Pharmacosomes as Unique and Potential Drug Delivery System

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Abstract

Many phospholipid based formulations are successfully used as drug delivery systems which deliver the drug at the site of action. Pharmacosomes are one of the unique and potential vesicular systems which contain drug phospholipid conjugate have more stable in comparison other novel approaches. There are many pharmaceutical agents such as proteins, peptides, nucleic acid, anticancer drugs, antihypertensive drugs and other agents that have less bioavailability, low solubility and permeability profile, rapid clearance, high GI toxicity, rapid elimination from body, adverse effects. Therefore, there are enormous requirements to develop drug delivery methods and also carrier for drug delivery that will provide essential and effective delivery for such therapeutic agents or drug molecules.

One of the promising novel approaches to promote the absorption as well as the bioavailability of pharmaceutical active compounds is the Pharmacosomes which are formulated via complexation of drug molecules with phospholipids. Pharmacosomes are able to lower the interfacial tension between the developed system and the GIT fluid, thus facilitate the permeability of active agents through tissues, membrane or cell wall; and also capable of promoting the absorption of drugs across the biological barrier. Drug- phospholipids complex is good alternative which is used to improve bioavailability of drugs having low solubility or less permeability, and also used for protection of active pharmaceutical agent from degradation in the gastrointestinal tract, reduction of gastrointestinal side effects of NSAIDs and other agents. The bitter taste of orally administered drug is overcome by this approach which also facilitates inhibition of P-GP (para-glycoprotein) efflux of the drug**s.**

Introduction

Oral administration is one of the convenient routes for all types of patients, especially employed in pediatrics and gediatrics. The drug delivers at the site of action and is the most commonly preferred route. More than 60% of marketed drugs are used orally. Traditionally drug phospholipid complex is employed to promote the oral bioavailability of phytoconsituents and other therapeutic agents and also used for increasing the solubility and permeability of BCS class II, III, IV drugs, which possess low solubility, permeability. **(Fan et al., 2017 and Jena et al., 2014)** Poorly aqueous-soluble drug candidates demonstrated in drug discovery having poor or variable bioavailability problems. **(Telange et al., 2016)** The bioavailability of oral drugs is strongly influenced by two important parameters which include solubility and permeability. **(Fricker et al., 2010**)

There are many approaches to overcome solubility and permeability related problems such as salt formation, micronization, nanocrystal, amorphous solid dispersion, cyclodextrin complex, solid lipid nanoparticles, liposome, micelles etc. **(Damle et al., 2016)** Singh et al., 2014 reported phospholipid being an essential unit of cell membrane which is mainly used to achieve goals such as improving the bioavailability of pharmaceutical agents and phytoconsituents via increased solubility and permeability thereby reducing their side effects. (**Singh et al., 2014**) Pharmacosomes one of the potential alternative to

enhance the solubility as well as the permeability of therapeutically active moiety of either natural or synthetic origin is the conjugation with phospholipid**. (Habbu et al., 2013 and Kassem et al., 2017)** Semalty et al., 2009 reported phospholipid based vesicular system such as pharmacosomes are used to improve the dissolution rate, solubility and permeability of poorly aqueous soluble drugs and also used in case of lipophilic agents with less permeability. **(Semalty et al., 2010)** Drug-phospholipid complex hypothesized enhancement or uptake and alteration release of drugs from the lipid based system**. (Semalty et al., 2009)**

Phospholipids - Phospholipids are lipid molecules, which are key components of all cell membranes. Phospholipids is zwitter ionic compounds (have a both charges) so it can form lipid bilayers in structure of various type of phospholipids. **(Bhattacharya S, 2009)** Phospholipids consists of both type properties, (a) lipid loving constituents i.e. fatty acid ('tail') and (b) water loving constituents i.e. phosphate ('head') joined together via alcohol or glycerol bridges. The 'head' is hydrophilic (water loving) which having negatively charged molecules (phosphate group) and glycerol while the Lipophilic 'tails' which is consisting 2 long chains of fatty acid which are repelled by water and are forced to aggregate. It is placed in water, to form of different type of structure of phospholipids depending on their specific properties**.** Various phospholipids are present in all type of cell membrane. Phospholipids are used as natural digestive aids.

They also work as carriers for delivery of both types of nutrients i.e. water miscible as well as fat miscible, in human and other higher animals. Phospholipids can be isolated from egg yolk and soy beans. Phospholipids are chemically extracted by using various solvent like hexane, cold chloroform and methanol (CH_3OH). Phosphotidylcholine is a molecule which has work as bifunctional, such as for the hydrophobic behavior use of Phosphatidyl moiety and the choline moiety being water loving in nature. **(Singh et al., 2012, Reis et al., 2013 and Changediya et al., 2011)**

Table: 1 Classification of Phospholipids. (**Babu et al., 2014)**

Phosphatidyl choline	1,2-Dilauroyl-sn-glycero-3-phosphocholine (DLPC) 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC)
Phosphatidyl ethanolamine's	1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE) 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine(DOPE)
Phosphatidyl glycerol	1,2-Dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG) 1,2-Distearoyl-sn-glycero-3-phosphoglycerol (DSPG) 1,Palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG)
Phosphatidic acid	1,2-Dimyristoyl-sn-glycero-3-phosphate (DMPA)

	1,2-Dipalmitoyl-sn-glycero-3-phosphate (DPPA) 1,2-Distearoyl-sn-glycero-3-phosphate (DSPA)

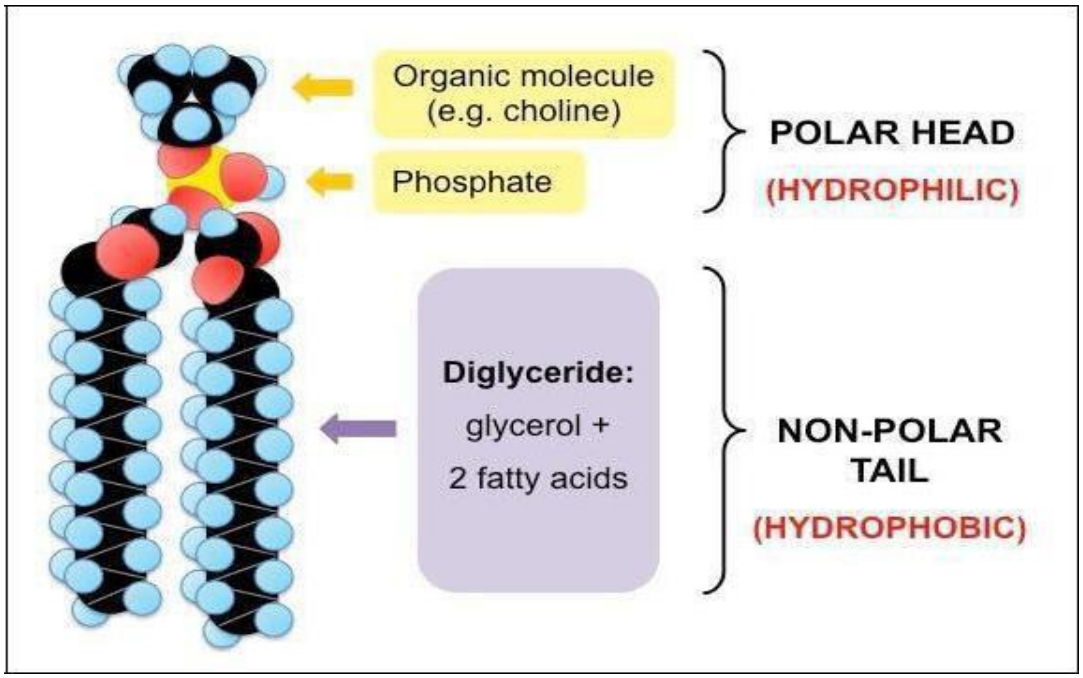

Figure1: Structure of Phospholipid **(Singh et al., 2012)**

Application of Phospholipids

Following are some of the applications of phospholipids:

