International Journal of Pharmacognosy and Pharmaceutical Research
www.pharmacognosyjournals.com
Online ISSN: 2664-7176, Print ISSN: 2664-7168
Received: 17-09-2021, Accepted: 01-10-2021, Published: 18-10-2021
Volume 3, Issue 1, 2021, Page No. 26-33

Phytosomes: An advance technique for herbal preparation

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Abstract
Now a days, Novel Drug Delivery System is most commonly used technique for the optimum understanding of Pharmacokinetic and Pharmacodynamic of any Drug. NDDS is a kind of system through which pharmaceutical compound is transported to the targeted location at right time in a required amount for optimum result. Phytosome is Novel Drug Delivery System results from the reaction of phospholipid and natural active ingredient which are extracted from natural sources. Phytosomes are used to increase the absorption of any drug especially lipid in-soluble drug orally as well as topically. Phytosomes are used to encapsulate various drug complexes and naturally isolated nutraceutical such as polyphenolic compounds, flavonoid and many more.

Keywords: novel drug delivery system, conventional drug delivery system, phyto-constituent, phytosomes, phospholipid, stoichiometric ratio

Introduction

Novel Drug Delivery System (NDDS) is a kind of approach, system or technique used to overcome the limitations faced by Conventional Drug Delivery system (CDDS). NDDS is a technique used to deliver API (active pharmaceutical ingredient) to the targeted organ or targeted area safely to achieve the desired result despite of any limitations faced by CDDS [1]. NDDS formulation is based on two different basic parameters such as route of entry and dosage form. Based on these parameters NDDS is categorized into large family such as control release drug delivery system, Targeted drug delivery system, Rate pre-programmed drug delivery system and Localized drug delivery device. If NDDS is applied to herbal medicines (that is an extracted pharmaceutical compound from natural sources) can enhance the efficacy and potency of drug. For such enhancement a new technique was merged with herbal medicine such as nanoparticle, micro emulsion, phytosomes, matrix system, liposomes and so on. Phytosomes is one of the widely used NDDS especially design to deliver Herbal Medicines [2]. Phytosomes seems to improve pharmacological parameter of phyto-constituent which amplify the bioavailability of them [10]
Phytosomes also has shown vast demand in cosmeceutical industry i.e. in cosmetic preparation. They are mainly used in preparation of lotions, ointment, sunscreen and many more as they increase the absorption of phyto-constituent through skin [9]. Hence by combining the magnifying and emulsifying action of phospholipid along with phyto-constituent provide dramatically enhanced bioavailability and improve absorption through skin [9]. Therefore natural molecules with the help of phytosomes can be used to improve complexity of skin or to nourish the skin. So, phytosomes are used topically as well as orally for various purpose [13].

What is phytosomes?

Phytosomes is a modern or advanced technique under NDDS which is used to enhance the bioavailability of constituent extracted from plant origin. Phytosomes act as phospholipid complex in stoichiometric ratio between phyto-constituent and soy lecithin compound such as phosphotidylcholine, phosphotidylethanolamine and phophotidylserine [5].
Where, Phyto stands for plant like and some stands for cell like [3]. This indicates that phytosomes are small cell-like structure composed of water soluble phyto-active constituent and phosphatidylcholine. Phytosomes are lipid based vesicle [3]. Phytosomes are unique NDDS in which there is conjugation between lipid vesicle and hydrophilic herbal extract. It means phytosomes are used for encapsulation of hydrophilic phyto-constituent.

What are Phyto-constituent?

Phyto-constituents may has poor bioavailability limited to their clinical usage [12]. Hence, by encapsulating these constituent bioavailability of such phyto-constituent can be enhanced. Many active phyto-constituent extracted from plant has wide application but are being limited because they lack in absorptive property as well as they are available in improper dosage form. There are two main causes behind such limitations, first- structure of constituent is so large to get absorb through cell membrane by passive diffusion these, phyto-constituents can be made into proper use by converting them into required appropriate dosage form. Second- poor lipid solubility these, Water soluble phyto-constituent can be converted to lipid soluble constituent with help of lipid complex phytosomes [4]. Water soluble phyto-constituent such as Flavonoid, Glycosides, Terpenoids, and Phenolic compounds can be destructed by gastric secretion and micro-flora of gut. This destruction can be prevented by encapsulating such phyto-constituent with phospholipid complex phytosomes [4].

