovarian cancer cells (OVCAR-3 cells). The study found that cytotoxicity was associated with enhanced permeability through the cells, apoptosis, and intracellular release of reactive oxygen species.[73] Moreover, Alkamy and coworkers found that thymoquinone-loaded phytosome possesses anticancer potential against human lung cancer cells (A549 cell line). The study reveals the importance of this novel phytosome approach helping a sustained release profile and cytotoxicity against lung cancer cells.[42] Resveratrol, effective for treating breast cancer,

acts through different mechanisms such as apoptotic, antiangiogenesis, and aromatase inhibitory activity. The study uses two synergistic combinations of anticancer drugs from the herbal and fungal sources that were selected and fabricated in a multicompartment nanocarrier system and finally encapsulated in a resveratrol phytosome. However, both monascin and ankaflavin were obtained from red mold rice combined in casein micelles and finally complexed in resveratrol phytosome as the phytoactive. The formulation's efficacy will be superior, as evidenced by the decrease of tumor volume and growth biomarkers.[21] Celastrol, also known as tripterine, is obtained from the medicinal plant *Trypterygium wilfordii*, possessing various therapeutical applications, including anti-inflammatory, neuroprotective, and antioxidant effects. The researchers found its potential effect on cancer, especially human prostate cancer, non-small-cell lung cancer A549 cells, pancreatic cancer cells, and breast cancer MCF-7 cells. Due to bioavailability issues of the celastrol, phytosomes of celastrol were formulated thereby improving its absorption issues. *In vivo* pharmacokinetics studies were conducted in healthy rabbits and confirming the increased oral availability using complexation, practical as the anticancer drug.[74] Diosgenin is a steroidal sapogenin, showing cytotoxic effects and is predominantly used as an intermediate in the production of steroidal hormones.

Moreover, the researchers modified the diosgenin structure to ensure new derivatives with enhanced cytotoxic effects. They screened FZU-0021-194-P2 (P2) for its antiproliferative effects against human lung cancer A549 and PC9 cell lines, human cervical cancer HeLa cell line, and human hepatoma HepG2 cell line via MTT assays. Its phytosomes (P2P) does formulate and test the antiproliferative effects. Similarly, the particle size of the P2P does found to be 53.6 ± 0.3 nm, which inhibited the proliferation of cancer cells, indicating an effective candidate for non-small-cell lung cancer.[75]