(a) Permeation enhancer

Unsaturated phospholipids are used to promote permeability of therapeutic agents because its structure constitutes an important and integral part of biomembrane in the epidermis and at 37^{o}C (body temprature) the phospholipids get in liquid crystalline state and modify and fludize the structure of the barrier layer, resulting is an enhanced permeability of bioactives. **(Babu et al., 2014)**

(b) Preparation of Liposomes

The preparation of liposomal drug delivery system involves formation of vesicles using soya lecithin and other phospholipids as the key components. The structure of phospholipid resembles that of cellular membrane. **(Li et al., 2014)** Multi-lamellar vesicles (MLVs) are able to carry maximum amount of active pharmaceutical agents, exclusively in cases where the mixture of drugs with phospholipid is being solubilized into volatile organic solvent. The entrapment efficiency is affected by the presence of negative charge at phospholipid molecules. **(Babu et al., 2014)**

(c) Solubility enhancer: The drug-phospholipid complex is used to improve the solubility of hydrophobic bioactive agents in water phase by using amphiphilic nature of phospholipids. (**Pathan et al., 2011)**

(d) Emulsion stabilizer: Phospholipid molecules are used to formulate microemulsion systems w/o (water in oil) as well as o/w (oil in water). Kale et al., 2017 prepared microemulsion containing curcumin

which is extract out from turmeric with the help of Tween80 as a surfactant and phospholipid is lecithin. The entrapment efficiency of microemulsion containing curcumin prevented from degradation by environmental factors and also increased the concentration of curcumin in aqueous solution. The microemulsion has an ability to be diluted without destroying its structure. **(Kale et al., 2017)**

(e) Mixed Micelles: At least two different type of moiety are need to formation of polymeric micelle, observed disk like structure in case of mixed micelles form by use of two different species which is phospholipid and detergent. Use of small scrap of phospholipid with surfactant to form of micelles come in contact with aqueous phase usually water lipophilic part of lipid is unfavourable exposure. **(Fricker et al., 2010)**

(f) Micro/Nanoemulsions: The phospholipid based some novel formulations such as micro- and nanoemulsion maintain their liquid state at room temprature with droplet sizes in the range of 50–500 nm are usually prepared by high pressure homogenization. **(Fricker et al., 2010)**

(g) Self-emulsifying Drug Delivery Systems (SEDDS): SEDDS are mixtures of oil and phospholipids, ideally identical, for the preparation of self-emulsifying drug systems sometimes co-solvent is used, which emulsify under conditions of agitation, like those who will be in the gastro-intestinal tract. **(Guo et al., 2011)**

(h) Solid lipid Nanoparticles (SLN) preparation: SLNs formulations depend on "melt-emulsified" method of lipids, resulting is solid form at room temperature. For e.g. repaglinide loaded solid SLN using cephalin and lecithin as phospholipid carriers and surfactant of (Tween 80) as a stabilizer. The loading efficiency of SLN containing repaglinide was increased from 82 to 92% as the concentration of lipid increased**. (Babu et al., 2014)**

(i) Drug phospholipid complex: Drug phospholipid complex formed by interaction between drug and compatible phospholipid reduce the side effects and enhance the bioavailability via increasing solubility of therapeutic agents. Phospholipids based drug delivery systems have promising, better and significant effects to deliver the drugs and provide appropriate systematic drug delivery **(Fricker et al., 2010)**

Type of Phospholipids

Following types of phospholipid discus here because these are used for preparation of pharmacosomes

Natural Phospholipids

In 1793, a scientist Fourcroy first found the existence of complex of aliphatic molecules. Then in 1812, Uauquelin found phospholipids presence in human brain. In 1846, the scientist of Gobley separated phospholipids from the egg yolk. The term "lecithin" is derived from the Greek word like *"lekithos,"* firstly it was used as a sticky orange material isolated from egg yolk. After the 20 years, choline component was found in presence in lecithin. **(Khan et al., 2014 and Li et al., 2014)**

Phospholipids are obtained from various animals, plants, and vegetable oils including soyabean, cotton seed, corn, sunflower and grapeseed) and animal tissues (e.g. egg yolk and bovine brain). Phospholipids solitude from varies plants and animals then purified into different levels, like food and pharmaceutical grade etc. For example, lipoid E80 is contains PC (phosphotidylcholine), PE (phosphatidylethanolamine), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), SM and trace amounts of triglycerides, cholesterol, fatty acid, d, L-α-vitamin E and water **(Li et al., 2014)**

To obtained a single phospholipid component with define structure and configuration researches focused on chemical synthesis or semisynthesis of phospholipids as it is still not possible to get single component of naturally occurring phospholipid using chromotagraphic techniques. Semi-synthetic molecules require less reaction steps as semisynthesis of glycerophospholipids involves changing of head and tail or both depends of natural phospholipids moiety. **(Li et al., 2014 and Khan et al., 2014)**

Use of phospholipids in pharmaceutical formulations:

Phospholipid compounds are used in pharmaceutical formulation emulsion as wetting agents, emulsifying agent also used non conventional formulation such as liposomes micelles etc as a builder. Phospholipids mainly use for promote the transport of active therapeutic agent acrose the biomembrane with low permeability and in case of poorly aqueous solubility of BCS class II, IV moiety also use for improve solubility as well as bioavailability.

Phospholipids are used in the preparation of many pharmaceutical formulations such as suspensions, emulsions, mixed micelles, liposomes, solid dispersions, drug–phospholipid complexes etc. Due to the physiological role of phospholipids they are having a very low toxicity profile so also given by any route of administration. Various categories of formulations for parentral administration, such as liposomes, o/w emulsion, mixed micelles etc are based on natural as well as synthetic phospholipids. For the delivery of lipophilic molecules, oil-in-water emulsions are commonly preferred as a carrier, which is prepared by using egg phospholipid as emulsifier. **(Hoogevest et al., 2014, Hoogevest, 2017 and Desai et al., 2000)**

Drug Phospholipids Interaction

Drug phospholipid interaction occurs by using polyphenolic compounds which contain constituent like carboxylic group or hydroxyl group and also contain amine group to form a covalent bond via interaction with phosphotidylcholine or some other fatty acid like oleic acid, linolenic acid etc by chemical bonding. **(Pathan et al., 2011)** This is evaluated by use of different type of techniques like thermal analysis, spectrophotometry of drug–phospholipid complexes with respect to the pure drug and the physical mixture of drug and phospholipids and complex correlated with respect to the use of DSC, FTIR**. (Semalty et al., 2010)** The complexation of herbs and synthetic moiety with phospholipids to improve their solubility, permeability and also bioavailability was first developed by Indena, an Italian pharmaceutical and nutraceuticals company. They patented the technology as ‘PHYTOSOME’ (**Khan et al., 2013)**

Vesicular System

Oral administration is most preferred route for most of the drugs and patient compiliance. **(Qin et al., 2018)** In case of oral ingestion a synthetic drug and phytoconsituents (herbs) are dissolved in to gastric fluid (hydrophilic environment), for reaching in the blood (i.e. systemic circulation) they follow permeation path across the cellular or biological membrane in the presence of hydrophobic environment. Many synthetic and natural drugs obtained from plants are associated with problems like poor solubility and permeability, poor bioavailability, gastric toxicity and P-GP efflux (para-glycoprotiene) which are not absorbed properly. **(Weinheimer et al., 2016; Gnananath et al., 2017)**