Phospholipid complexes

Amphipathic phospholipids such as phytosomes shows activeness for both components i.e. for hydrophobic as well as

hydrophilic. Hence, they act as 'USHER' of main active phyto-constituent by helping them to penetrate through bilayer cell membrane or outer membrane of gastrointestinal cell to reach the blood stream so, as to exhibit their required therapeutic action [4].

Formation of phospholipid complexes can improve the oil-water partition coefficient and membrane permeability [13].

Structure of phyto-phospholipid complex

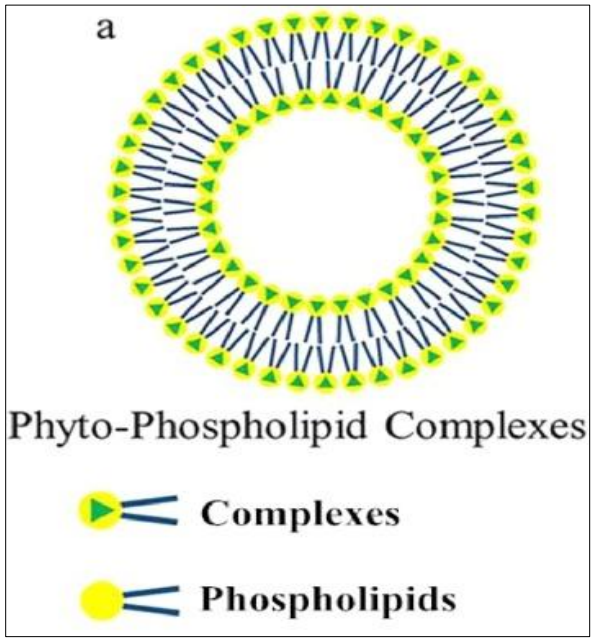

Fig 1

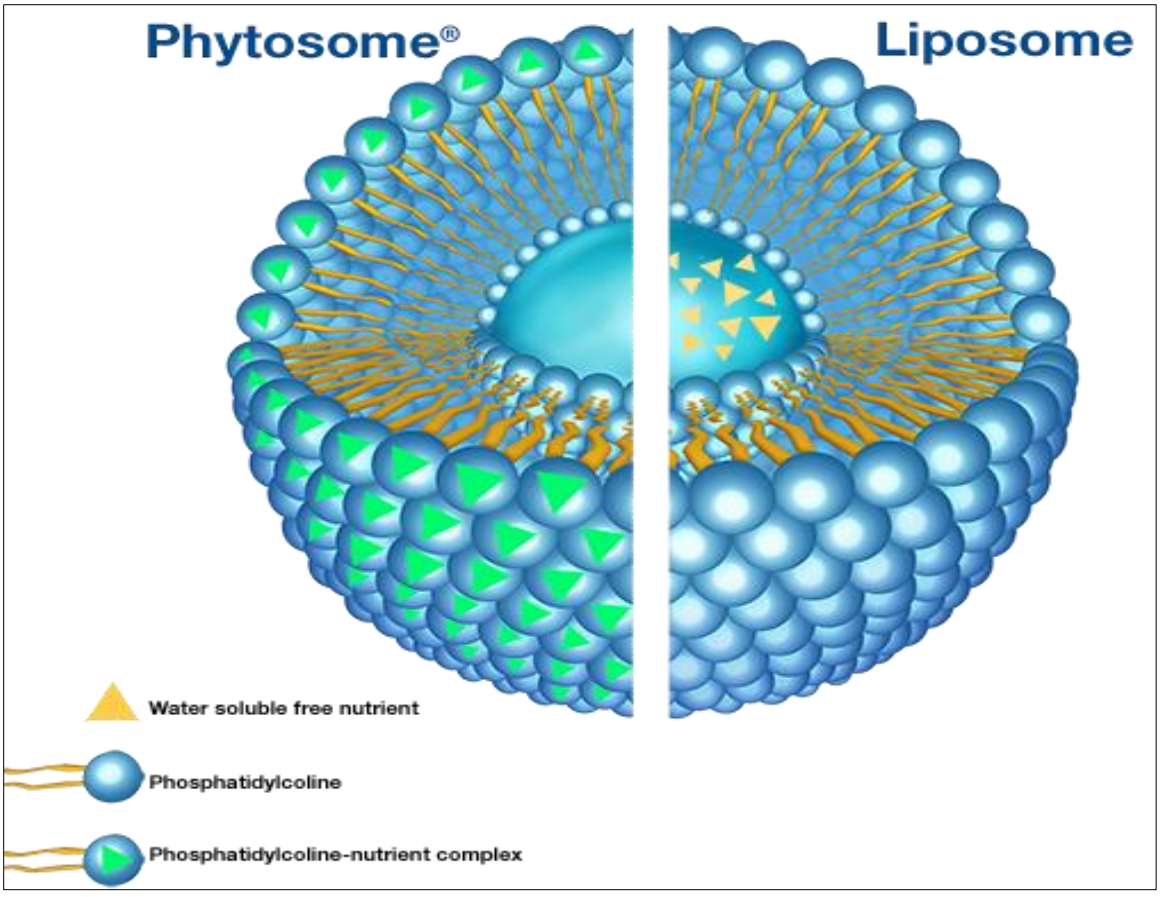

Fig 2

This structural elucidation predicts that there is chemical interaction forming hydrogen bond between polar sides of the constituents i.e. phospholipid and phyto-constituent via specific pattern [5] which leads to phyto phospholipids complex which is an integral part of phytosomes. Phospholipids polar head is anchored but the non-polar tail group not participate in complex formation. This two long fatty acid chains can move and encapsulates the polar part of complex to form the lipophilic surface [17]. Fig. 1: Structure of phyto-phospholipid complex [6].

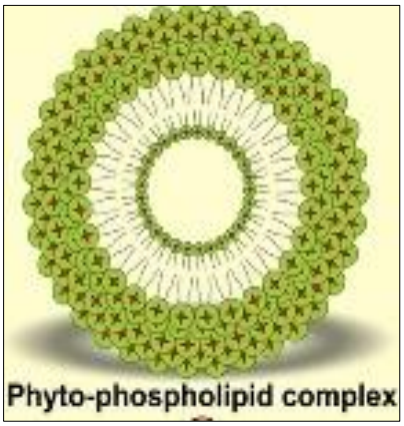

Fig 3: Phyto-phospholipid complex [33].

Phospholipids used in for Phyto-phospholipid complex preparation are given below

Phospholipids can be divided into Glycerophospholipids and Sphingolipids depending on the backbone.

1. **Glycerophospholipids Include:** Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylserine (PS), Phosphatidic acid (PA), Phosphatidylinositol (PI), Phosphatidylglycerol (PG).
2. **Sphingolpids Include:** Ceramide phosphorylcholine (Sphingomyeline), Ceramide phosphorylethanolamine and Ceramide phosphorylipids [34].

Solvents used for Phyto-phospholipid complex preparation

1. Different types of solvents have been utilized as reaction medium i.e. as mediator for preparing phyto-phospholipid complexes.
