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PHYTOSOMES, A NOVEL STRATEGY TO IMPROVE THE BIOAVAILABILITY OF FLAVONOIDS: A REVIEW

[1*]Pankaj Sharma, [2]Lakshita Dogra, [3]Diksha Thakur, [4]Oshina, [5]Smily and [6]Dolly Chauhan

Government College of Pharmacy, Rohru, Distt-Shimla Himachal Pradesh-171207, India.

***Corresponding Author: Pankaj Sharma**
Government College of Pharmacy, Rohru, Distt-Shimla Himachal Pradesh-171207, India.

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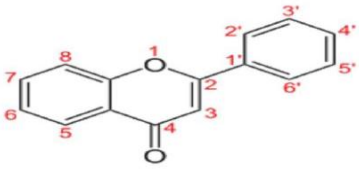
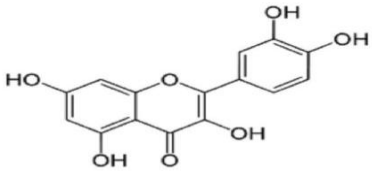
ABSTRACT
Phytosome are the small cell like structure which serve as a intermediate between conventional and novel drug delivery system. Phytosome are able to carry drug themselves from to the hydrophilic to the lipophiliic environment of the cell membrane, which ultimately reaches to blood. The hydrophilic constituent has find their application in the various disorder treatment such as skin disorder, anti-ageing process, antimicrobial, antitumor which cannot be treated with conventional delivery system and hence transformed into phytosomes. Phytosome complex is to be prepared by mixing of polyphenolic constituents and phosphoatidylcholine in molar ratio. Phytosome are superior to the conventional drug delivery system in term of pharmacokinetics and pharmacodynamic properties. Phytosomal delivery of extract of silybin, grape seed, hawthorn, centella, olive oil etc. has been profitable use. Phytosomes have been refined the therapeutic uses like hepatoprotective, cardioprotective, antihypertensive, anti-inflammatory, vein and skin disorder etc. or for preventive of health reasons. In the ever-expanding horizon, phytosome as a modern technology increasing for drug bioavailability as well as efficiency of drug delivery system.

KEYWORDS: Phytosomes, flavonoids, bioavailability, phospholipids.

INTRODUCTION
Flavonoids are to be isolated from botanical origin used to treat various diseases since our past history. Flavonoids extracted from ginkobiloba prepared as a ginko phytosome[1] has been used Anti-aging, Protects brains & vascular lining. Polyphenol from turmeric plant prepared as a curcumin phytosomes[2] which is to be reported to exhibit improved antioxidant, anticancer properties. Moreover, a number of the foremost wide studied active constituents are polyphenols, like flavonoids, terpenoids, and phenolics.[3] However, flavonoids containing active constituents extracted from botanical origin are poorly absorbed once administered orally, that limits their widespread uses. The poor absorption of these compounds results from two properties. First, more than one ring structures of polyphenols (containing flavonoids) are too large in size to be absorbed by passive diffusion or non-energy dependent absorption.[4] Second, the poor water or macromolecule (Lipids) solubility of those compounds prevents them from passing across the outer membrane of GIT cells. Active constituents extracted from natural plants are shown to exhibit strong in vitro medical specialty effects, however poor in vivo absorption.[5] A number of preparation are developed to resolve the matter of poor absorption, like the preparation of emulsions, micro-emulsion, liposomes, and nanoparticles, niosome as well as the modification of chemical structures and delivery as prodrugs. Among the potential strategies lipid-drug complexes referred to as phytosomes have emerged as a promising strategy to bolster the bioavailability of active drug complexes. Phytosome are ready by complexing active constituents at outlined molar ratios with phospholipids beneath bound conditions. Amphipathic phospholipids principally act as "ushers" of flavonoids to assist them go through the outer membrane of duct cells, eventually reaching the blood. After forming lipoid complexes, the membrane porosity (liphophilicity) and oil–water partition constant of constituents are increased. Thus, lipid-drug complexes are additional without delay absorbed and generate higher bioavailability compared to free active constituents. Encouragingly, the method of lipid-drug complexes has reduce the problem of poor bioavailability of many flavonoids like Flavones, Quercetin, Flavanones, Catechins, Anthocyanins & Cyaniding etc.[6,7] Therefore, the development of lipid-drug complexes has now a days received much more attention.