Poor absorption may be due to the poor water solubility, whereas poor permeation may be due to the structure of the drug natural plant origin which contain multiple ring on their structure, this molecules is too large and if possess poor miscibility with lipophilic material, this molecules are absorbed by use simple

diffusion mechanism. **(Zhou et al., 2017)**

Many phospholipid-based preparations have been investigated to promote the absorption as well as permeation of pharmaceutically active constituents of synthetic and natural origin. These approaches include development of more soluble prodrugs, solid dispersions and complexation with agents such as metals, cyclodextrin and phospholipids. Therefore, developing pharmacosomes (drug phospholipid conglomerate) is a potential approach for amplify the bioavailability and removing the problems like gastric toxicity and P-GP efflux (Para-glycoprotein). **(Semalty et al., 2009)**

Pharmacosomes

Different types of novel phospholipid-based systems have been developed for delivery of active moiety at targeted site with controlled and sustained release of the pharmaceutical moieties. Pharmacosomes, a novel vesicular drug delivery system, having unique advantages in comparisons liposome and niosome, have emerged as a potential approach to traditional vesicles. In contrast to other formulations pharmacosomes are better for increasing the solubility as well as permeability; Pharmacosomes are nanometric colloidal dispersion system have drug bound with phospholipids and form ultrafine hexagonal micellar aggregates depending on the chemical structure of the drug–lipid complex. **(Bombardelli et al., 1991 and Semalty et al., 2009)**

Various alternative phospholipids based vesicular drug delivery systems are associated with problems of drug entrapment, leakage from the formulation, or insufficient shelf life of drug, which can be avoided by the use of unique phospholipid based pharmacosomes approach. Development of the vesicular pharmacosomes system is depends on surface and bulk interactions of lipids with therapeutic agents. If the drug molecules possess an active free hydrogen atom (-COOH, -OH, -NH2, etc.) can be esterified with the lipid, with or without spacer chain, resulting to produce strongly amphoteric compound known as pro drug, which will facilitate biomembrane, tissue, or cell wall transfer. **(Rewar et al., 2014)**

These Prodrug exhibit both hydrophilic and lipophilic properties and thus manifests amphiphilic characteristics upon dilution with water and also phosphate buffer to form drug enclosed by lipidemic environment known as pharmacosomes. **(Biju et al., 2006)** Pharmacosomes being neutral molecules promote membrane, tissue, or cell wall transfer in the organism. The pharmacosomes have an amphiphilic characters these characters help to reduce interfacial tension between hydrophilic and lipophilic environment and at higher concentrations exhibit mesomorphic behavior. This decrease in the interfacial tension leads to an increase in the contact area thereby increase permeability lead to bioavailability of drugs and herbal constituents. (**Chauhan et al., 2012)**

Limitation of pharmacosomes

- **a.** The complete leaking of drug is prevented from pharmacosomes by the formation of covalent bond between active moiety and phospholipids
- **b.** The formation of pharmacosomes require lipid which have amphiphilic property.
- **c.** For the preparation of pharmacosomes require synthetic active moiety
- **d.** Pharmacosomes undergo fusion, hydrolysis as well as aggregation during storage. **(Rangha et al., 2013)**

Advantages of Pharmacosomes

a. Pharmacosomes are suitable for both types of drug either water soluble or lipid soluble.
b. Leaking of drug is not possible because formation of covalent bonding between dug and lipid component
c. Drug entrapment efficiency is high.
d. Volume of inclusion does not influence entrapment efficiency.
e. Pharmacosomes are used for improving the bioavailability of poorly water soluble drugs.
f. Reduction in adverse effects and GIT toxicity of active pharmaceutical agents.
g. Reduced cost of therapy.
h. Avoid the problem like drug have a P-GP efflux.
i. Reduce the GIT Toxicity.
j. Avoid first pass metabolism.
k. Improve the solubility in case of poorly soluble drugs.
l. Used as targeted drug delivery system directly at the site of infection

Disadvantages of Pharmacosomes

a. Preparation of pharmacosomes is based upon phospholipids' amphiphilic nature.
b. Requires surface and bulk interaction of active agents with phospholipids
c. Drug leakage protection of drugs requires covalent bonding between drug and phospholipid.

Formation of Pharmacosomes

Mechanism of formation of pharmacosomes

The drug molecules are present in complex form in the pharmacosomes by sharings of electron pair and electrostatic forces or by forming a hydrogen bond between lipid and drug molecules or covalent bonding can also occur. The lipid based complexed vesicles may exist as nanometric colloidal size micelles, the vesicles or may be in the form of assembled hexagone possess a functional hydrogen, amine and carboxy atom banking upon the architecture of the complex. **(Kavitha et al., 2010)**

If the drug molecules possesing free carboxylic, amino, and hydroxyl groups, so these groups are converted to ester group with the help of the hydroxyl moiety which is present in lipid component, resulting in the formation of covalent bond between drug and lipid component termed as prodrug. The prodrug on contact with aqueous phase, arrange in single or multiple layers resulting in the formation of pharmacosomes. These formulations are based on surface and bulk interaction phenomena. **(Pandita et al., 2013)**

Table 2: Comparison from other vesicular systems (Kumar et al., 2016)

Liposomes	**Niosome**	**Transferosomes**	**Pharmacosomes**
1. Expensive 2. Degradation by oxidation 3. Lack of purity of natural phospholipids 4. Chances of leaching of drug	1. Time consuming preparation 2. Comparatively less efficient 3. Instability	1. Expensive 2. Chemical instability	1. Cheaper 2. Oxidation resistant 3. Pure natural phospholipid not needed 4. Covalent linkage prevents drug leakage 5. Less time consuming prepn. 6. More efficient 7. More stable 8.Cheap 9.Chemically stable

