AAPS PharmSciTech (2019) 20: 43
DOI: 10.1208/s12249-018-1252-4

Review Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery
Guest Editor: Sanyog Jain

Drug-Phospholipid Complex—a Go Through Strategy for Enhanced Oral Bioavailability

Kaushik Kuche,[1] Nallamothu Bhargavi,[1] Chander Parkash Dora,[1] and Sanyog Jain[1,2]

Received 10 September 2018; accepted 16 November 2018; published online 4 January 2019

Abstract. Among many, the oral route of delivery is considered to be the most favorable route with the highest patient compliance. The main issue with oral delivery is the environmental vulnerability of gastro intestinal tract (G.I.T). The bioavailability could further decrease when drug has poor aqueous solubility and permeability through biological membrane. This drawback could be resolved by employing drug-phospholipid complex strategy, as they utilize mechanism which is similar to the absorption mechanism of nutritional constituents form G.I.T. The drug-phospholipid complexes are considered ideal for oral delivery as they are biodegradable and non-toxic, which enable them to be employed as solubilizer, emulsifier, and as a matrix forming excipient for dugs with poor solubility and/or permeability. The present review compiles the basic know how about the phospholipids and the mechanism through which it improves the bioavailability of drugs. Further, it also compiles the crucial formulation aspects and methods of preparations of drug-phospholipid complex along with its physical and *in silico* characterization techniques. The increase in number of recent reports involving the utilization of drug-phospholipid complex to improve oral bioavailability of drugs thus explains how vital the strategy is for a successful oral delivery.

KEY WORDS: oral bioavailability; phospholipid; drug-phospholipid complex; characterization.

INTRODUCTION

In the present scenario, there are several drugs either from the synthetic or natural origin which suffers from low oral bioavailability. This might be attributed either due to the limited solubility and/or due to permeability of drug across the biological membrane (1). Several approaches have been applied to increase bioavailability of drugs which are well reported in the literature (2–6). Out of this lipid-based approaches, *i.e.*, liposomes, solid lipid nanoparticles (SLNs), phytosomes *etc.*, have gained more attention as they can resolve major drawbacks related to drug delivery. However, there are some drawbacks, like in case of liposome problems like drug leakage, low drug loading capacity, and poor stability that remain to be a major concern. Similarly, low inherent incorporation rate, high tendency for drug expulsion, and unpredictable gelation tendency of SLNs restrict their overall utilization (7). Among these, the drug-phospholipid complexes serve to resolve major drawbacks of the existing methodology. For instance, entrapment efficiency is not a major concern because drug itself forms a complex with the lipids *via* covalent or non-covalent interactions. Since the drug is complexed with lipids, possibility of drug leakage is not common; also, physicochemical stability of formed complex depends upon the strength of drug lipid interaction. Such interactions can be screened *in silico* prior to the development of a stable delivery system (8,9).

Although many reviews already exist in literature which embark on several lipid delivery systems, reports focusing on drug-phospholipids are scarcely found (10). Thus, the current review emphasizes on mechanism of absorption, pre-formulation aspects, method of preparation, characterization, and current trends involved in drug-phospholipid complex formation. The manuscript also expounds on advance strategies to overcome the lacuna's associated with drug-phospholipid complexes.

Kaushik Kuche and Nallamothu Bhargavi contributed equally to this work.

Guest Editor: Sanyog Jain

[1] Centre for Pharmaceutical Nanotechnology, Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Sec-67, S.A.S., Nagar, Punjab 160062, India.
[2] To whom correspondence should be addressed. (e–mail: sanyogjain@niper.ac.in; sanyogjain@rediffmail.com)

PHOSPHOLIPIDS: A BRIEF DISCUSSION ON ITS STRUCTURAL AND PHYSIOLOGICAL ATTRIBUTES

The phospholipids are amphiphilic and zwitterionic molecules and are considered to be an important component of cell plasma membrane (11). It gains its amphiphilic nature due to the presence of hydrophilic (head region) region composed of phosphate group which is negatively charged and a hydrophobic region (tail region) composed of long chain fatty acids. These head and tail regions are connected by glycerol or alcohol group, thereby allowing them to form a lipid bilayer in biological systems. Broadly, the phospholipids can be bifurcated in two types, *i.e.*, glycerophospholipids and sphingophospholipids based on the alcohol it possesses. Glycerophospholipids possess glycerol in the neck region whereas sphingomyelins possess sphingosine as their alcoholic moiety (12). Glycerophospholipids can be further classified based on to the length and saturation of hydrophobic group, head group, type of bond present, and upon the number of fatty acid chains attached as depicted under the Fig. 1**.**