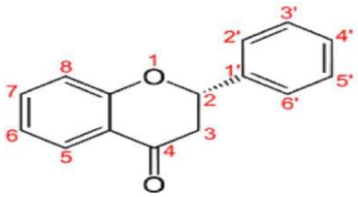
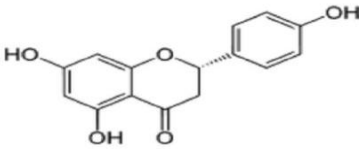
CHEMISTRY OF FLAVONOIDS

One of the largest categories of present polyphenolic compounds are the flavonoids.[8] This cluster of plant pigments is essentially answerable for the colour of the many fruits and flowera and over four thousand flavonoids compounds are characterised and classified per chemical structure.[9] The word flavonoids origin from the latin term flavus which implies yellow; but some flavonoids are redish, blue, purple or white. Chemically they are C_6 - C_3 -C_6 compounds in which the two C_6 groups are substituted benzene rings, and the C_3 is an aliphatic chain which contain a pyran ring. Flavonoids occur as O or C-glycosides or in free state as aglycone with hydroxyl or methoxy groups present on the aglycone.[10] Flavonoids are often divided into numerous categories on the idea of their molecular structure. The flavonoids are also divided into seven types: flavones, flavonols, flavononones, chalcones, xanthones, isoflavones and biflavones.[11] The molecular structure of four main type of flavonoids together with the foremost effective far-framed member of each type is as follow: flavones are defined by planar structure due to because the covalent bond within the central aromatic ring.[12] One in each of the foremost effective described flavonoids, quercetin is member of this type.

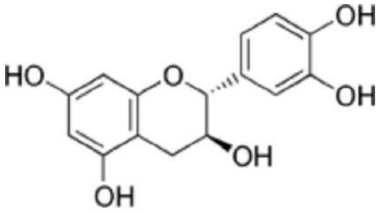
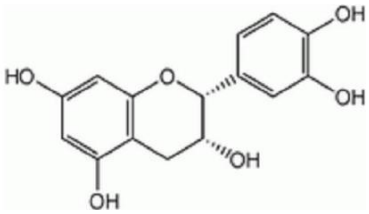



Flavone Quercetin

The second group is the flavononones; narigin is the example of this group of flavonoids.

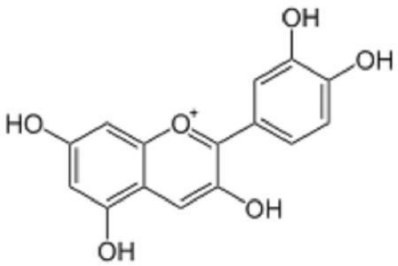



Flavanones Naringenin

Catechins Epi-cathechins

The third group is the catechins, epicatechins is the example of this group of flavonoids.

Anthocyanins Cyanidin

The fourth group is the anthocyanins, cyanidin is the example of this group of flavonoids

Flavonoids are shown to be smart categorisation markers for family Asteraceae. Flavonoids have largest structural diversity and are isolated in good scale from family Asteraceae species. They'll be used as categorization markers at lower hierachial levels.[13] Sometimes terpenes, edusmocoides, ginsenoside, flavonoids, epigallocatechi-3-o-gallate, procyanidins, flavones polyphenols are crucial candidates of phytosome. Relative lipophilicity and capability constant K, hydroxylation pattern of C_2-C_3 is taken into thought for final selection of most acceptable biomolecule as phytosome. These distinctive chemical characteristic and structure of flavonoids produce major challenge for the employment of them in higher

absorption through tissues. The employment of phytosomes is also a unique formulation herbal technology that helps to reduce most of the problems arises in pharmacodynamics and bioavailability of medication.[14]

STRUCTURE OF PHYTOSOME

Phytosomes are developed by interactions between active plant constituents and also the polar head of phospholipids.[15] Interactions between active plant constituents Associate with phospholipids modify phospholipid complexes to be an integral half within which the phospholipids head cluster is anchored; however the two long carboxylic acid chains don't participate in complexes formation. The two long carboxylic acid chains will move and encapsulate the polar a part of complexes to make a lipotropic surface. Phytosomes complexes type agglomerates once diluted in water, that they resemble small cell that shows some similarity to liposomes; the variations between liposomes and phytosome[16] complexes are shown in Fig. 1. As are often seen from Fig. 1, the largest distinction between phytosomes and liposomes is that, in liposomes, the active ingredient is distributed within the medium contained the cavity or within the layers of the membrane, whereas in phytosomes, it's Associate with integral a part of the membrane, being the molecules stabled through hydrogen bonds to the polar head of the phospholipids.