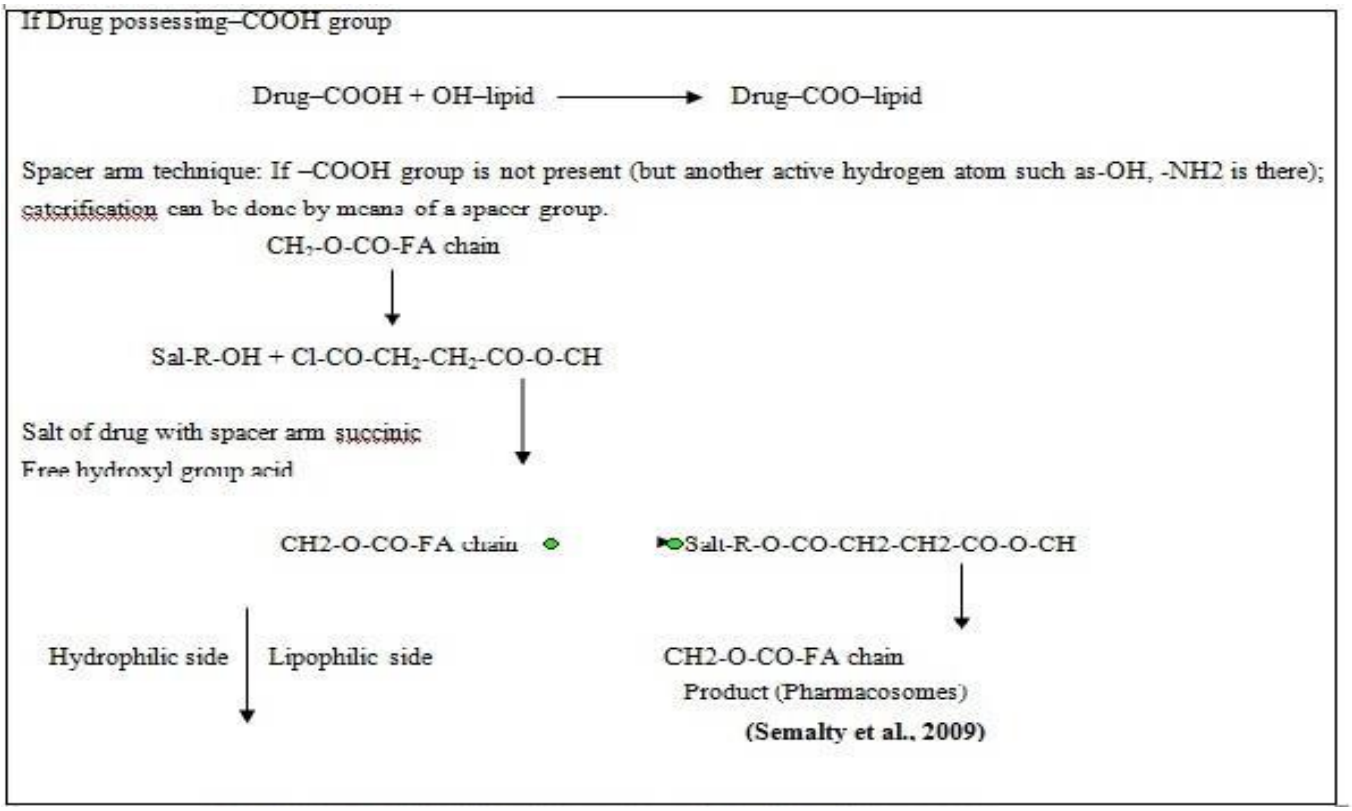

Figure 2: Basic mechanism for formation of Pharmacosomes

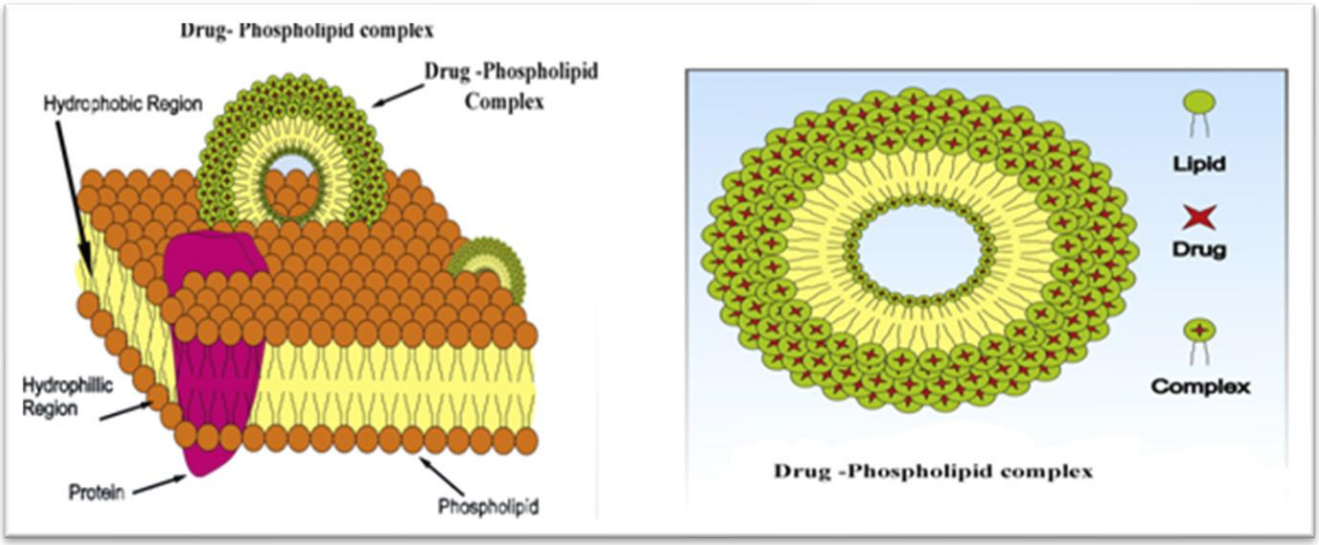

Figure 3: Drug phospholipid complex (Khan et al., 2013)

Material used for the preparation of pharmacosomes

(i) Drugs

Any synthetic active pharmaceutical moiety having active hydrogen atom (-COOH, OH, NH_2) can be esterified with the lipid like (phosphotidylcholine etc), with or without spacer chain to form amphiphilic complex which in turn facilitates crossing of membrane, tissue, cell wall transfer in the organisms. **(Al kaf et al., 2017)**

(ii) Lipids

Phospholipids are the similar structural component to biological membranes. For the preparation of pharmacosomes different types of phospholipids are used. Generally two types of phospholipids are used **i.e.** phosphoglycerides and sphingolipids. Phosphotidylcholine is mostly usable molecule for preparation of pharmacosomes. Phosphotidylcholine is zwitter ionic molecule, in which a linkage of a pair of hydrophobic acyl -hydrocarbon chains i.e. 'non polar tails', with a hydrophilic 'polar head' group by glycerol bridge. **(Rewar et al., 2014)**

Table 3: Composition by weight of refined and unrefined soya lecithin (Ranga et al., 2013)

S. No.	Oil-Free Compound	Refined Lecithin	Unrefined Lecithin
1.	Phosphatidyl choline	23%	17.5%
2.	Phosphatidyl ethanolamine	20%	15.0%
3.	Phosphatidyl inositol	14%	10.0%
4.	Unrefined soy oil	0-3%	31-34%
5.	Glycolipids	13-16%	13-16%
6	Neutral lipids (mostly triglycerides)	-	2-4%

(iii) Solvents

The required solvents for the preparation of vesicular system like pharmacosomes should have analytical grade, solvent should be high purity and having property of high volatility in nature because during complexation solvent is evaporated on contact with aqueous medium to form unilayer and multilayer of vesicles. A solvent selected for the preparation of pharmacosomes should have intermediate polarity. (Venkatesh et al., 2014)