Transdermal applications of phytosomes

The carotenoids is essential for dlaying aging process. A. Naik and colleages investigated *Nyctanthes arbor-tristis* L. and the petals of *Tagetes patula* L. loaded phytosome for studying D-galactose induced aging mice model. The prepared phytosome was incorporated into a gel base. The entrapment of phytoconstituents into a phytosome not only provides stability but also improves the systemic availability as evidenced in the range of 99.98 % w/w to 99.85 % w/w of carotenoids at the end of three months. The formulation results in enhancement of dermal and epidermal layers, and an increase in GSH (glutathione) levels of skin.[76] Sinigrin, a significant glucosinolate, has been studied for its wound-healing abilities (both sinigrin and its phytosome formulations). The sinigrin-loaded phytosome was prepared, and observed the skin permeability of the phytosome complex of sinigrin using the Franz diffusion cell. Tape stripping study results show enhanced delivery of sinigrin phytosome (0.5155 µg/ml) into the stratum-corneum as linked to the sinigrin alone (0.0730 µg/ml).[77] Rutin is a polyphenolic flavonoid, possessing various clinical effects, for example, antioxidant, anti-inflammatory, antineoplastic, and antithrombotic effects. Rutin has low bioavailability via the oral route. However, the rutin phytosomes can cross the biomembrane in oral and topical applications. In 2014, Das and Kalita observed that the complex increases the skin permeability to relieve arthritis and rheumatism and helps in a sustained effect.[78] Kumari and coworkers[79] formulated and evaluated the soy-phytosome cream for the topical delivery and conducted a skin-irritation study, and the results obtained were promising as the complex enhances the permeation.Nanosized soy phytosome-based thermogel helped reduce obesity, as found by El-Menshawe *et al.*[80] in the year 2018. The observation comprises that the complex has a potential anti-obesity effect on the abdomen of albino rats with a negligible effect on the lipid profile. The entrapment efficiency (>99%)and drug release (77.61-99.78%) of the phytosome will be high.Extracts of *Vitis vinifera* (grapes) seed (GSE) comprises many phenolic compounds showing good free-radical scavenging properties. However, owing to its polar character, its applicability in the topical routes is inadequate as it has low permeability through the epithelial barriers. The present investigation focussed on the formulation of phytosomes of GSE and, finally, the serum formulation prepared from the phytosome. The *in vitro* permeability report shows that the permeation of total phenolic compounds from the serum phytosome has found to be 27.25 ± 0.67%, which was elevated as compared to its serum formulation (11.97 ± 0.49%) and GSE solution (10.63 ± 0.41%), assuming that the phytosome loaded serum enhances the permeation of phenolic compounds.[81] Rhein is an anthraquinone derivative used to treat various skin disorders due to its enormous therapeutic properties, including antimicrobial, antioxidant, and anticancer effects. The improvement in permeation through the skin layers and improved solubility in water and oil does produce by its phytosome complex.[82] Albash and colleagues formulated a bergamot oil-loaded nano-phytosomes, combined with spironolactone to compare the pharmacological effect against acne vulgaris. Molecular simulations study also reveals the significance of constituents as linking platforms for the preferred anchoring of spironolactone on the Phosphatidylcholine (PC) interface. Clinical efficacy study also reports the beneficial effects of the combination of spironolactone-incorporated bergamot oil-loaded nanophytosome over bergamot oil-loaded nano-phytosome.[83]

Physicochemical Properties of Phytosomes

Mixing polyphenols or polyphenol extracts with phospholipids in a hydrophobic solvent is the usual preparation method at the beginning of its synthesis.[84] Later, it found that hydro-alcoholic solvents have been used to make the phytosome. Because of its amphiphilic nature, it helps improve the biopharmaceutical aspects

of phytomedicines. In the presence of water, phytosomes assume the shape of the micelle; thus, its systemic availability is increased as related to the traditional herbal extracts,[85] framing a liposome-like structure. Basal differences exist well amongst both phytosome and liposomes. In liposomes, the active moiety is dissolved in the central portion so that no interaction occurs between the nearby lipid and the polar ingredient. Instead, the complex produced in the phytosome can be an essential component of the lipid membrane, where the hydrophilic functional group of lipophilic moiety interacts by hydrogen bonds with the hydrophilic head of a phospholipid (i.e., phosphate and ammonium group), building an exclusive arrangement and thus helps significantly in the permeability of biomembranes with improved systemic availability too. A review found that the phytosome formulation increases systemic availability, as evidenced from the comparative study of systemic availability of curcumin and curcumin-loaded with phosphatidylcholine when administered orally. The concentration of curcumin phytosome and curcumin alone in plasma was evaluated and showed that the curcumin phytosome significantly elevated the plasma concentration.[86] Puerarin is an isoflavone obtained from *Pueraria lobata*, showing enormous health benefits such as cardiovascular, neurological, and hyperglycemic disorders. Nevertheless, to improve the puerarin's absorption, the phospholipid complex was prepared by traditional methods such as solvent evaporation, freeze-drying, micronization, and supercritical fluid (SCF) technology compared to the physicochemical characters. Solid-state characterization such as differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), dissolution, solubility, and scanning electron microscopy (SEM) need to examine. The gas antisolvent (GAS) technique for the puerarin showed a better crystal shape, which DSC, XRPD, and I.R. established. The phytosome prepared through supercritical fluid technology enhances *in vitro* dissolution characteristics. However, the highest solubility does observe using the solvent evaporation technique with the complex prepared. It does demonstrate that supercritical fluid technology provides a better preparation method for the puerarin phytosome than the other conventional method such as the solvent evaporation method.[41]