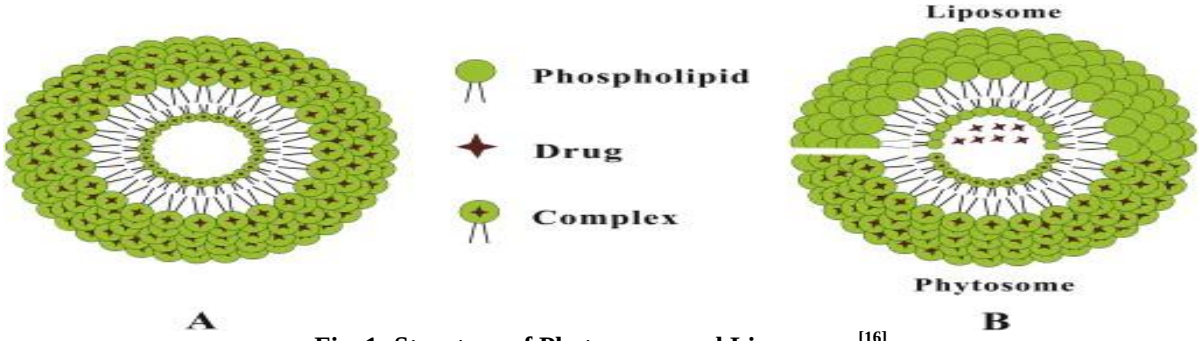


Fig. 1: Structure of Phytosomes and Liposomes.[16]

Liposomes are closed vesicles fashioned by phospholipids bilayers which will encapsulate compounds among associate binary compound compartment of multiple lipoids bilayers however do-not combine with compounds phytosome.

FORMULATION COMPONENTS

Bombardelli[17] projected that phyto-phospholipid complexes may be created from the reaction of phospholipids at a ratio quantitative relation with active constituents that are extracted from plants. Supported later studies, this first description of phytosomes has been challenged. As per the literature, we've projected an updated list of the four essential parts needed: phospholipids, phyto-active constituents, solvents, and therefore the ratio quantitative relation concerned within the formation of phytosomes.

1). Phospholipids

Egg yolk and plant seeds are the most common natural source of phospholipid.[18] Currently, industrially prepared phpholipids are available in market. Phospholipids can be divided into glycerophopholipids and sphingomyelins depending on the backbone. additionally glcerophopholipids include phosphatidyl choline (PC), Phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), Phophatidylinositol (PI) and phosphatidylglycerol (PG), Phosphatidyl choline (PC), Phosphatidylethanolamine (PE), and Phosphatidylserine(PS) are the major phospholipids used to prepare complexes that are composed of a hydrophilic head group and two hydrophobic hydrocarbon chains. Among these phospholipids, PC (phosphatidyl choline) is the most frequently used to prepare phospholipid complexes. The structure of the Phosphatidyl choline (PC) is as follows **(Fig. 2)**. The benefits of phosphatidyl choline PC include its amphipathic properties that give it moderate solubility in water and lipid media. Moreover, Phosphatidyl choline (PC) is an essential component of cell membranes, and accordingly it exhibits robust biocompatibility and low toxicity. Phosphatidyl choline (PC) molecules exhibit hepatoprotective activities, and have been reported to show clinical effects in the treatment of liver diseases, such as hepatitis, fatty liver, and hepatocirrhosis. Phospholipids in phytosomes formulation is used as a vehicle creating component.