Considering the physiological actions of phospholipids, they are widely spread in mammals and plants as they are among the essential components of cellular membrane (13). Among the essential activities which phospholipid play is assembling the formation of circulating lipoproteins, which functions to transport the triglycerides and cholesterols through the blood (13). Phospholipids along with cholesterol and bile salts can act as emulsifiers and can form mixed micelles which in turn enhance the absorption of lipophilic substances (14,15). This emulsifying properties of phospholipids are being utilized in the pleural layers and alveoli of lung, pericardium, joints, *etc.* (16). However, each phospholipid has its unique physiological implications which can be employed depending on the required morphology and physical attributes.

Discussing the physical aspects of phospholipids, they show different types of the assembly which is characteristic of their molecular conformation and shape (17). For instance, the lysophospholipids have single long chain fatty acid and a bulky head group which provides it an inverted cone like molecular shape. The glycerol-phospholipids (Fig. 1b) show cylindrical molecular contour which allows them to attain a suitable geometry to form lipid bilayers. Similarly, other phospholipids like unsaturated phosphatidylcholine, phosphatidic acid, and phosphatidylserine below pH 3 tend to show cone-shaped molecular geometry (18). Thus, a thorough understanding of crucial geometry and morphology of involved lipids is essential in developing desired lipid-based delivery system.

In our literature review, it was observed that researchers have majorly employed phosphatidylcholine to form drug complex. The probable reason apart from the abundance of choline phospholipids in eukaryotic cells and its ability to from covalent or ionic drug complex is the morphology of phosphatidylcholine. As the presence of choline increases the volume of the head group which enables it to attain truncated cone (critical packing factor lies between ½ to 1) like shape. Thus, possession of such truncated geometry assists them to form flexible bilayer like structures. Also, due to the fact that phosphatidylcholine being a zero curvature lipid, this helps it in forming a stable bilayer vesicle in GIT, thereby enhancing the solubility and permeation of complexed drug (19). In case of ethanolamine phospholipids, they have a small head group which enables it to be a non-bilayer prone lipid with a negative curvature. Such conformational characteristics restrict it to form stable micelles alone in GIT (20,21). Although other phospholipids like phosphatidylserine and phosphatidylinositol show similar morphology as that of phosphatidylcholine, seldom reports using these phospholipids are found.

A COMPARISON-LIPOSOME/PHYTOSOMES/ PHARMACOSOMES

Whenever phospholipids as a delivery vehicle are talked about, there is a general misunderstanding about liposomes, phytosomes, and pharmacosomes. Thus, in order to avoid such foul conception, a brief comparison between the three is mentioned in this section. Broadly, phytosomes are considered to be a herbal drug-based delivery vehicle in which the phytoconstituent extracted from the crude drug is chemically bonded to the phosphatidylcholine (head region) part. This bonding enables the phytoconstituent to show better absorption than its conventional form (22). In case of pharmacosomes which also fall under the category of phospholipid complexes in which the drug (having –COOH, –NH2, and –OH as active groups) is covalently linked to lipid molecules, which then assembles to form vesicles (8,23). The main concept of pharmacosomes to form lies on the surface and bulk interaction of phospholipids with water (24). Thus, in pharmacosomes, the attached drug moiety itself behaves as a polar head and the lipid moiety as a lipophilic region which enables them to form micelles and enhance dissolution and permeation profile of drugs (25). Although the liposomes, phytosomes, and pharmcosomes are technically similar, the crucial parameters on which they differ are listed in Table I.