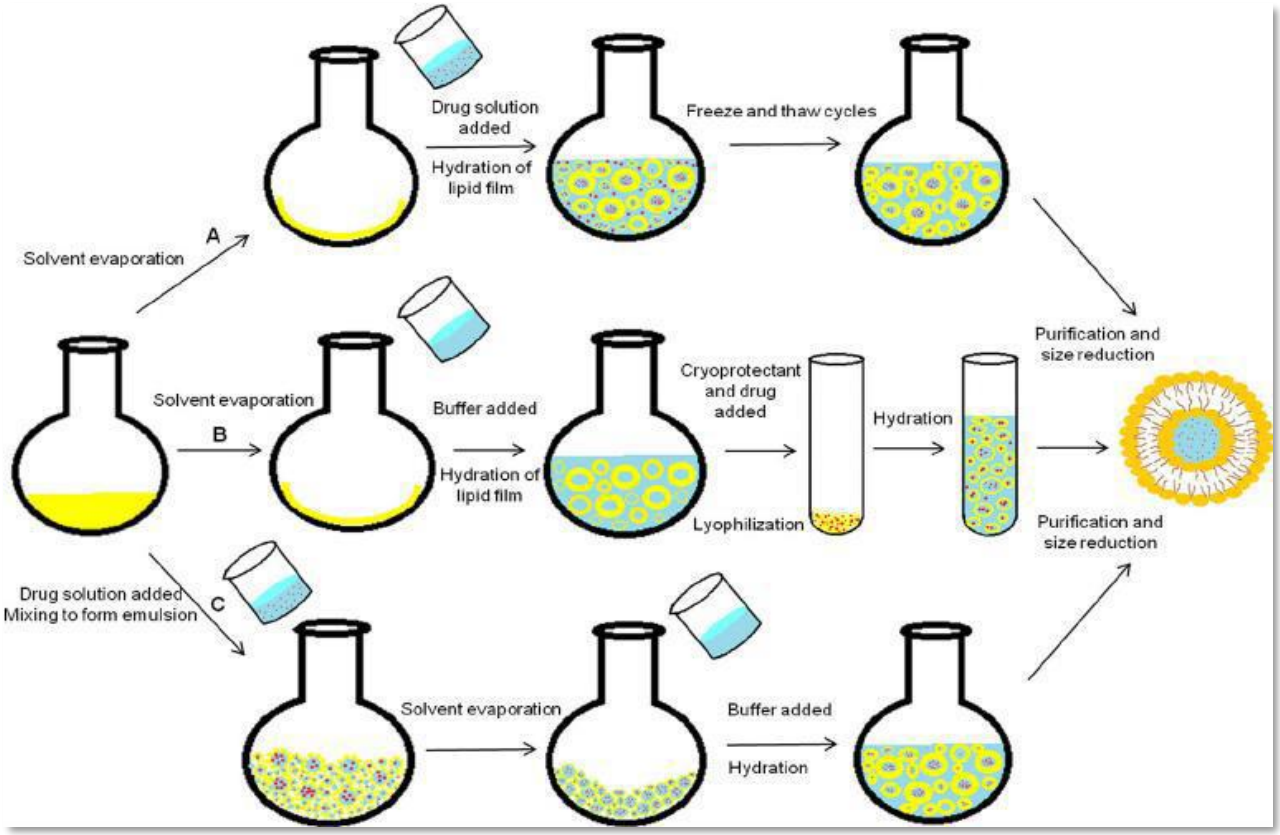

Figure 4: Schematic presentation of handshaking method. (Sailaja Krishna A, 2016)

Methods for preparation of pharmacosomes

Following methods are used for the preparation of pharmacosomes

A. Hand shaking method

In this method, the complex with phospholipid is dissolved in organic solvent (solvent or mixture of solvents), which should be highly volatile in nature, and taken in round bottom flask then rotated continuously clockwise or anticlockwise until the complete solvent is evaporated to form of uniformity layer under the conditions of vacuum, results in deposition of a thin layers form on the walls of round-bottom flask. Then the film is hydrated by adding aqueous medium by rotating opposite side for the formation of film to yield a vesicular suspension known as pharmacosomes. **(Pandita et al., 2013)**

B. Ether injection method

In this method, organic solution of drug lipid complex is injected slowly into warm aqueous medium. The complex is injected dropwise in to aqueous medium generally water. However sometimes phosphate buffer (pH 6.8) can also be used. Vesicles get readily formed. Here the drug lipid complex is dissolved in ether which acts as a volatile solvent. **(Sharma et al., 2014)**

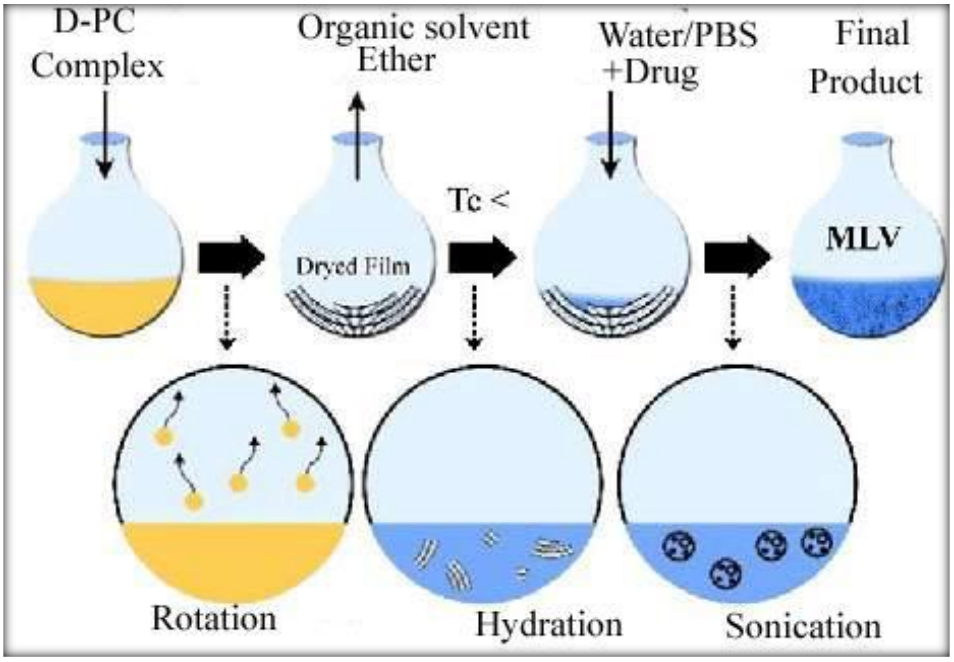

Figure 5: Schematic presentation of ether injection method (Sailaja Krishna A., 2016)

C. Super critical fluid process

In this method two types of techniques are used for preparation of pharmacosomes.

a. Gas anti solvent method (GAS)

b. Solution-enhanced dispersion by supercritical fluid (SEDS)

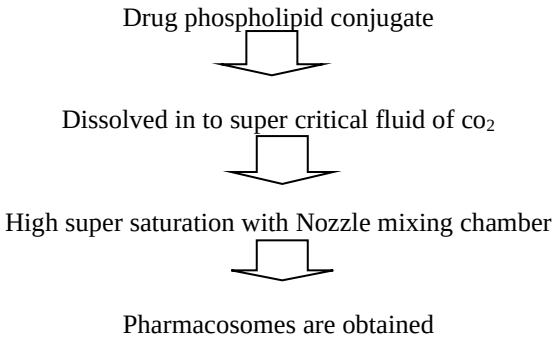

Figure 6: Flow diagram of super critical fluid process

In this method drug phospholipid complex is dissolved in super critical solvent of CO_2. Then mixed with CO_2 in nozzle chamber then obtain single or multi-layer of vesicles of nanometric size micelle known as pharmacosomes. **(Kaur et al., 2017)**

D. Anhydrous co-solvent lyophilization method (for insulin powder)

This technique is generally used for insulin powder. In this method the insulin powder is co-dissolved with phospholipid into any volatile solvent generally dimethyl sulfoxide (DMSO), dichloromethane (DCM)) containing 5% of glacial acetic acid to form clear mixture by gentle agitation then freeze dried for overnight at condenser temperature to obtain clear product known as complex store at 4^oC flushed

with nitrogen. Then vesicles are formed by solvent evaporation method is known as "pharmacosomes" **(Chauhan et al., 2012)** the steps involved in this method are as following:

Drug powder + phospholipid Add in 1ml of DMSO containing 5% glacial acetic acid

↓

Formation of clear mixture by agitation

↓

Freeze dried at condenser for overnight

↓

Resultant complex store at 4°C

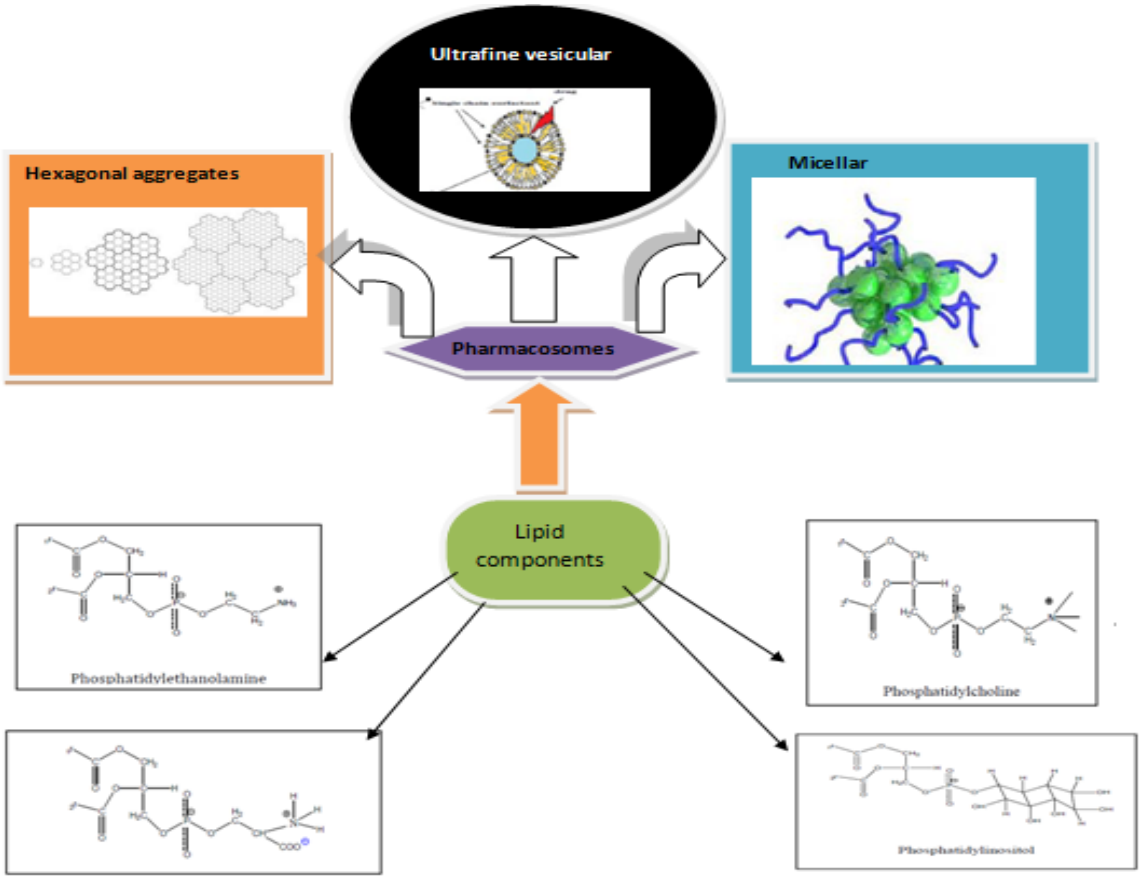

Figure 7: Basic mechanism of formation of Pharmacosomes (Chauhan et al. 2012)

2.1.7 Characterization of pharmacosomes

A. Complex determination

Complex determination is important parameter to estimate which type of bonding occur between drug and phospholipids by using FTIR, by correlating between spectra of drug, phospholipid, and physical mixture with the spectra of drug phospholipid complex. **(Pandita et al., 2013)**

B. Determination of solubility

Mali et al., 2014 was demonstrated, to determine the change in solubility due to complexation, between drug and phospholipid, the solubility of pharmacosomes is determined by adding excess amount of drug, physical mixture and pharmacosomes are dissolved into 10 ml of distilled water and different type of buffer solution at different pH 1.2, 1.4, 4.5, and 6.8 phosphate buffers in vials closed by cap. The vials are allowed to shake at room temperature for 24 hrs at wrist shaker. After the equilibrium is maintained, the saturated solutions are taken then centrifuged to remove excess drug undissolved in solution. The supernatant part is taken then filtered rapidly through a 0.45 mm membrane filter. Then filtrate solutions are analyzed by UV spectrophotometrically and HPLC. **(Mali et al., 2014)**

C. Surface morphology by (SEM/TEM)

SEM and TEM are used for determining surface morphology of complex between drug and Phospholipid. Surface morphology of pharmacosomes is also determined by using scanning electron microscope and transmission electron microscope. **(Thakur et al., 2017)** Surface morphology determination is important because surface morphology of vesicular system is depends on purity of phospholipid and process variables such as application of vaccum, speed of rotation, or method used which affect the size, shape of vesicular system. **(Bhingare et al., 2014)**

D. Drug content

Drug content is also an important parameter determined by taking equivalent weight of conjugated drug. Then complex is dissolved in those solvent having suitable solubility, in volumetric flask with pH 6.8 buffer solution then withdrawing the sample after dissolving in phosphate buffer. Suitable dilution is made by same solvent, then analyzed by UV spectrophotometer in suitable Lamda max and HPLC is also used. **(Rangha et al., 2013)**

E. Differential scanning calorimetry (DSC)

Differential scanning calorimetry is known as thermal method used for study of drug excipient compatibility. This method is also used for determining interaction of drug molecules and phospholipid. **(Venkatesh et al., 2014)** Interaction is measured by disappeared endothermic peak, appearance of new peak with respect to temprature and melting point and change in shape of peak, which confirms the presence/absence of interaction **(Kumar et al., 2012 and K. Maiti et al., 2006)**

F. Powder X ray diffraction (PXRD)

PXRD is an important parameter for determining crystalline and amorphous nature of drug by checking the changes in crystal morphology of drug bound with phospholipid in form of complex. X-ray diffractometry is also a useful technique to study complex in the solid state. The peak position (angle of diffraction) indicates the crystal structure and heights and shape of peaks are the measures of sample crystallinity (crystallite size). **(Cheng et al., 2017)** The area under the curve of powder x-ray differaction

is used to observe intensity of peaks of all combined reflection intensity of peak that specifies by specimen attributes. **(Kaur et al., 2017)**

G. Complexation Rate

The suitable amount of drug phospholipid complex is taken which is equivalent to 50mg of drug bound with phospholipid. The complex is dissolved into those solvent (2ml) select are having property like purity and drug insolubility but complex solubility. After the solublization of complex then withdraw 1ml of solution and dilute up to 10ml then take absorbance by UV spectrometry. **(Tan et al., 2012)** The complexation rate is determine by using this formula

$$\textbf{Complexation rate} = M_1/M_2 * 100$$

Where is

M_1 is amount of drug present phospholipid complex.