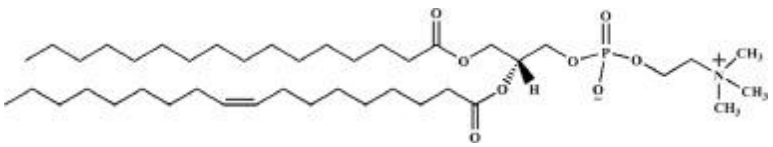
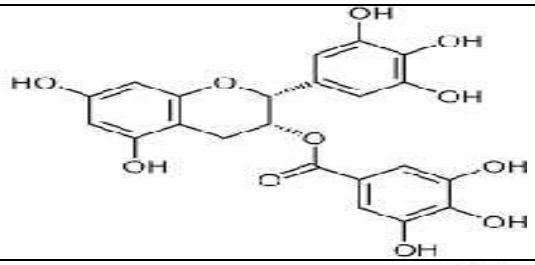
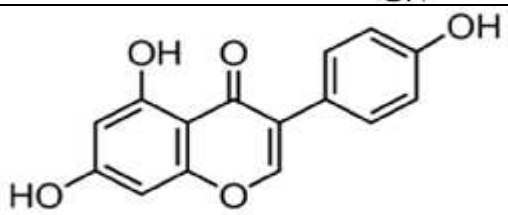
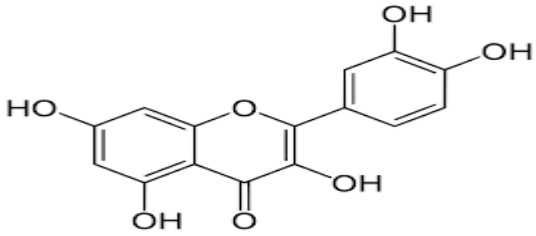
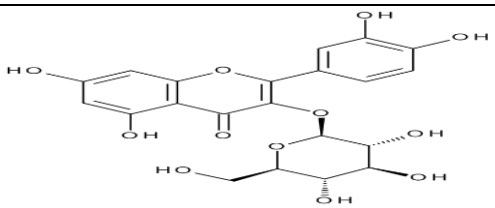
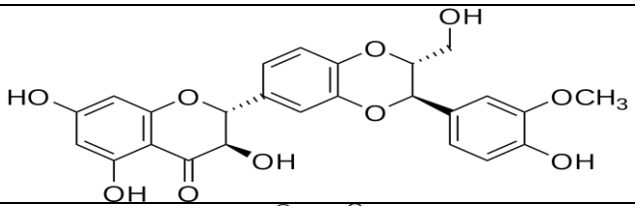
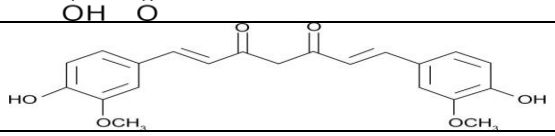
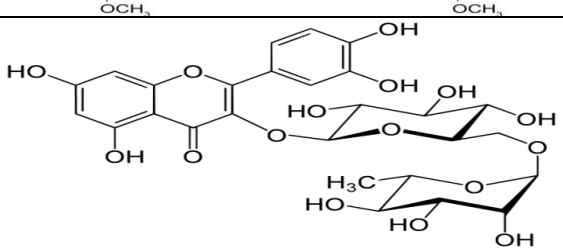


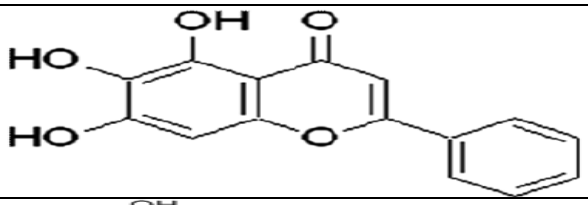
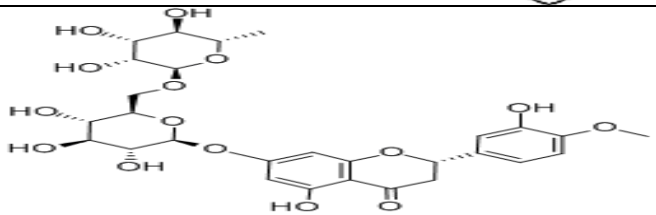
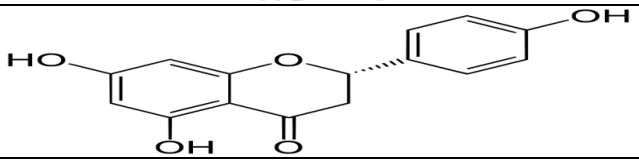
Fig. 2: Structure of phosphoatidylcholine (PC).

2). Phyto-active constituents

The active constituents of herbal extracts identified by researchers are generally defined based on robust in vitro pharmacological effects, rather than on in vivo activities. Most of these compounds are flavonoids. Some of the flavonoids structural drugs are shown below (Fig. 3). Some of the biologically active flavonoids constituents of plants show affinity for the aqueous phase and cannot pass through biological membranes, such as quercetin, cathechin & silibinin etc. By contrast, others have high lipophilic properties and cannot dissolve in aqueous gastrointestinal fluids, such as curcumin and rutin. Phyto-phospholipid complexes cannot only improve the solubility of lipophilic flavonoids in aqueous phase but also the membrane penetrability of hydrophilic ones from aqueous phase. Furthermore, the production of complexes can protect flavonoids from destruction by external forces, such as hydrolysis, photolysis, and oxidation.