Both particle size and zeta potential are the two vital properties as it determines the stability and reproducibility of the drug-phospholipid complexes. Usually, the average particle size of the complex ranges from 50 to 100 nm. Tan and the team prepared an evodiamine-phospholipid complex. The particle size was analyzed, dispersed in a small range of about 246.1 nm through a polydispersity index Anwar and Farhana formulated and evaluated the phytosome loaded with a maltodextrin-gum Arabic microsphere system for the delivery of *Camellia sinensis* extract. The zeta potential of the nanoparticles, above + 30 mV and below −30 mV, should be highly stable as the presence of the charge on the surface prevents the accumulation among particles. The formulated phytosome has the zeta potential values of −41.3, −47.9 ± 3.43, −48.2 ± 1.78. The polydispersity index of the formulated phytosomes was 0.194, 0.227, and 0.276, which indicates that the first formulation had the maximum particle size homogeneity; meanwhile, the polydispersity index value is closest to zero.[87]

Instrumental techniques such as H1-NMR (Proton nuclear magnetic resonance) (Gabetta, Zini GF, 1989), 13C-NMR (Carbon-13 nuclear magnetic resonance), 31P-NMR (Phosphorous-13 nuclear magnetic resonance), and by I.R. (Infrared) spectroscopy are the commonly used methods giving primary information about complexation as well as molecular connections between phytoconstituents and phosphatidylcholine in solution. In addition, the phytosome complex had confirmed by the FT-IR spectra, and the presence of different functional groups can be interpreted based on their corresponding wavenumber.[88] For example, the work done by Wina Maryana and the team established the presence of chemical interactions between silymarin and phospholipids. In which bands at 3550 (O.H.), 3001 (C.H.), and 1776 (C=O) cm-1 whose intensity is reduced or missing in complex form.[27]

Industrial Applications of Phytosomes

Mixing phospholipids with plant extracts like glycosides, flavonoids, and terpenoids in a suitable ratio results in a phytosome, an advanced form of a liposome. Owing to better permeation through the epidermis and its high lipid content acts both as an herbal carrier and nourishes the skin, often used in herbal cosmetics.[89] Furthermore, due to their rapid absorption, the dose need appears to be reduced, and they have a higher stability pattern as chemical connections establish among phosphatidylcholine and phytoconstituents. The Ginkgo biloba terpenes phytosome, a commercial product that offers anti-inflammatory and soothing effects, is in a topical formulation.[89] Indena, an Italy-based pharma company, is the leading manufacturer of skincare and health care phytosome products. The phytosome formulation uses standardized plant extracts, mainly containing polyphenolics and terpenoid fractions, to cure various health problems. Also, Natural Factors (Canada) and Nature's Herbs (USA) are manufacturers of phytosome formulations.[90] Table 2 shows the phytosome-based commercial products with their source, dose, and pharmacological activity.

Significance of Phytosome Over Other Conventional Formulations

Although the phytosome approach has established both the nutraceutical and herbal drug industries, it offers significant advantages against complicated delivery systems that require extensive methods and techniques.

- Their binding significantly improves the bioavailability of phytoconstituents with phospholipids which enhances gastrointestinal intake.[15] The bioavailability of domperidone is low, and its absorption is enhanced using piperine (a P-glycoprotein inhibitor) phytosome.

Table 2. Phytosome based commercial products available in the market with their source, dose, and pharmacological activity.