2. Previously, aprotonic solvents such as aromatic-hydrocarbons, halogen derivatives, methylene chloride, ethyl-acetate or cyclic ethers any many such solvents have been used to prepare phyto-phospholipid complexes but they have been largely replaced by protonic solvents like ethanol, methanol and etc., [26, 25] because this solvents are readily available and much harmless as compare to other solvents. This protonic solvents are even easily removed under vacuum at 40°C [25]
3. Different types of solvents were successfully studied. When the yield of phospholipid complexes is sufficiently high, ethanol can be a useful and popular solvent that seems leave less residue and cause negligible damage [27, 25].
4. Some Phyto-phospholipid complexes works efficiently in the presence of water or buffer solution, where the phytosomes interact with a solvent with a reduced dielectric constant [25, 27].

Stoichiometric ratio of Phyto-constituent and phytosomes

1. Phyto-Phospholipid complex are formulated by reacting a synthetic or natural phospholipid with the active constituents in a molar ratio ranging from 0.5 to 2.0 [22].
2. A stoichiometric ratio of 1:1 is considered as the most appropriate ratio for preparing phospholipid complexes [21] but not necessarily essential to obtain optimum effect at this ratio until and unless different experiments are conducted.
3. Example: Quercetin-phospholipid complex was prepared by mixing of Lipoid S 100 and Quercetin at a stoichiometric ratio of 1:1 [11].
4. However, various stoichiometric ratios of active constituents and phospholipids were used. i.e., Silymarin-phospholipid complexes with different stoichiometric ratios of 1:5, 1:10, and 1:15 were prepared, it was 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% [23].
5. A comparative study using different stoichiometric ratios such as 1:1, 1.4:1, 2:1, 2.6:1, and 3:1 for oxymatrine-phospholipid complexes was carried out and it concluded that an optimal quantity was obtained at a ratio of 3:1 [24].