Some of flavonoids (Phyto-active constituents) used in phytosome preparation (Mukherjee 2001).[19]

S. no.	Flavonoids	Structure
1.	Epigallocatechin- 3-gallate (EGCG)	
2.	Genistein	
3.	Quercetin	
4.	Isoquercetin	
5.	Silibinin	
6.	Curcumin	
7.	Rutin	

8.	Baicalein	
9.	Hesperidin	
10.	Naringenin	

3). Solvents

Different solvents have been utilized by different researchers as the reaction medium for formulating phytosome complexes. Traditionally, aprotic solvents, such as aromatic hydrocarbons, halogen derivatives, methylene chloride, ethyl acetate, or cyclic ethers etc. have been used to prepare phyto-phospholipid complexes but they have been largely replaced by protonic solvents like ethanol. Indeed, protonic solvents, such as ethanol and methanol, have been more recently been successfully utilized to prepare phospholipid complexes. For example, Xiao prepared silybin-phospholipid complexes using ethanol as a protonic solvent; subsequently, protonic solvent was removed under vacuum at 40°C.[20] Various types of solvents have been successfully studied. When the yield of phospholipid complexes is sufficiently high, ethanol can be a useful and popular solvent that leaves fewer residues residual and causes minimal damage. Some liposomal drug complexes operate in the presence of water or buffer solution, where the phytosomes interact with a solvent with a reduced dielectric constant. Recently, many studies have used the supercritical fluid (SCF) process to control the size, shape, and morphology of the material of interest.[21] Supercritical anti solvent process (SAS) is one of the SCF technologies that are becoming a promising technique to produce micronic and sub-Micronics particles with controlled size and size distribution. In this technique, a supercritical fluid (usually CO_2) will be chosen as an anti-solvent to reduce the solute's solubility in the solvent.

4). Stoichiometric ratio of active constituents and phospholipids

Normally, phyto-phospholipid complexes are prepared by reacting a synthetic or natural phospholipid with the active constituents in a molar ratio ranging from 0.5 to 2.0. Whereas, a stoichiometric ratio of 1:1 is considered to be the most efficient ratio for preparing phytosome complexes.[22] For example, quercetin-phospholipid complexes were prepared by mixing Lipoid S 100 and quercetin at a molar ratio of 1:1. However, different stoichiometric ratios of active constituents and phospholipids have been used. Maryana et al. prepared silymarin-phospholipid complexes with different stoichiometric ratios of 1:5, 1:10, and 1:15; they found that the complexes with a stoichiometric ratio of 1:5 showed the best physical properties and the highest loading capacity of 12.18% ± 0.30%. Yue et al.[23] conducted a comparative study using the stoichiometric ratios of 1:1, 1.4:1, 2:1, 2.6:1, and 3:1 to generate oxymatrine-phospholipid complexes; they determined that optimal quantity was obtained at a ratio of 3:1. Therefore, a stoichiometric ratio of 1:1 is not always optimal for the formulation of phospholipid complexes. For different types of drugs, we should experimentally adjust the stoichiometric ratio of active constituents and phospholipids according to distinct purposes, such as the highest drug loading.

Mislllaneous agent

Buffering agent used to maintained the P^H of the preparation. The commonly used buffering agent are saline phosphate buffer (p^H 6.5) 7%V/V and ethanol Tris buffer (p^H 6.5). It is used as a hydrating medium. The dye is to be used in the phytosome formulation Are Rhodomine-123, Rhodamine-DHPE, Flourescien-DHPE, Nile-red 6, caboxy fluorescence. It is used for CSLM Study.

INTRACTIONS BETWEEN ACTIVE CONSTITUENTS AND PHOSPHOLIPIDS

In 1989, Bombardelli[24] reported a chemical bond between a flavonoid molecule and a phospholipidmolecule. In the past, there was controversy about the formation of phyto-phospholipid complexes. A study on the interaction of the 20(S)-protopanaxadiol phospholipid complexes at the molecular level using molecular docking showed that a hydrogen bond formed between one of the –OH group in 20(S)-protopanaxadiol and the —P═O group in the phospholipids[25] (figure 4). Phyto-phospholipid

complexes, which empolyed by reaction of stoichiometric amount of phospholipids and the phytoconstituents complex, are revealed by the spectroscopic data that the phopspholipid – active ingredient interaction is due to the formation of hydrogen bond between the polar head and the polar functionalities of the active ingredient. The ^{1}HNMR and ^{13}CNMR data show that, the signal of the fatty chain has not changed both in free phospholipids and in the complex, which suggested that long aliphatic chains are wrapped around the active principle, producing lipophilic envelope. The same conclusion can also be drawn from the thermal analysis in other studies that, the interaction between the two molecules had been attributed to formation of hydrogen bond or hydrophobic interaction.