M_2 is amount of drug initial used. **(Cheng et al., 2017)**

H. Entrapment efficiency

The unentrapped drug from Pharmacosomes vesicles are separated by centrifuging at 10,000 rpm at 4°C for 1 hour. The supernatant was diluted with pH7.4. The concentration is assayed by spectrophotometrically at suitable absorbance. The values are express as mean standard deviations. The percentage of drug entrapped in pharmacosomes is calculated by the using following formula, **(Letha et al., 2017)**

Percentage Entrapment Efficiency = [(Ct – Cf) / Ct] x 100 (1)

Here is,

Ct = concentration of total amount of drug added

Cf = concentration of free drug

I. *In vitro* release study

This technique is also known as Reverse bag dialysis for estimation of release of drug from Pharmacosomes. Pharmacosomes formulation introduced inside the dialysis bag and receiver is known as continuous phase which is placed outside the assembly. Dialysis bags containing the continuous phase (receiver phase) with the suspended vessel containing the diluted formulation (donor phase) and the system is homogenously stirred. **(Sharma et al., 2014)** At predetermined time intervals, sample is withdrawn from the dialysis bag and the contents are analyzed for released drug. The advantage of this technique involves increase in the membrane surface area, which is helpful to provide transport of sample from the donor to the receiver phases. The advantage of this technique is reduction of steps which increases the efficiency. **(Rewar et al., 2014)**

J. Stability study

Pharmacosomes are characterized for size and size distribution, entrapments and bound of drug with lipids. The approach has successfully improved the therapeutic activity of various drugs like pindolol

maleate, bupranolol hydrochloride, taxol, acyclovir, etc. **(Sharma et al., 2014)** Letha et al., 2017 was reported the optimized formulations are maintained at different temperature for different time duration like refrigeration temperature (4±2°C), room temperature (30±2°C) and at elevated temperature (40±2°C) for 3 months and 6 months to determine physical and chemical stabilities. Sample is collect at fixed time intervals then sample is analyzed for their organoleptic behavior, drug entrapment, drug content and therapeutic efficacy. **(Letha et al., 2017)**

Applications of Pharmacosomes

a. Pharmacosomes have demonstrated greater stability profiler shelf life than other vesicular system.

b. Pharmacosomes have the ability to accelerate drug absorption and its distribution.

c. Pharmacosomes can enhance the rate of permeation of drug across biomembrane by enhancing the fluidity of cellular membrane. Because the transition temperature of vesicles and micelles might pose an evident effect interaction of vesicular system with biomembrane, hence promote the transfer of drug across biomembrane. **(Al kaf et al., 2017)**

d. Bupranolol hydrochloride- The prodrug comprise of bupranolol (β-blocker) which is covalently bound with 1, 3-dipalmioyl-2-succinyl-glycerol to formed prodrug, it is ampiphilic nature and dissolved readily in warm water above 30°C forming a smectic lamellar phase. The solublization of prodrug is similar to phospholipids which have charges showed continuous swelling with increasing hydrophilicity (water content) and so in excess water region, the structure of unilamellar vesicles is form, which is thermodynamically most stable while oligomeric vesicles also form. The intraocular effect is increased in rabbits after this drug entrapped in vesicular system (pharmacosomes). **(Jadhav et al., 2011)**

e. The formation of pharmacosomes has improved the therapeutic activity of various therapeutic agents like pindolol maleate, Bupranolol hydrochloride, taxol, acyclovir, etc. The phospholipid complexes of proteins such as insulin and salmon calcitonin have been developed successfully and have been reported to improve their bioavailability. **(Semalty et al., 2009)**

f. Pharmacosomes are also used for the development of novel ophthalmic dosage forms. **(Sailaja Krishna A, 2016)**

g. Pharmacosomes possess wider degree of selectivity for effect on specific target cells. **(Sailaja Krishna A, 2016)**

h. Pharmacosomes are used to improve bioavailability and avoid P-GP efflux and also used to improve of Solubility as well as permeability. **(Bhingare et al., 2014)**

i. Development of Pharmacosomes can improve the therapeutic efficacy of various drugs like pindolol diglyceride, amoxicillin etc. **(Rangha et al., 2013)**

Table 4: Therapeutic activity of drugs after incorporation in Pharmacosomes (Jha et al., 2011 and Chandra et al., 2014)

S.No.	Drug	Effect of drug in Pharmacosomes
1.	Amoxicillin	Improved cytoprotection and treatment of *H.pylori* infections in male rats
2.	Dermatan sulphate	Improved biological activity
3.	Bupranolol hydrochloride	Enhanced effect on intraocular pressure Enhance lymph transport
4.	Pindolol diglyceride	Three to five fold increase in plasma concentration Lower renal clearance
5.	Cytarbin, taxol	Improved biological activity

Conclusion

Pharmacosomes are unique potential novel vesicular system. They reduce toxicity, side effect having prolongation existence to give a sustained drug release and also used as targeted drug delivery system. Pharmacosomes are not only having high entrapment efficiency but also having predetermined release of drug. Because drug covalently bound with phospholipid, pharmacosomes having unique advantage over than other vesicular system like liposomes, niosomes, and transferosomes. Pharmacosomes have unique as potential system, and convenience of the vesicular system can be exploited by extending this approach to additional drugs. They are physically and chemically more stable than other conventional vesicular systems due to linkage of drug (therapeutic agent) with carrier like Phospholipids. These systems help to improve the biological activity of drug to achieve therapeutic goal.

References

[1] Fan J, Dai Y, Shen H, Ju J, Zhao Z. Application of Soluplus to Improve the Flowability and Dissolution of Baicalein Phospholipid Complex. Molecules. 2017;22:776

[2] Jena S, K, Singh C, Dora C, P, Suresh S, Development of tamoxifen-phospholipid complex: novel approach for improving solubility and bioavailability. Int J Pharm. 2014;473:1-9.

[3] Telange D R, Patil A T, Pethe A M, Fegade H, Anand S, Dave V S. Formulation and characterization of an apigenin-phospholipid phytosome (APLC) for improved solubility, in vivo bioavailability, and antioxidant potential. Eur J Phar Sci. 2016;108:36-49.

[4] Fricker G, Kromp T, Wendel A, Blume A, Zirkel J, Rebmann H, Müller-Goymann C. Phospholipids and lipid-based formulations in oral drug delivery. Pharm Res. 2010;27:1469-1486.

[5] Damle M, Mallya R. Development and evaluation of a novel delivery system containing phytophospholipid complex for skin aging. AAPS Pharm Sci Tech. 2016;17:607-617.

[6] Singh C, Bhatt TD, Gill MS, Suresh S. Novel rifampicin–phospholipid complex for tubercular therapy: synthesis, physicochemical characterization and in-vivo evaluation. Int J Pharm. 2014;460:220-227.

[7] Habbu P, Madagundi S, Kulkarni R, Jadav S, Vanakudri R, and Kulkarni V. Preparation and evaluation of Bacopa–phospholipid complex for antiamnesic activity in rodents. Drug Invention Today. 2013;5:13-21.

[8] Kassem AA, El-Alim SHA, Basha M, Salama A. Phospholipid complex enriched micelles: a novel drug delivery approach for

promoting the antidiabetic effect of repaglinide. Eur J Phar Sci. 2017;99:75-84.

[9] Semalty A, Semalty M, Singh D, Rawat M S M. Development and characterization of aspirin-phospholipid complex for improved drug delivery. Int J Pharm Sci and Nanotech. 2010;3:940-947.

[10] Semalty A, Semalty M, Rawat B S, Singh D, Rawat MSM. Pharmacosomes: the lipid based new drug delivery system. Expert Opinion Drug Deliv. 2009;6:599-612.

[11] Bhattacharya S. Phytosomes: the new technology for enhancement of bioavailability of botanicals and nutraceuticals. Int J Health Res. 2009;2:225-232.

[12] Singh DSM, Rawat M, Semalty A, Semalty M. Quercetin-phospholipid complex: an amorphous pharmaceutical system in herbal drug delivery. C Drug Dis Tech. 2012;9:17-24.

[13] Reis A, Rudnitskaya A, Blackburn G J, Fauzi N M, Pitt AR, Spickett CMA. comparison of five lipid extraction solvent systems for lipidomic studies of human LDL. J Lipid Res. 2013;54:1812-1824.