Therefore, we can conclude that a stoichiometric ratio of 1:1 is not always effective for the formulation of phyto-phospholipid complexes. So, for various types of drugs, we should always experimentally adjust the stoichiometric ratio of active constituents and phospholipids according to distinct purposes, such as the highest drug loading [25].

Advantages of phytosomes

1. Phytosomes are used to amplify the absorption of lipid insoluble phyto-constituents via topical as well as oral route of administration [8].
2. They have been widely used in cosmeceutical industries as well in preparation of many cosmetic formulations such as lotion, ointment, sunscreen as they enhance the absorption of natural moiety via skin [9].
3. The exact amount of dose is entrapped for required therapeutic effect [10].
4. Phytosomes seems to enhance the pharmacokinetic and pharmacodynamics parameter of any phyto-constituent which immensely used in treatment of chronic or acute diseases [7].
5. Phytosomes shows benefit of phyto-constituent along with its phospholipid property [8].
6. Phosphotidylcholine used in the formation of phytosomes not only act as carrier but also possess several therapeutic properties so, gives the synergistic effect when particular substance is given in addition to it [20].
7. Drug entrapment is not a problem with herbo-some as the complex is biodegradable; it improve solubility of bile to herbal constituent [36].
8. Phytosomes are used to immensely intensify the effect of Phyto-constituent by not only improving absorption but also enhancing biological activity, and delivering it to the target tissue; therefore, phytosomes are suitable for a targeted drug delivery system as well [36].

Mechanism of phytosome technology

Many Phyto-constituents has poor bioavailability as they lack in absorption/ permeation via biological membrane. This is mainly due to two different factor, initially because of their particle size and in addition to that is their solubility. These are the limitation that inhibits their absorption. So, phytosome technology is mainly used to overcome such a limitations. In this technology Phospholipids are complex with phyto-constituent in 1:1 or 1:2 ratio depending on their necessity, results in formation of phytosomal complex [38].

Strength of phytosome

Phytosomes shows immense stability as phyto-constituent and phytosome molecules are chemically linked with each other forming strong bond. Hence bioavailability of phyto-constituent is enhanced, so as concluded dose is reduced to required amount. This complex formations are more stable and convenient to obtain desired results without begin affected by gastro-intestinal micro-flora. Absorption of lipid insoluble polar phyto-constituents via various routes such as oral, parenteral or topical shows better absorption, hence shows significantly required therapeutic effect. Phosphatidylcholine used in the formation of phytosomes not only act as carrier but also possess several therapeutic properties, hence gives the synergistic effect when given along with particular substance. Drug entrapment is not a problem with phytosome as the complex is biodegradable and more enhanced [39]. So, Phyto-phospholipid complex shows enhanced effect.

Method for phytosome preparation

1. Phytosomes are prepared by reacting 1 to 3 mole of phospholipids with 2-3 mole of bioactive components such as Flavonoid, Terpenoids, and Alkaloids etc. in an aprotic solvent such as dioxane, acetone, methylene chloride, or ethyl acetate etc.
2. Then the solvent is allowed to evaporate under vacuum or it is being precipitate with non-solvent i.e., aliphatic hydrocarbons, Lyophilization, or spray drying.
3. After which complex is isolated.
4. Phytosomes encompasses bioactive components, inorganic solvents and phospholipids until clear solution is formed by solvent evaporation and formation of thin layer by sonication [36].

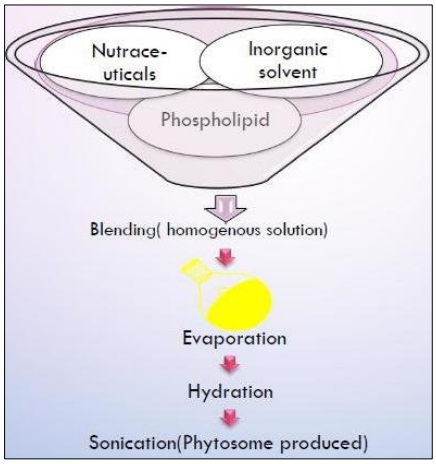

Fig 4

Different methods for Phyto-Phospholipid complex preparation

1. Anti-solvent precipitation process.
2. Rotary Evaporation technique.
3. Solvent Ether Injection process.
4. Novel Method.