Commercial product	Active ingredient	Dose and dosage form	Category	Pharmacological activity	Ref.
Casperome® Boswellia Phytosome®	≥25.0% of boswellic acids by HPLC, from *Boswellia serrata* Roxb. ex Colebr.-Resin	-	Therapeutic	Healthy inflammatory response, joint and gut health	91
Ginseng Phytosomes® Phytosomes	Ginsenosides from *Panax ginseng*	150 mg	Cosmetic, nutraceutical	Improves elastic strength of the skin	91
Lecoselect®	≥25.0%≤30.0% of proanthocyanidins by GPC, obtained from *Vitis vinifera* (Grapes)	50-100 mg	Nutritional food, cosmetic purpose	Antioxidant, anticancer, U.V. protective	91
Siliphos®	Silybin (≥29.7%≤ 36.3% by HPLC) from *Sylibin marianum*	120 mg	Pharmaceutical, health food, cosmetic	Liver protective, antioxidant	91
Cucurbita Phytosome™	Tocopherols, steroids, carotenoids from *Cucurbita pepo* (Pumpkin)	Face powder, cream	Cosmetic.	Anti-inflammatory, benign prostatic hyperplasia	91
Ecsinbeta-sitosterol Phytosome™	Ecsinbeta-sitosterol from horse chestnut fruit	30% gel, shampoo, hair conditioner, toothpaste, lotion	Cosmetic use	Antiedema, shining of the skin	92
Oleaselect Phytosomes ™	Polyphenols from *Olea europea*	-	Therapeutic	Antihyperlipidemic, anti-inflammatory. More bioavailable than crude extract.	92
Centella triterpenoid Phytosome ™	Terpenes from *Centella asiatica*	60-120 mg	Cosmetic, health food	Skin disorders, anti-ulcer, wound healing, brain tonic	92
Glycyrrhetinic acid Phytosome ™	18-beta glycyrrhetinic acid from *Glycyrrhiza glabra*	-	Cosmetic	Anti-inflammatory, anti-irritant, skin infection	92
Zanthalene Phytosome™	Hydroxy-a-sanshool of *Zanthoxylum bungeanum*	Emulsion and lotion	Cosmetic.	Soothing and anti-reddening	92
Soyselect Phytosome™	Genistein and daidzein of *Glycine max* (Soy)	400 mg/day	Therapeutic	Antiangiogenic, anticarcinogenic, cardioprotective, immunostimulatory, and hypocholesterolemic	92
Madeglucyl Phytosome™	Tannins from *Syzygium cumini* (Jamun)	3 g/day (Suggested)	Therapeutic	Anti-hyperglycemic, anti-inflammatory, antioxidant	92

Table 2. Continued.

Pycnogenol Phytosome™	Procyanidins from *Pinus maritima* (Pine)	-	-	Anti-inflammatory, antiwrinkle, antiallergic	92
Ruscogenin Phytosome ™	Ruscogenin, neoruscogenin from *Ruscus aculeatus* (Butchers' broom)	Topical preparation	-	Antioxidant, anti-inflammatory	92
Millet Phytosome™	Mineral salts, vitamins, unsaturated fatty acids, amino acids of *Panicum miliaceum* (Millet)	-	Topical use	Antistress, beauty food for skin, nails, hairs	92
Millet Phytosome™	Mineral salts, vitamins, unsaturated fatty acids, amino acids of *Panicum miliaceum* (Millet)	-	Topical use	Antistress, beauty food for skin, nails, hairs	92
VitaBlue Phytosome™	Anthocyanosides, tocotrienol complex, alpha-lipoic acid from *Vaccinium angustifolium* (Blueberry)	-	-	Anti-oxidant, memory enhancer, improves vision	93
Visnadex® Phytosomes	Visnadine from *Ammi visnaga*	-	Cosmetic (Emulsion, lotion, gel)	Vasokinetic	93
Lymphaselect® Phytosome	Triterpenes from *Meliotus officinalis*	2-60 mg	Therapeutic	Hypotensive, insomnia	94
Merivaselect® Phytosomes, Curcumin Phytosome ™	Polyphenols from *Curcuma longa*	250-360 mg	Health food, cosmetic use	Anti-inflammatory, osteoarthritis, anticancer	95
PA_2 Phytosomes	Proanthocyanidins A_2 from horse chestnut bark	-	Health food, cosmetic use	Anti-wrinkle, UV protectant	96
Ximilene and Ximenoil Phytosome ™	Ximenynic acid, ethyl ximenynate of *Santalam album*	Emulsion, lotion, gel	Cosmetic	Microcirculation improver	96
Green tea Phytosome™	Epigallocatechin, catechin, epicatechin-3-O-gallate of *Camellia sinensis* (Tea)	400 mg capsule	Nutraceutical	Anticancer, nutraceutical, antioxidant, liver protectant, atherosclerosis, antidiabetic, anti-inflammatory	97
Gingkoselect Phytosome™	Gingko flavonoids, gingoic acids of ginkgo flavonglucosides ginkgolides, and bilobalide from *Gingko biloba* (Mai den hair tree)	120 mg; Emulsion, solution, conditioner, shampoo.	Cosmetic	Cognition enhancer, Raynaud's disease, antiaging, anti-asthmatic, soothing, anti-inflammatory, antidepressant	98