ORAL BIOAVAILABILITY ENHANCEMENT USING A PHOSPHOLIPID COMPLEX: A MECHANISTIC OUTLOOK

Generally, drugs with poor solubility (BCS class II) or with poor permeability (BCS classes III and IV), when given orally, show relatively low bioavailability (26). Additionally, other reasons for low bioavailability are presence of P-gp pump which causes the efflux of naked drugs, the presence of metabolizing enzymes, and the environmental pH-mediated degradation (27). Thus, delivery vehicles are essential for drugs so as to attain a desired level in the systemic circulation (28). Phospholipid-drug complex could be used for the same, in which the absorption process is similar to the process through which the triglycerides and essential phospholipids are absorbed. The mechanism for absorption of the phospholipid-drug complex is similar to that of the endogenous absorption of phospholipids through enterocytes (29). Structurally, the phospholipid comprises two fatty acid chain attached to the glycerol (diacyl glycerol) moiety, which undergoes hydrolysis to release fatty acid, which triggers its absorption. Similarly, the drug-diacyl glycerol complex when taken *via* oral route undergoes hydrolysis. Minor hydrolysis occurs in the stomach at pH of ~ 1.5, and majority of it occurs in the intestine starting from duodenum in which secretions from the liver, bile bladder, and pancreas in the form of juice are secreted (30,31).

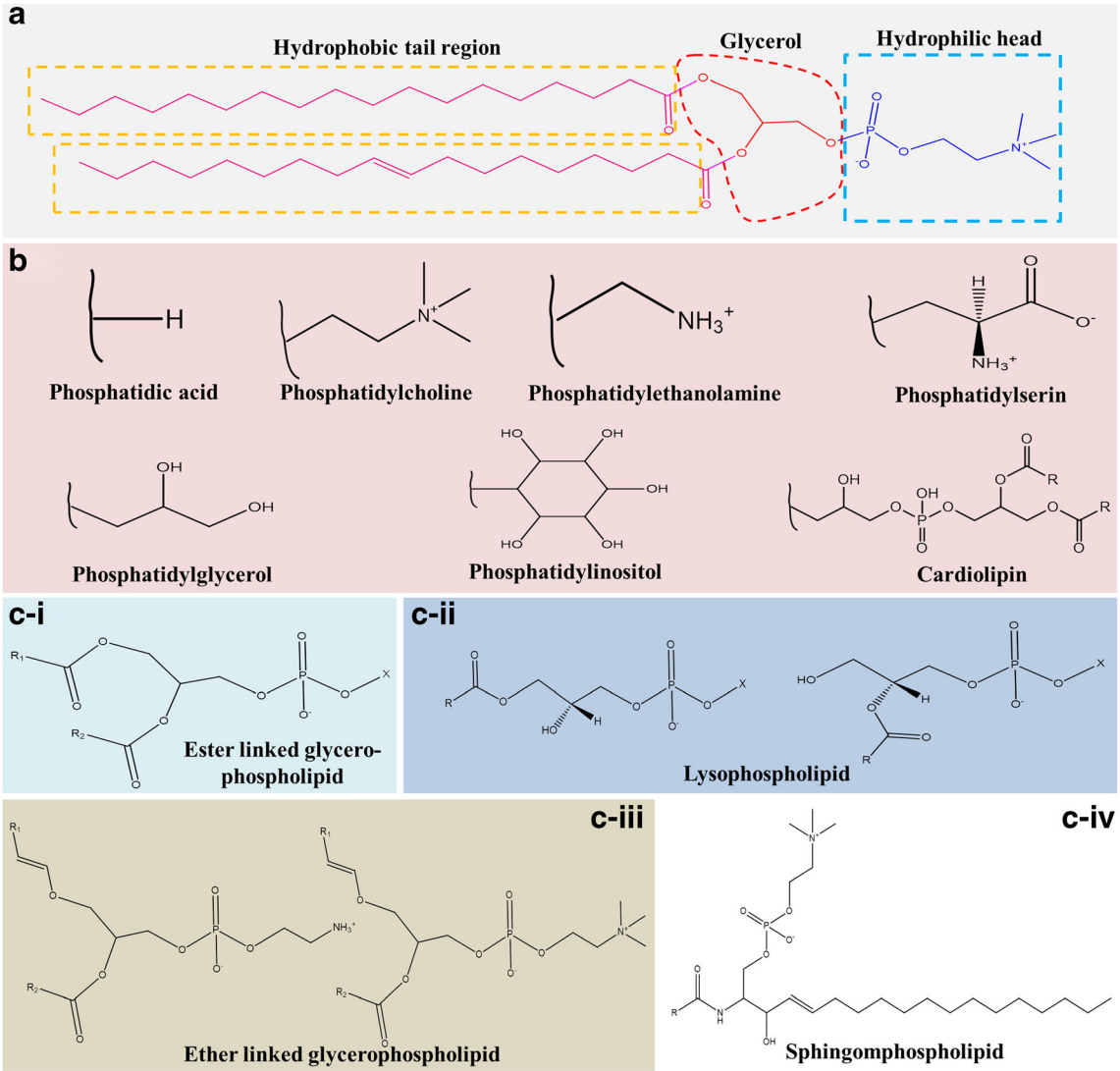

Fig. 1. Structural aspects of glycerophospholipids. **a** The polar head and hydrophobic tail groups attached via glycerol to form glycerophospholipid. **b** The types of phospholipids based on the polar head groups. **c-I**, **II**, **III** and **IV** The types of phospholipids based on the type of bond present in glycerophsopholipids