1. Anti-solvent precipitation process

Anti-solvent precipitation process is mostly used technique for preparation of ultrafine drug particles. In this technique the herbal drug is dissolved in organic solvent and that solution is then mixed with an anti-solvent means in which the drug is insoluble. Anti-solvent such as acetone and dichloromethane are most commonly used in this technique [16].

Method

The specified quantity of herbal extract i.e. phyto-constituent and phospholipids like soya-lecithin such as phosphatidylcholine were refluxed with 20 ml of organic solvent such as acetone at specified experimental conditions not exceeding above temperature 60°C for 2-3 hours. Then this reaction mixture is concentrated up-to 10 ml of volume. After completion of this reaction add carefully low polarity solvent such as n-hexane (20ml) with continuous stirring to get precipitates. Filtered the above solution and filtered precipitate were collected and stored in desiccators. Dried precipitates is pulverized in mortar and pestle and sieved through #100 mesh. Powdered complex are stored in amber colored glass bottle at room temperature in order to prevent it from external environment [15, 17].

2. Rotary evaporation technique

Rotary evaporation is the process of reducing the volume of a solvent by distributing it as thin film across the interior of the vessel at elevated temperature and reduce the pressure [18].

Method

The specific amount of herbal extract and phospholipids were taken in rotary round bottom flask with addition of 30 ml of water miscible organic solvent like tetrahydrofuran, acetone followed by continuous stirring fir 2-3 hours at 40-50°C temperature. Addition of anti-solvent such as n-hexane to the obtained thin film of sample with continuous stirring using magnetic stirrer. Then obtained precipitate was collected and stored in amber colored glass bottle at room temperature [15, 17].

3. Solvent ether-injection process:

This method involve injection of ether lipid solution into an aqueous phase of drug solution warmed above the boiling point of ether. The ether get vaporized when comes in contact with aqueous phase and lipids gets dispersed after which drug molecule is encapsulated by same [19].

Method

In these technique, there is addition of lipid dissolved in organic solvent and aqueous herbal extract. Then initially prepared phospholipids solubilized in diethyl ether were slowly injected to an aqueous solution of phyto-constituents which is to be encapsulated. It leads to evaporation of solvent under reduce pressure with formation of cellular vesicles i.e. Phytophospholipid complex [14, 15, 17].

4. Novel method

Novel methods for the preparation of Phytophospholipid complex include Supercritical Fluids which involves Gas Anti Solvent technique (GAS), Compressed Solvent Process, Supercritical Anti-solvent method (SAS) [14, 15].