The enhanced absorption occurs due to inhibition of the P-glycoprotein transporter, which minimizes the side effects of a high dose of domperidone.[99]

- The small nanophytosome protects the drug from gastric secretions, gut bacteria, or secretory chemicals.
- It can deliver hepatic protectant flavonoids due to the high bioavailability of phytosome formulation, and also phosphatidylcholine is a liver protectant and thus provides a synergistic effect as a liver protectant. El-Gazayrly and companion investigated *Silybum marianum* (milk thistle), a powerful liver protector with low bioavailability at 20-50 %. However, its bioavailability has increased, and loading efficiency was >85% in phytosomal formulation.[20]
- It also enhances the drug loading capacity and increases bioavailability, which decreases the drug required to produce the pharmacological effect.
- Good entrapment of phytoactive occurs where the choline part of phosphatidylcholine binds to the phytoactive molecule, whereas the tail portion encapsulates the polar portion of the complex to give rise to the lipophilic surface and thus aids in easy permeation through biomembranes.[22]
- The high stability of phytosome formulation is due to chemical interaction between phosphatidylcholine and phytoactive constituents.[100]
- Phytoconstituents can have delivered to multiple desired locations for pharmacological properties at a low cost. When employed as functional cosmetics, phytosomes provide synergistic benefits by protecting the skin from exogenous and endogenous risks in stressful and typical environments.
- The broad applicability in the cosmetic industry is because of the enhanced skin permeation with a high lipid profile.[10]
- The phytosome's active component, phosphatidylcholine, works as a carrier and nourishes the skin. During formulation development, drug entrapment is not an issue. Because the drug forms vesicles after conjugation with lipid, the drug entrapment efficiency using phytsome is high and predictable.
- Dose reduction is feasible due to the enhanced systemic availability of the phytoactive.
- Low-risk profile: This technology offers no risk; meanwhile, the pharmacological profiles of the phytosomal components are well recognized.
- No complicated technical investment is needed to produce the phytosome as it is easy to formulate.

Clinically Approved Phytosome-based Medications and Recent Advancements

Many research activities for phytosome technology are due to its good pharmacokinetics and pharmacodynamic properties. The growing need for herbal medicine designed to treat various illnesses necessitates the development of a phytosome approach, a revolutionary drug delivery method. Some phytosome-based formulations have advanced to the clinical trial stage during the preclinical phase, providing more research into medication safety and how a drug interacts with the human body; this is a critical step in seeking FDA approval. In 2007 the first clinical trial on phytosome-based formulation was carried out.[101]

The below-mentioned literature review focus on the current status of this technology:

Ali and his colleagues investigated effect of phytosomal curcuminoids in non-alcoholic fatty liver disease (NAFLD). Seventy-two patients were selected for the randomized double-blind placebo-controlled trial for two months with a daily dose of 250 mg. There was an improvement of catalase activity alongwith significant reduction in monocyte chemoattractant protein-1 (MCP-1).[102] Cicero and a coworker conducted a double-blind crossover study on healthy, non-smoking young volunteers to check the effectiveness of coenzyme Q_{10} phytosome on the endothelial reactivity. The endothelial reactivity improved compared to baseline and placebo.[103] Sbrini and the team studied the phytosome of Centella asiatica and Curcuma longa, obtained from Indena, and evaluated the chronic oral treatment to find out the effect on the brain-derived neurotrophic factor (Bdnf). Bdnf is essential for proper brain development and maintenance. The phytosome improved the Bdnf levels in the prefrontal cortex of the adult male Sprague Dawley rats. Also found that an increase in expression of the eukaryotic elongation factor (eEF2) leads to improvement in memory function and cognitive abilities.[56]