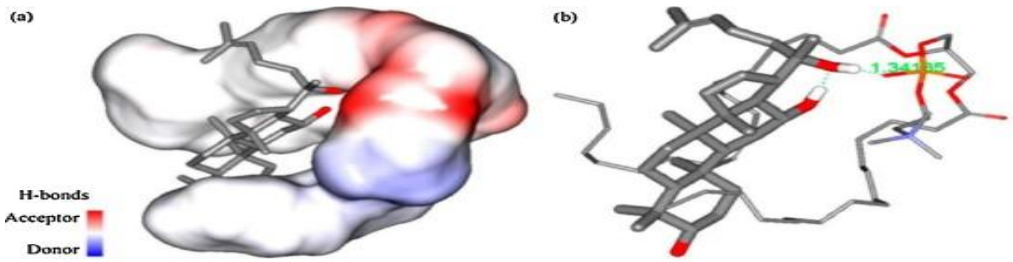
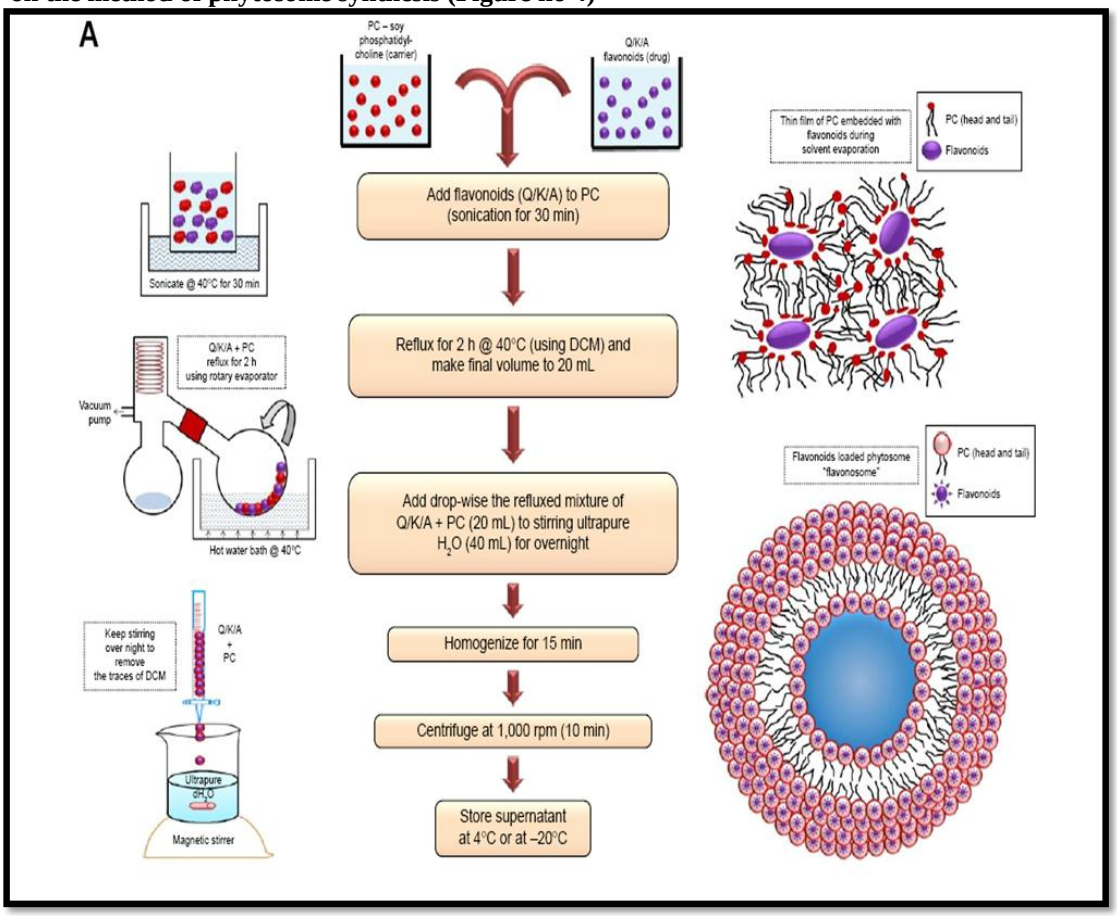


Figure 3: Interaction of the 20(S)-protopanaxadiol phospholipid complexes.[25]

In summary, most researchers agree that interactions between active constituents and phospholipids occur via hydrogen bonds to generate intermolecular force rather than chemical or hybrid bonds.