1. In detail about Supercritical anti-solvent technology [32].

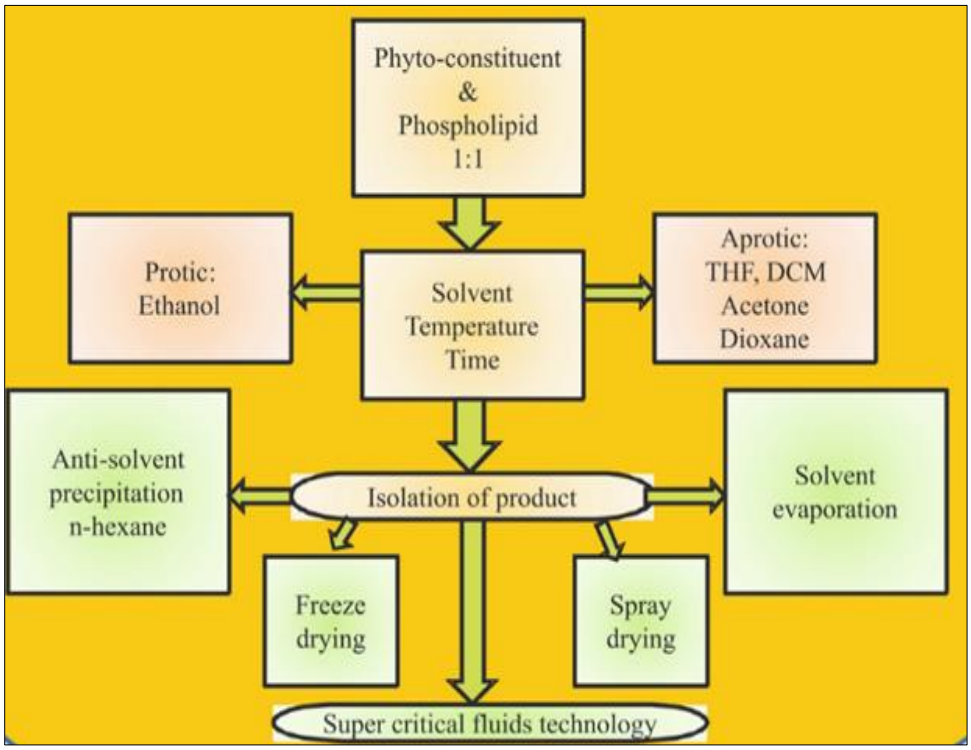

Fig 5

2. In detail about Gas Anti-solvent Technique: A technique where a solid sample is first partially dissolved in organic solvent in a vessel. The solution is then pressurized with dense gas or supercritical fluid resulting in precipitation of solid as a fine powder [35].

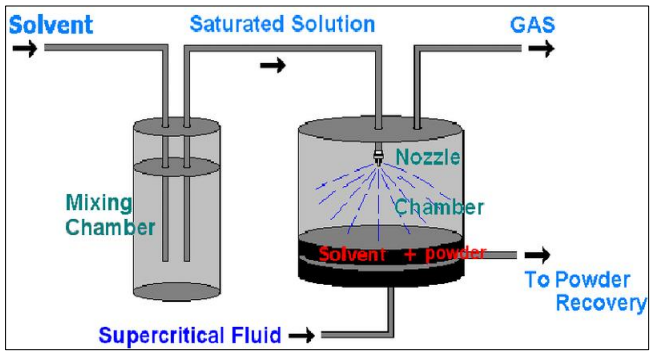

Fig 6

Example of Phyto-phospholipid Complex

Curcumin preparation using phytosome

Curcuma longa is rhizomatous herbaceous plant, Curcumin as a phyto-constituent is obtained from this rhizomes belonging to Zingiberaceae family. Turmeric powder has many medicinal properties and begin used from ancient time. It has been considered as a best Ayurvedic medicine because of its various therapeutic properties and activities such as Anticancer, Anti-inflammatory, Wound healing, Immunity booster, Anti-fungal, Treatment of blemishes etc [37].

Material and method used for Curcumin-phytosome preparation

Materials

Curcumin powder, Soy lecithin Phospholipid and Dichloromethane is used as solvent [37].

Equipment

Particle size analyzer, FTIR (Agilent Technologies), UV-Visible spectrophotometer [37].

Method (Rotary evaporation)

Curcumin Phytosomes were prepared by using Rotary Evaporation method. The Specific quantity of Curcumin powder and Soya lecithin were dissolved in Dichloromethane in Rotary round bottom flask with continuous stirring for 1 hour at the temperature not more than 40°C. Thin film of the mixture was obtained in which the n-hexane was added and stirred this continuously until the monolayer of Phospholipid is formed. Then add the phosphate buffer of 6.8 to obtain the precipitate. Precipitate was collected, pulverized and stored in amber colored glass bottled at room temperature [37, 18].

Analytical evaluation for Phyto-phospholipid complex

To confirm the formation of Phyto-phospholipid complex and to check its optimum effectiveness, few analytical procedures are used:

Determination of Encapsulation Efficiency (EE)

The efficiency of encapsulation (EE %) of plant extract in the Phytosomes is identified by evaluating the fraction of the non-encapsulated extract.

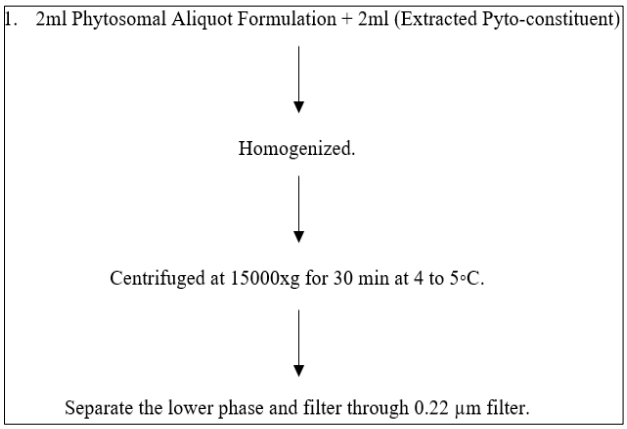

Fig 7

2. Blank sample was prepared by following same procedure as mentioned above but instead of 2ml polysomal aliquot, use 2ml water in formulation.