In the intestine, the hydrolysis of drug-diacyl glycerol occurs due to the presence of phospholipases (particularly phospholipase A_2) which leads to release of fatty acid and form drug-monoacyl glycerol. The former drug-monoacyl glycerol along with the bile salts then forms micellar vehicles. For these micelles to form, excretion of bile into the duodenum is crucial which is regulated by a hormone called cholecystokinin (CCK) which is released when a higher concentration of fatty acids are formed by hydrolysis of diacyl-glycerophospholipids and triglycerides (30,32). However, the minor hydrolysis which occurs in the stomach tends to release the fatty acid which initially triggers the CCK release which further regulates the release of bile acids and salts. Once the hydrolysis is done, the drug-monoacyl phospholipid vesicles are then taken up by passive diffusion by enterocytes. The enzymes in smooth endoplasmic reticulum of enterocytes, *i.e.*, acyl-CoA convert drug-monoacyl phospholipids and endogenous diglycerides to diacyl phospholipids and triglycerides, respectively. Further, in golgi apparatus, apoportein B-48 is integrated into the phospholipid vesicle to form nascent chylomicron (33). The chylomicron exits enterocyte *via* exocytosis through the basal membrane and enters lacteal (lymph capillary) which then transports it away from the intestine and bypasses the first pass metabolism. The chylomicrons deliver the drug complex into systemic circulation at thoracic duct connection with a left subclavian vein (34). Once nascent chylomicron enters the systemic circulation, it is converted to mature chylomicron when high density lipoprotein transfers apolipoprotein C-II and apolipoprotien E to the nascent one (33–35).

After the triglycerides are stored, the matured chylomicron returns back the apolipoprotein C-II, and then, they are termed as chylomicron remnant, which is generally present in the liver for endocytosis and breakdown (35). Hence, through the chylomicron, the drug phospholipid complex enters the systemic circulation and bypasses the first pass metabolism. This mechanism of the drug-phospholipid complex allows the absorption of drugs which

Table I. Parameters Based on Which the Liposomes Phytosomes and Pharmacosomes Are Differentiated

Parameter	Liposomes	Phytosomes	Pharmacosomes
Chemical bonding	Not present	Covalent bonding with the head group	Strong hydrogen or a covalent bond
Formation time	Time consuming	Time consuming	Less time consuming
Drug leakage	Yes	No	No
Entrapment efficiency	Low	High	High
Stability	Less	High	High
Drug release	Diffusion	Hydrolysis	Hydrolysis and/or diffusion
Mode of administration	Topical or oral	Topical or oral	Topical, oral or intravascular
Components for formualtion	Phospholipids (natural or synthetic) + cholesterol	Phospholipids + phytoconstituent	Phospholipid (natural or synthetic)

are either not soluble or has shown extensive first pass metabolism. The schematic illustration of the same is depicted in Fig. 2.

FORMULATION ASPECTS

Drug

For an active moiety (drug) to form phospholipid complex, it should have active hydrogens like COOH, NH_2, OH, and NH bonds to form ester bond with the lipid. However, reports are also found for hydrophobic interaction between the drug and phospholipid to form complex. Experimental studies have revealed the fact that molecules possessing conjugated systems of π electrons are capable of forming different type of complexes with phospholipids. For example, Afanaseva et al. have studied the interactions of different flavonoid derivatives with phospholipids using ^{13}C NMR and quantum calculations. They suggested that flavonoids tend to interact with phospholipids through π electron systems (36–39).