A novel, Curcumin-decorated nanophytosomes (Cur-NPhs) have been prepared to use photo-sonodynamic antimicrobial chemotherapy to decrease the pathogenicity of *Aggregatibacter actinomycetemcomitans*. The antimicrobial study of Cur-NPhs-PACT, including cell viability, biofilm killing, metabolic study, quorum-sensing-associated *qseB* and *qseC*, and biofilm-associated *rcpA* gene under blue laser irradiation, have been conducted. The appearance of a sphere-shaped vesicle and the self-closed structure of Cur-NPhs suggest a high drug-loading and an enhanced entrapment efficiency. The investigation demonstrated that the phytosome had an antimicrobial efficacy with substantial cuts in cell viability (13.6 $\log_{10}$ CFU/mL), biofilm degradation (65%), metabolic activity (89.6%), mRNA levels of pathogenic determinant genes.[104]

Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble molecule found in all cell membranes, responsible for electron transfer in the mitochondrial respiratory chain. The CoQ_{10} synthesis in humans decreases after the twenties, forming decreased tissue concentrations. The primary malfunction may be due to a defect in the gene encoding its biosynthesis and the development of severe abnormalities such as encephalomyopathy, cerebellar ataxia, and severe infantile multisystemic disease. The secondary malfunction may be due to dietary deficiency or medicines such as statins. Bergamini and Fato investigated the bioenergetic and antioxidative properties of a CoQ_{10} phytosome product

(UBIQSOME, UBQ) in 1407 and H9c2 cells. After incubating with UBQ, the cellular and mitochondrial content of CoQ10 and its redox state were examined. The UBQ upgraded the cellular bioenergetic parameters in *in vitro* methods. These findings indicate that CoQ_{10} administration causes a distinct intracellular disposition, preferring CoQ_{10} accumulation in fat droplets instead of mitochondria.[105]

Hatami investigated the cisplatin and glycyrrhizic acid-loaded phytosomal nanoparticles on colon and liver cancer (*in vitro* model). In combination with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and sonication, the thin-layer hydration approach proves to be a suitable method for generating nano phytosomes. Compared to non-pegylated nanophytosomes, pegylated nanophytosomes assume the shape of the sphere as per the SEM and TEM data, as confirmed by the particle size, zeta potential, and drug-loading efficiency. The cell lethality effect on DLD-1 and LIM-2405 shows that the pegylated nanophytosomes show enhanced cytotoxicity, and its IC_{50} value does found to be lower.[106]

One of the research works of Sbrini and companions observed the influence of Centella phytosome (CENTEVITA, provided by Indena S.P.A) on the cognitive performance through promoting Bdnf expression in the prefrontal cortex of male rats. Similarly, a novel object recognition test does perform to evaluate the cognitive performance, and eventually, it seems that increased performance does produce using Centella phytosome.[55]

Tripterine, also known as celastrol, is a herbal drug obtained from *Tripterygium wilfordii*, having biological activities such as antioxidant, antitumor anti-inflammatory, and immunoregulatory activities. It has known that selenium reduces the free radicals in the body and aids in relieving the symptoms of arthritis, such as joint pain reliever, reducing swelling, and reducing joint stiffness. In this research, selenium-deposited tripterine phytosomes were formulated and studied the synergistic effect of tripterine-loaded phytosome and selenium-deposited tripterine for arthritis intervention. *In vivo* anti-

Table 3. Phytosomes formulations under clinical trial: www.clinicaltrials.gov are the source of data, accessed on 17-12-2021.