Review on the method of phytosome synthesis (Figure no 4)



Schematic representations (A) of conventional phytosome synthesis including thin-film formation and typical phytosome structure.[26]

METHOD FOR THE PREPERATION OF PHYTOSOME COMPLEXES

There are three primary methods for preparation of phyto-phospholipid complexes, including solvent evaporation, mechanical dispersion method, and anti-solvent precipitation. Solvent evaporation is a traditional and frequently used method for preparing phospholipid complexes. Shan and colleagues[27] applied the solvent

evaporation method to prepare oleanolic acid-phospholipid complexes. Yu et al[28] prepared a berberine-phospholipid complexes (P-BER) by a rapid solvent evaporation method followed by a self-assembly technique, for the sake of developing a more efficient berberine drug delivery system. Briefly, discuss about the these three method is as follow in mechanical dispersion method[29] the working principle is as follow Phytosome Complex can be prepared by mechanical dispersion method. 100 mg weighed soy lecithin was dissolved in 2 ml of diethyl ether in beaker and put into bath sonicator. 50 mg of drug was dissolved in 20 ml double distilled water and this solution was added drop by drop into the beaker containing soy lecithin while sonicating and then left for 15 min for further sonication. The resultant formulation was kept in refrigerator. in second method is to be a anti-solvent precipitation method[30] is as follow the Specific amount of drug and phospholipid were refluxed with suitable solvent. The mixture so formed was concentrated and another solvent was then added for precipitation with continuous stirring. Precipitates thus formed were then filtered and collected and stored in vacuum desiccators overnight. The third method used is solvent evaporation method[31-32] the procedure of the method is as follow The specific amount of phyto-constituent and soya lecithin were taken into a round bottom flask and refluxed with acetone at a temperature 50 – 60^{o}C for 2 h. Precipitates thus formed were then filtered and collected and stored in vacuum desiccators overnight. Out of these, some methods described in various studies are discussed here. Li et al.[33] developed supercritical antisolvent precipitation to produce puerarin phospholipid complexes and proposed that supercritical fluids were superior to conventional methods for drug phospholipid complexes preparation. Damle and Mallya[34] used Salting-Out Method and Film Formation Method to prepared a phyto-phospholipid complexes.

Factors influencing the phyto-phospholipid complexes

The factors that influence the formation of phyto-phospholipid complexes are mainly included solvent, stoichiometric ratio of active constituents, reaction temperature and reaction time. Depending on the desired target, different process variables can be selected. For maximum yield, Saoji et al.[35] studied the influence of process variables such as the phospholipid-to-drug ratio, the reaction temperature and the reaction time, and used a central composite design to acquire the optimal formulation. For best solubility and skin permeation, Das and Kalita[36] prepared a rutin phytosome in different stoichiometric ratios. According to a recent report, by changing stoichiometric ratios and reaction temperature, the highest yield apigenin-phospholipid complexes are prepared by Telange and his colleagues.[37]

Yield (complexation rate) of phyto-phospholipid complexes

The yield of active constituents in complex with phospholipids represents an extremely important index for prescription screening. The weight difference between the initial active constituent and free compounds is the amount of active constituent in complex with phospholipids. The formula is as follows:

Yield (%) = [(a−b)/a]×100%

Where "*a*" is the weight or content of initial active constituent, "*b*" is the weight or content of free active constituent, and "(*a* – *b*)" is the weight or content of the phospholipid complexes.[38] Based on the properties of the active constituents, high-performance liquid chromatography (HPLC) or ultraviolet spectrophotometry can also be used to calculate the yield. The choice of solvent, temperature, duration, drug concentration, and stoichiometric ratio of active constituents to phospholipids are the main factors that affect the yield of phospholipid complexes.

A. Characterization of phyto-phospholipid complexes

1) Solubility studies

Determinations of solubility characteristics of drug (methanolic extract), physical mixture of drug & phospholipid and phytosomes were obtained by adding excess of the samples to 10 ml of water and n-octanol in sealed glass container at room temperature.[39] The liquids were shaken for 24 h and centrifuged at 5000 rpm for 10 min. The supernatant was filtered, and the concentration of drug in water and n-octanol was determined spectrophotometrically using UV double beam after appropriate dilutions.

2) Determination of partition coefficient[40-41]

The apparent partition coefficients were measured by the shake-flask method. In this method the two phases were mutually saturated before use. Equal volumes of water and n-octanol containing pure drug and phospholipid complex were mixed in the different volumetric flask and equilibrated under constant shaking at 37°C for 24 h. Both phases were then separated by using separating funnel and the concentration of drug was determined spectrophotometrically using UV double beam after appropriate dilutions. The partition coefficients were determined by using following equation: **Partition coef icient (P) = concentration of drug in n − octanol/ concentration of drug in water**

3) Particle size and zeta potential

Particle size and zeta potential are important properties of complexes that are related to stability and reproducibility. In general, the average phospholipid complexes particle size ranged from 50 nm to 100 µm. Mazumder[42] prepared sinigrin phytosome complexes, and the average particle size and zeta potential of the complex were 153 ± 39 nm and 10.09 ± 0.98 mV, respectively.

4) Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

SEM has yielded important insights into the solid state properties and surface morphology of complexes. TEM is often used to study the crystallization and dispersion of nano-materials and to measure the particle size of nano-particles. SEM has shown that active compounds can be visualized in a highly crystalline state, but the shaped crystals disappeared after complexation. When diluted in distilled water under slight shaking, TEM showed that phyto-phospholipid complexes exhibit vesicle-like structures.

5) Drug content

Phytosomes equivalent to 10 mg of drug was accurately weighed and taken into a 100 ml volumetric flask. The contents of the flask was dissolved in small quantity of methanol and sonicated for 30 minute. Volume was adjusted to 100 ml with methanol. Contents of the flask were filtered and drug content was determined by the spectrophotometrically using UV double beam after appropriate dilutions.[43]

B) Structural verification of phyto-phospholipid complexes

1) Ultraviolet spectra (UV-spectra)

Samples that reflect different absorption in the UV wavelength range can be used to characterize own structural properties Most studies have revealed no differences in the UV absorption characteristics of constituents before and after complexation. Xu et al.[44] prepared luteolin-phospholipid complexes and found that the characteristic peaks of luteolin remained present Therefore, we conclude that the chromophores of compounds are not affected by being complexed with phospholipids.

2) Differential scanning calorimetry (DSC)

In DSC, interactions can be observed by comparing the transition temperature[35], appearance of new peaks, disappearance of original peaks, melting points, and changes in the relative peaks area. Phyto-phospholipid complexes usually display radically different characteristic peaks compared to those of a physical mixture. It is assumed that, in addition to the two fatty chains of phospholipids, strong interactions occur in the active ingredients and the polar part of phospholipids also inhibits free rotation. Das and Kalita[45] prepared phyto-phospholipid complexes that contained rutin and the resulting DSC thermogram showed two characteristic peaks that were lower than that of the physical mixture and the peaks of rutin and PC disappeared.

3) Fourier transform infrared spectroscopy (FTIR)[46]

FTIR is a powerful method for structural analysis, and yields different functional groups that show distinct characteristics in band number, position, shape, and intensity. The formation of phyto-phospholipid complexes can be verified by comparing the spectroscopy of phospholipid complexes to that of physical mixtures. Separate studies may show different results. Indeed, Das and Kalita[43] 5prepared phyto-phospholipid complexes composed of rutin. The FTIR of a physical mixture of rutin and phyto-phospholipid complexes was superimposable with that of pure rutin . When Mazumder et al[42] prepared sinigrin-phytosome complexes, the FTIR of phytosome complex showed different peaks from that of sinigrin, phospholipids and their mechanical mixtures.

4) X-ray diffraction

Currently, X-ray diffraction is an effective method to examine the microstructure of both crystal materials and some amorphous materials. X-ray diffraction[47] is usually performed on either active constituents or active constituent phyto-phospholipid complexes, PCs and their physical mixtures. X-ray diffraction of an active constituent and physical mixture shows intense crystalline peaks that indicate a high crystal form. By contrast, active constituent phyto-phospholipid complexes do not exhibit crystalline peak, which suggests that the constituents in complex with phospholipids exhibit a molecular or amorphous form. That may account for the observation that phyto-phospholipid complexes have better lipophilicity and hydrophilicity than active constituents.

5) Nuclear magnetic resonance (NMR)

The ^{1}H NMR and ^{13}C NMR techniques[48] have major application in the identification of the structures of the complexes. As noted above, interactions between flavonoids and phospholipids are created by hydrogen bonds rather than chemical bonds. Angelico et al.[49] established based on NMR data that hydrogen bonds can form between some polar phenolic functional groups of silybin A and phospholipids. The spectra of different phyto-phospholipid complexes suggest that the hydrophobic side of lipids can act to cover the envelope on the central choline-bioactive parts of these complexes.

CONCLUSIONS

Phospholipids show affinity for active constituents through hydrogen bond interactions. In theory, a phospholipid complex strategy should be suitable for any active molecule not limited to flavonoids. With advances in research, we update the newest advances in phospholipids, phyto-active constituents, solvents, and stoichiometric ratios that are essential to prepare phospholipid complexes. The characterization and structural verification of phospholipid complexes has been well established. Bioavailability can be significantly improved with the help of phospholipids compared with chemically equivalent non-complexed forms. The potential of phyto-phospholipid complexes, with the effort of clinicians and other researchers, has a bright future for applications in the pharmaceutical field.

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