Drug/Phospholipid Ratio

The ratios in which the drug and phospholipid are mixed sometimes depend on the interacting groups or functional

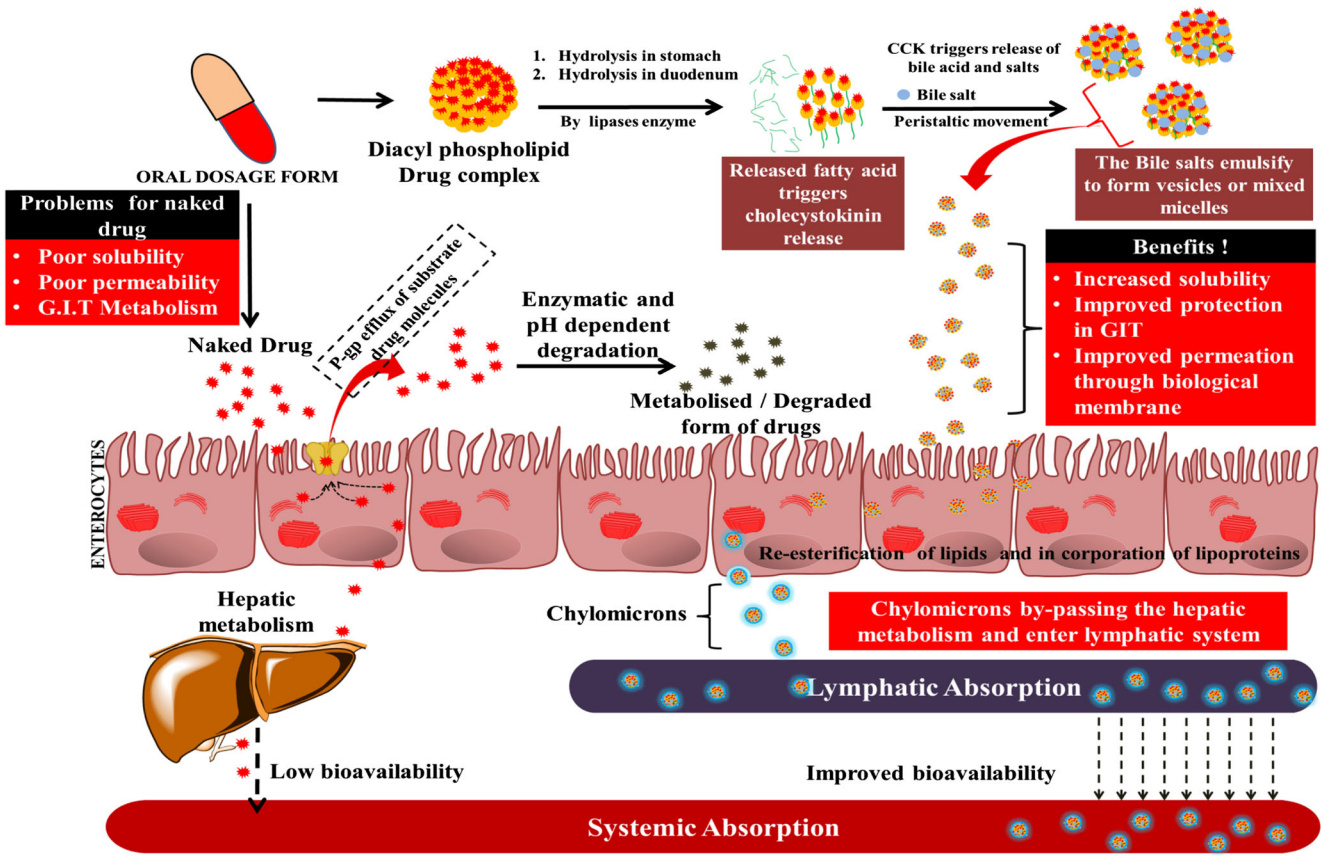

Fig. 2. The general mechanism of drug phospholipid complex to enhance bioavailability. This is the brief illustration of phospholipid-drug complex absorption through which it enhances the oral bioavailability of drugs

groups involved in complex formation. The stoichiometric ratios of drug/phospholipid should be selected appropriately in order to achieve higher drug loading. An ideal ratio of 1:1 has been reported almost in all cases (40). However, different stoichiometric ratios of drug-phospholipid have been tried in order to achieve stability and the highest drug loading of the complex (41,42).

Solvents

Selection of solvent is one of the critical parameters while preparing drug and phospholipid complex. The solvent should be selected based on the solubility of both drug and phospholipid. Moreover, sometimes, it is difficult to dissolve the drug and the phospholipid in the same solvent; in that case, the mixture of solvents should be used to prepare the complex. It has been reported that the solvents which have high dielectric constant may actuate a greater change in electric potential and can lower the interactions between the molecules. However, phospholipid complexes can be prepared by using methanol and ethanol as solvents (which have dielectric constants of 24.5 and 32.7, respectively) (43).