Title	Conditions	Interventions	Study Design	Clinical Trial Phase and Number	Status	Study results	Ref.
Trial to Study the Adjuvant Benefits of Quercetin Phytosome in Patients With COVID-19	COVID-19	Drug: Standard COVID-19 care Dietary Supplement: Quercetin Phytosome (400 mg oral)	Randomized Intervention Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Treatment	Phase 3 NCT04578158	Completed	Under investigation	-
A Phase II Study to Assess Efficacy of Combined Treatment With Erlotinib (Tarceva) and Silybin-phytosome (Siliphos) in Patients With EGFR Mutant Lung Adenocarcinoma	Carcinoma, Non-Small-Cell Lung	Drug: Erlotinib Dietary Supplement: Silybin-phytosome	Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment	Phase 2 NCT02146118	Unknown	Under investigation	-
Artichoke and Bergamot Phytosome	Hyper-cholesterolemia	Dietary Supplement: Combined Bergamot phytosome and Artichoke leaf dry extract Combination Product: Placebo	Randomized Intervention Model: Parallel Assignment Masking: Triple (Participant, Care Provider, Investigator) Primary Purpose: Treatment	Not Applicable NCT04697121	Completed	Anticholesterolemic	-
Leucoselect Phytosome for Neoadjuvant Treatment of Early Stage Lung Cancer	Early Stage Lung Cancer (I and II)	Drug:leucoselect phytosome	Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment	Phase 2 NCT04515004	Not yet recruiting	Delay in planned surgery > 14 days	-

Table 3. Continued.

Effects of Greenselect Phytosome® on Weight Maintenance After Weight Loss in Obese Women	Obesity	Dietary Supplement: Globes® Dietary Supplement: Placebo	Randomized Intervention Model: Parallel Assignment Masking: Single (Participant) Primary Purpose: Treatment	Phase 4 NCT02542449	Completed	Maintaining weight subsequent weight loss	
The Effect of High-dose Silybin-phytosome in Men With Prostate Cancer	Prostate Cancer	Drug: Silibin-Phytosome	Non-Randomized Intervention Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Basic Science	Phase 2 NCT00487721	Completed	Enhanced concentration of silybin	
Effects of Short-term Curcumin and Multi-polyphenol Supplementation on the Anti-inflammatory Properties of HDL	Inflammation Atherosclerosis Cardiovascular Disease	Dietary Supplement: Polyresveratrol Supplementation Dietary Supplement: 500 mg of Curcumin phytosome twice daily for one week	Randomized Intervention Model: Crossover Assignment Masking: Single (Participant) Primary Purpose: Basic Science	Phase 2 NCT02998918	Unknown	Inflammation, cholesterol lowered	-
Assessment of the Anti-inflammatory Effects of Norflo Oro in Acute Relapses of HLA-B27 Associated Autoimmune Uveitis	Uveitis	Meriva (Norflo Oro is highly bioavailable curcumin complexed into phytosomes)	Randomized Interventional Masking: Quadruple	Early Phase 1	Active, not recruiting	The cell damage and inflammation reduction	-
Coenzyme Q10 phytosome (Ubiqsome)	Presenescent athletes	500 mg daily dose of CoQ10 phytosome for 30 days (50-65 years of age)	Randomized, intervention-controlled, single-center trial trial	-	-	Increased CoQ10 levels in plasma and muscle.	[107]
Curcumin Phytosome	Nonalcoholic fatty liver disease	Number of patients- 80 Dose 250 mg for two months	Randomized clinical trial	-	-	Fat in the liver and serum aspartate aminotransferase (AST) decreased	[108]
Anthocran® Phytosome®: Prevention of Recurring Urinary Infections and Symptoms after Catheterization	Recurrent-urinary tract infections (R-UTIs)	Anthocran® Phytosome® at the dose of 120 mg/day or 240 mg/day. Nitrofurantoin (50 mg three-times/daily) for 4weeks	64 healthy subjects were selected	-	-	The hematuria and urine bacterial contamination were decreased	[109]
The Effects of Propolis on Viral Respiratory Diseases	Nonstreptococcal and viral pharyngitis caused by paramyxo-viruses, rhinoviruses, adenoviruses	Propolisina® holding a 75 mg/ sachet of pure propolis	Open-label, retrospective, controlled clinical study	-	-	It decreased the symptoms such as sore throat, fever, and pharyngeal erythema.	[110]

arthritis effect of tripterine was studied using male rats. Tripterine-loaded phytosome has a less anti-inflammatory effect than selenium-deposited tripterine phytosomes, which decrease the thickness of synovial cell lining and a low degree of bone damage.[111]