The encapsulated extract (%) was calculated by difference between the total Phyto-constituent encapsulated area and the total chromatogram area of the supernatant (corresponding to non-encapsulated phenolic compounds).

The encapsulation efficiency (EE %) of Phyto-constituent in the phytosomes was calculated by using Equation (1) and it was determined at 280 to 400nm UV range as per the different individual injections injected into the HPLC column were performed and analyzed.

$$EE\ (\%) = \frac{W\ (\text{added extract}) - W\ (\text{free extract in supernatant})}{W\ (\text{added extract})} \times 100 \quad (1)$$

Hence total phyto-constituent in supernatant was also calculated by using spectrophotometric quantification [31].

UV Spectroscopy

Samples that shows different absorption i.e. λ_{max} at different UV/visible ranges can be used to characterize their own structural elucidation. Most studies have revealed that there is no comparison in the UV absorption characteristics of constituents before and after completion. Prepare Phyto-phospholipid complexes and found that the characteristic peaks of Phyto-constituent seems to be present [28, 25]. So, from this observation it can be concluded that chromophores of any particular compound begin examined remains unaffected by complex formulation.

Differential scanning calorimetry (DSC)

In DSC, interactions is observed by comparing two or more different transition temperature of different compounds, appearance of new peaks with different intensities, disappearance of original peaks may occur, melting points (MP), and changes in the relative peaks area [29, 25]. Phyto-phospholipid complexes usually display radically different characteristic peaks compared to those of original physical mixture. It is claimed that, in addition to two fatty chains of phospholipids the strong interactions may occur in active ingredients and the polar part of those phospholipids also inhibits free rotation. Phyto-phospholipid complexes that contain flavonoids and the resulting DSC thermo-gram showed two different characteristic peaks that were lower than that of actual physical mixture [30, 25].

Fourier transform infrared spectroscopy (FTIR)

FTIR is an analytical technique used for the structural analysis of any compound. In case of phyto-phospholipid complex, these complexes can be verified by comparing different spectroscopy of phospholipid complexes to that of physical mixture of same. Various studies may show different results. Phyto-phospholipid complexes containing flavonoids was formulated and studied. It was observed that both the complexes as well as phytosomes show different peaks and structure was identified [25].

Nuclear magnetic resonance (NMR)

The ^{1}H NMR i.e. Hydrogen NMR and ^{13}C NMR i.e. Carbon NMR techniques are important for identification of the structures of the complexes (Phyto-phospholipid complex). As it is assumed that there is interaction between Polyphenols and phospholipids by hydrogen bonds rather than chemical bond because, few researcher developed an NMR data showing hydrogen bond can form between polar phenolic functional group of phyto-constituent and phospholipid. The spectra of different phyto-phospholipid complexes concludes that the lipophilic side of lipids can act to form an envelope on the central Phyto-bioactive parts of these complexes [25].

Scanning electron microscopy (Sem) and transition electron microscopy (TEM)

SEM is an analytical technique used to determine particle size and its appearance. Dry sample was placed on an electron microscope brass stub coated with gold in an ion sputter. Random scanning of the complex was carried out at 100x.

TEM is a technique used to detect size of phytosomal vesicle with 1000x [38].

Conclusion

Phytosomes is an advance technique used for encapsulating Phyto-constituent and exhibits its optimum effect. Phospholipids shows affinity for various constituent extracted from plant through hydrogen bond. Stoichiometric ratio plays at most important role in formation of these complexes. Phytosomes, phyto-constituents and solvents be used must be selected as per their compatibility.

Phytosomes are usually used to enhance the bioavailability of various hydrophilic phyto-constituent. Phytosomes are not only used for herbal constituent but they are also used in many cosmeceutical preparation as well. The efficacy and potency of phyto-constituent is enhanced by using this technique so, phytosomes technique has showed bright future in pharmaceutical industry.

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