Method of Preparation

The following section contains widely used preparation methods for drug and phospholipid complexes. A brief information about their advantages and limitations is mentioned in the Table II.

Solvent Evaporation Method

Widely used preparation method for drug phospholipid complex is solvent evaporation method. In this method, the compound of interest and phospholipid are dissolved in solvent or mixture of solvents which are then refluxed for a certain period of time and then evaporated by using rota evaporator (47). The solvent evaporation by rota evaporator works on the principle of boiling point reduction by application of vacuum, followed by rotation to increase the heating surface area to the solution. Its speed and ability to handle a large volume of solvents make rota evaporator a suitable method to cause complex formation. However, for evaporating high boiling point solvents like DMSO and DMF *etc.*, high pressure vacuum system is required to get desired boiling point depression.

Co-grinding

This method mainly involves external mechanical force to knead the drug and phospholipid together for complex formation. An elucidation for this is probucol phospholipid complex which was prepared by co-grinding method and was compared with solvent evaporation method. It was observed that solvent evaporation gave a high degree of drug complexation as compared to co-grinding method. Nevertheless, this method was found suitable for scale-up production (44).

Mechanical Dispersion Method

In mechanical dispersion method, the phospholipid is dissolved in solvent and is subjected for sonication for few to several minutes. Then, the drug solution is added drop wise into the solution continuously while sonication. The example illustrating the application of mechanical dispersion to form drug-phosphoipid is well depicted in an investigation done by Sikarwar *et al.* (45). The authors developed marsupin phospholipid complex using mechanical dispersion which proved to be stable and efficient in improving the bioavailability.

Super Critical Fluid Process

SCF technologies are a promising technique as they can be used to produce particles of controlled size and distribution. This process can be performed at mild conditions of temperature and pressure. Apart from this, it is also environment friendly as compared to the process involving organic solvents. Carbon dioxide is the most widely used supercritical fluid because it has critical temperature of 31°C and critical pressure of 74 bar, allowing it to be used at mild

Table II. Methods to Prepare Drug Phospholipid Complex

Technique used	Methodology	Remarks	Ref
Solvent evaporation	Drug and phospholipids are mixed in solvent/mixture of solvents in different ratios followed by solvent evaporation by rota evaporator	Simple technique and high yield Selection of solvent is difficult	(42)
Super critical fluid (SCF) process	Using Supercritical fluids	Green technique, super critical fluid CO_2 causes plasticization of lipid structures; thermodynamically stable dispersion and costly	(44)
Co-solvent lyophilization	Solvent evaporation by freeze drying	High yield and costly	(45)
Anti-solvent precipitation	Use of anti-solvent to precipitate drug phospholipid complex	Low yield	(46)
Mechanical dispersion/sonication	Sonicator/High shear homogenizer	Low yield and energy consuming	(47)
Co-grinding	Drug and phospholipid are simply grinded in motar	Lower degree of complexation than other methods	(43)

temperature conditions (40–60°C). However, this technology has certain limitations like limited solubility of polar compounds in supercritical CO_2. Li et al. (46) reported puerarin phospholipid complex by this method and compared it with conventional methods like solvent evaporation, freeze drying, and gas anti-solvent crystallization. They claimed that the phospholipid complex formed by supercritical fluid technology showed more dissolution efficiency as compared to the other three methods due to their higher ability to cause amorphization of the drug.

Co-solvent Lyophilization

The principle involved in the lyophilization method is sublimation (removal of water from the frozen state without liquid phase). Lyophilization performed at temperature and pressure conditions below triple point, which enables sublimation of ice. Three steps involved in lyophilization process include freezing stage, primary drying, and secondary drying. An example describing the use of co-solvent lyophilization to form drug-phospholipid complex is explained in a study conducted by Cui *et al.* (48). In their investigation, authors developed insulin phospholipid complex which was then characterized by solubilization, IR, and X-ray diffraction. These mentioned characterization studies cumulatively helped in confirming the formation of drug-phospholipid complex.