Saudagar and colleagues developed and characterized the *Terminalia arjuna* phospholipid complex. A QbD-based approach uses a central composite design to systematically study the formulation's mutual effect and the production parameters such as the reaction temperature, entrapment efficiency, and phospholipid-drug ratio. The phytosome prepared using the solvent evaporation technique has shown good entrapment efficiency of 97.9 ± 0.4%. Also, different batches of the phytosome tablets have shown hardness, thickness, friability, and weight variation within the Pharmacopeial limits.[94] Table 3 represents the current status of phytosome research, including clinical trials.

Why the Constraints for Clinical Translation?

The vast applications of herbal drugs for various diseases emerge from nanosized herbal carriers' development to deliver actives efficiently. Moreover, the existing literature designates ongoing research on innovative drug dispositions. However, the obstacle in the preformulation and formulation studies, the clinical translation of phytosome into a product level, is missing. Investigators need to consider the drug disposition of herbal extracts to improve the phytoactive's therapeutic potential. The careful selection of phytoactive for complexation with phospholipids provide the development of phytosomes; thus, the compounds' anti-inflammatory, cardiovascular, and immunomodulatory effects do study. Conversely, this area's progress is stagnant in the preliminary stage.[112]

Moreover, several productions, stability, and therapeutic feasibility techniques need improvement over other conventional delivery systems. Also, replacing organic solvents with hydrophilic solvents such as ethanol helps to provide safety. Other problems faced by the researchers include the practical yield of the phytosome, the time required, the temperature, the preparation method used, and the difficulties in drying. These factors often affect the quality of the phytosome produced.

The stability of the phytosome is another limitation. The phospholipids, an essential component in phytosome production, affect oxidation, agglomeration, and chemical instability, providing instability in the final product. More research is needed to develop a stable phytosome, improving therapeutic efficacy. The pH susceptibility of phospholipids due to their zeta potential monitored through preparing phytosome formulations is the main disadvantage of phytosome formulation.[19] The formation of drug-lipid interaction at both surface and bulk leads to incompatibility, deteriorating the product. Moreover, chemical hydrolysis on preservation is another factor for the limited use.[23]

As a delivery channel, the phytosome is a good concept for improving the bioavailability of herbal actives and plant extracts. However, preparation for an industrial scale and clinical trial level is predictable in the upcoming years.

Conclusion

Phytosome is an advanced form of herbal drug delivery technology, capable of dramatically eliminating the drawbacks associated with conventional dosage forms, such as bioavailability issues, dose dumping, and site-specific delivery. Initially, this concept has used by the cosmetic industry. Nowadays, its importance as an herbal carrier has emerged in the pharmaceutical, pharmaceutical, and preservatives industry. Even though many herbal drugs have eliminated the root cause of the ailments, it is challenging to attain therapeutic efficacy. Thus, the small-sized phyto-phospholipid complex delivered the active moiety directly to the required area and acted with minimal side effects, often associated with a synthetic drug. Phospholipid forms a complex with the phytoactive constituent and protects the active by forming a bond, thus helping deliver lipophilic and hydrophilic constituents with nourishment to the membrane. The formulation technique for the phytosome is easy and can quickly scale up to a commercial level. It is often used for oral and topical drug administration and will produce better drug entrapment, improving stability.

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Author Contributions

Vijayakumari Mahadevan Hari Priya: Investigation, Writing - Original Draft. Alaganandam Kumaran: Conceptualization, Supervision, Writing - Review & Editing.

Conflict of Interest

The authors report no conflicts of interest.

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