[14] Changediya V, Khadke M, and Devdhe S. Phytosomes: new approach for delivering herbal drug with improved bioavailability. Res J Pharm. Bio Chem Sci. 2011;3:57-68.

[15] Suriyakala PC, Satheesh babu N, Senthil rajan D, Prabakaran L. Phospholipid as versatile carrier in drug delivery system. Int J Pharm Sci. 2014;6:8-11.

[16] Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, and Deng Y. A review on phospholipids and their main applications in drug delivery systems. Asian J pharm. sci, 2015;10:81-98.

[17] Pathan R, Bhandari U. Preparation characterization of embelin phospholipid complex as effective drug delivery tool, J. Incl. Phenom. Macrocycl. Chem.2011;69:139–141

[18] Kale S N, Deore, S L. Emulsion micro emulsion and nano emulsion: A review. Systematic Rev. Pharm, 2017;8:39.

[19] Wu Z, Guo D, Deng L, Zhang Y, Yang Q, Chen J. Preparation and evaluation of a self-emulsifying drug delivery system of etoposide–phospholipid complex. Drug dev. Ind. Pharm. 2011;37:103-112.

[20] Khan M S, Krishnaraj K. Phospholipids: a novel adjuvant in herbal drug delivery systems. Critical Reviews™ Thera. Drug Carrier Systems, 2014;31:5.

[21] Hoogevest V P. Review–An update on the use of oral phospholipid excipients. European J Pharm Sci, 2017;108:1-12.

[22] Van Hoogevest P, Wendel A. The use of natural and synthetic phospholipids as pharmaceutical excipients. European journal of lipid science and technology. 2014;116(9):1088-1107.

[23] Desai N R, Agha B J, Kale K M. US6074666 2000.

[24] Khan J, Alexander A, Saraf S, Saraf S. Recent advances and future prospects of phyto-phospholipid complexation technique for improving pharmacokinetic profile of plant actives. J Control rel. 2013;168:50-60.

[25] Qin L, Niu Y, Wang Y, Chen X. Combination of Phospholipid Complex and Submicron Emulsion Techniques for Improving Oral Bioavailability and Therapeutic Efficacy of Water-Insoluble Drug. Mol pharm. 2018;15:1238-1247.

[26] Weinheimer M, Fricker G, Burhenne J, Mylius P, Schubert R. The application of P-GP inhibiting phospholipids as novel oral bioavailability enhancers an in vitro and in vivo comparison. Eur J Phar Sci. 2017;108:13-22.

[27] Gnananath K, Nataraj KS, Rao BG. Phospholipid Complex Technique for Superior Bioavailability of Phytoco. Adv Pharm bull. 2017;7:35.

[28] Zhou Y, Dong W, Ye J, Hao H, Zhou J, Wang R, Liu Y. A novel matrix dispersion based on phospholipid complex for improving oral bioavailability of baicalein: preparation, in vitro and in vivo evaluations. Drug del. 2017;24:720-728.

[29] Bombardelli E, Spelta M. Phospholipidpolyphenol complexes: a new concept in skin care ingredients, Cosm Toil. 1991;106:69-76.

[30] Rewar S, Mirdha D, Rewar P. A vital role of pharmacosomes on controlled and novel drug delivery. www ijapbs com. 2015;4:163-170.

[31] Biju SS, Talegaonkar S, Mishra PR, Khar RK. Vesicular systems: an overview. Ind J Pharm Sci. 2006;68:141-153.

[32] Goyal T, Rawat AS, chauhan M. Pharmacosomes: opening new doors for drug delivery. Int J Pharm Sci. 2012;4:25-29.

[33] Rangha S, Kumar A. “A review on pharmacosomes”. Research and review: J pharm Sci. and nanot 2013;2:2347-7849.

[34] Kumar PT, Mishra J, Podder A. Design, fabrication and evaluation of rosuvastatin pharmacosome-a novel sustained release drug delivery system. World Health Organization (WHO) et al. General notes on Biopharmaceutics Classification System (BCS)-based biowaiver applications. Accessed November. 2016;23:332-350.

[35] Kavitha D, Naga Sowjanya J, Panaganti S. Pharmacosomes: an emerging vesicular system. Int J Pharm Sci Rev Res. 2010;5:168–171.

[36] Pandita A, Sharma P. Pharmacosomes: an emerging novel vesicular drug delivery system for poorly soluble synthetic and herbal drugs. Hindawi ISRN phar. 2013;1-10.

[37] Al-kaf AGA, Othman AM. A review on pharmacosomes: an emerging novel vesicular drug delivery system. Uni J Pharm res. 2017;2:33-36.

[38] Venkatesh DN, Kalyani K, Tulasi K, Priyanka VS, Ali SA, Kumar SS. Pharmacosomes: a potential vesicular drug delivery system. Int J Pharm. Sci Drug Res. 2014;6:90-94.

[39] Sharma PH, Powar PV, Sharma SS. Pharmacosomes: A novel drug delivery system. The Pharma Innovation. 2014;3:94.

[40] Sailaja AK. Pharmacosomes: A novel carrier for drug delivery. Innoriginal: Int J of Sci. 2016;8-10.

[41] Kaur C, Goel V, Devi R, Kumar A, Nautiyal U. A review on current scenario on pharmacosomes. Int J Pharm Med Res. 2017;5:412-419.

[42] Thakur S, Tibrewal R. Pharmacosomes: an overview. Int J Pharm and biol sci archive. 2017;5:1-7.

[43] Bhingare U, Khadabadi SS, Shinde N. Pharmacosomes: A Novel Drug Del. System. Int J. 2014;3:14-20.

[44] Mali K, Diraj DB, Kiran B, Wagh K. Formulation and evaluation of pharmacosomes of ketoprofen. Indo Ame J of Pharm Res. 2014;4:1363-1368.

[45] Kumar DP, Pharmacosomes AD. A potential vesicular drug delivery system. Int Res J Pharm. 2012;3:102-105.

[46] Maiti K, Mukherjee K, Gantait A, Saha B P, Mukherjee PK. Curcumin–phospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm. 2007;330:155-163.

[47] Cheng W, Li X, Zhang C, Chen W, Yuan H, Xu S. Preparation and In Vivo-In Vitro Evaluation of Polydatin-Phospholipid Complex with Improved Dissolution and Bioavailability. Int. J Drug Del Res. 2017:9:39-43.

[48] Tan Q, Liu S, Chen X, Wu M, Wang H, Yin H, Zhang J. Design and evaluation of a novel evodiamine-phospholipid complex for improved oral bioavailability. Aaps Pharm sci tech. 2012; 13:534-547.

[49] Letha S, Shammika P, Viswanad V. Available Online through Research Article.

[50] Taskintuna I, Banker AS, Flores-Aguilar M, Bergeron-Lynn G, Aldern KA, Hostetler KY, Freeman WR. Evaluation of a novel lipid prodrug for intraocular drug delivery: effect of acyclovir diphosphate dimyristoylglycerol in a rabbit model with herpes simplex virus-1 retinitis. Retina (Philadelphia, Pa). 1997;17(1):57-64.

[51] Jadhav SM, Morey P, Karpe M, Kadam V. Novel vesicular system: An overview. J of applied pharm sci.2012;2:193-202.

[52] Chandra D, Yadav KM K, Singh Kumar V, Patel A, Chaurasia Shashi. .An overview: the novel carrier for vesicular drug delivery system.World J phar res. 2014;3:1299-1322.

[53] Kumar R, Kumar S, Jha SS, Jha AK. Vesicular system-carrier for drug delivery. Der Pharmacia Sinica. 2011;2(4):192-202.