Anti-solvent Precipitation

In anti-solvent method, the drug and the phospholipid are dissolved in solvent and refluxed for particular time followed by precipitation using anti-solvent, which has limited solubility for the formed complex. Anti-solvent precipitation method can be performed at ambient temperature and pressure without using expensive equipment. This process is well elaborated in a study reported by Murugan et al. (49). The authors reported ellagic acid phospholipid complex by anti-solvent precipitation method using DCM as solvent and n-hexane as anti-solvent to precipitate complex. Both DSC and TEM analysis collectively confirmed the formation of drug-phospholipid complex.

Characterization

There are several characterization tools available for characterizing drug-phospholipid complexes. Few of them are enlisted in Fig. 3 and discussed below.

Morphology

Electron microscopy techniques, scanning electron microscopy, and transmission electron microscopy (SEM and TEM) provide qualitative information about size and shape of self-assembled drug phospholipid complex. For example in a study conducted by our group to develop gemicitabine-phospholipid complex (50), SEM and TEM were used as an analyzing tool to confirm the morphology of prepared complex (Fig. 4). The SEM analysis revealed rough and porous images of the formed complex when compared to the images obtained for pure crystalline drug and lipid (Fig. 4a (i-iv)). Similarly, the TEM images revealed the formation of uniform micellar shape vesicles with an inner dark core surrounded by lighter striations which were composed of phospholipids (Fig. 4b (i and ii)).

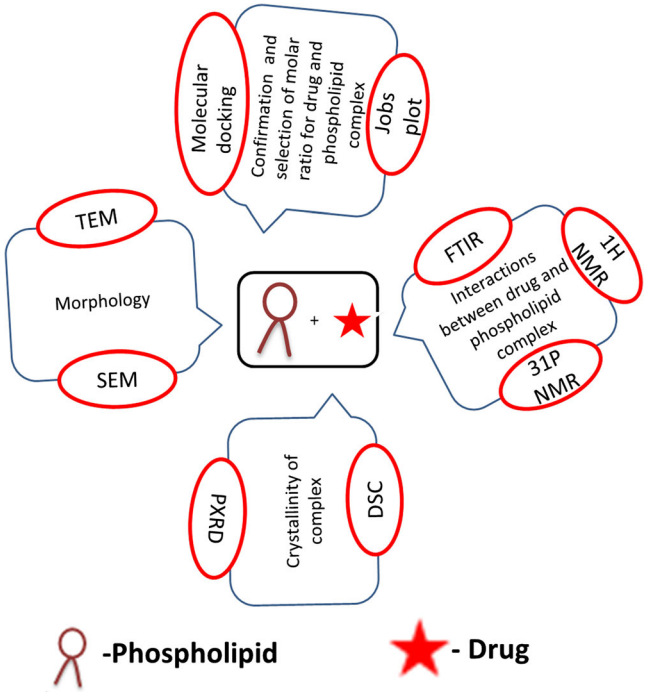

Fig. 3. Characterization of drug and phospholipid complex

Interaction Between Drug and Phospholipid Complex

Infrared Spectroscopy. IR is the primary tool to predict any interactions between the drug and phospholipid on the basis of functional groups (52). While comparing the change in functional groups, the spectra of individual components and physical mixture are superimposed to predict the complex formation. Generally, the drug phospholipid complex formation is indicated by broadening and shifting of characteristic peaks. Such shifting of peaks suggests that there are typical interactions between them while such type of interactions will not be possible in case of physical mixture. For better understanding, consider a case wherein rifampicin-phospholipid complex formation was confirmed using FTIR spectroscopy. The intensity of characteristic absorption peaks of drug at 1655 cm^{-1}, 1566 cm^{-1}, 1252 cm^{-1}, and 1430 cm^{-1} were shifted, whereas such changes were absent in case of physical mixture (53). This assured the presence of physical interactions between rifampicin and phospholipid to form a stable complex.

Nuclear Magnetic Resonance Spectroscopy. NMR is an important tool to confirm the complex formation by studying the magnetic properties of various nuclei like hydrogen, carbon, and phosphorous. Changes in the chemical shift of the important protons which involved in interactions can be observed in ^{1}H-NMR. The ^{31}P NMR chemical shifts are measured relative to phosphoric acid. The chemical shift of phosphorus group indicated interactions between the molecules. Apart from these, ^{31}P-NMR also reveals about

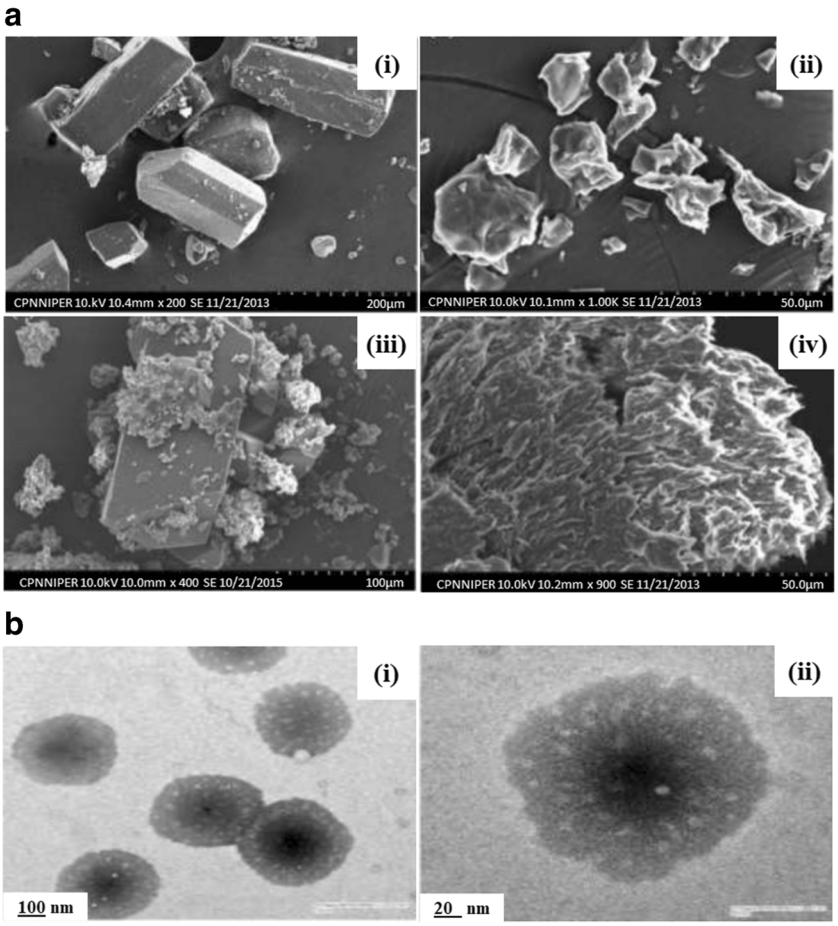

Fig. 4. SEM/TEM characterization images of gemcitabine drug-phospholipid complex. **a** The SEM images of (i) crystalline form of gemcitabine, (ii) phospholipid, (iii) physical mixture of drug and phospholipid, and (iv) is the drug-phospholipid complex. **b** (i, ii) The TEM images of inverted micelles of drug-phospholipid complex dispersed in aqueous solution (Reproduce with permission from (51) © Elsevier 2017)

the bilayer conformations which can differentiate the lipid system with phospholipid complex (54). For example, a study reported by our lab in which we employed ^{1}H-NMR and ^{31}P-NMR to confirm the formation of gemcitabine-phospholipid complex (50). The chemical shift of protons of methylene group attached to N-atom P–O–group (–P–O–CH2–) and –C (=O) –C group showed shift values of 3.45 δ (ppm), 3.62 δ (ppm), and 2.33 δ (ppm), respectively. Also, the aromatic pyrimidine protons of drug (gemcitabine) revealed chemical shift of δ 3.47 and 3.74 ppm, which were deshielded to show shifts at 3.79 δ and 3.82 δ (ppm), respectively, upon complex formation. This downfield shift ensured the possibility of interaction between gemcitabine and phospholipid. To further support the ^{1}H-NMR data, ^{31}P-NMR also revealed a phosphorous chemical shift from 0.5856 to 0.5976 upon the complex formation.

Crystallinity of Complex

In DSC, interaction is determined by elimination of endothermic peak, appearance of new peak, and change in peak onset, change in the melting point, and relative peak area or enthalpy (55). The crystalline drugs exhibit characteristic intense peaks in X-ray diffraction pattern while phospholipids, being amorphous, show wide peaks. The physical mixtures exhibit both sharp and wide peaks due to presence of both free drug and phospholipids. The disappearance or reduction in intensity of sharp peaks indicates the formed complex and reduction in crystallinity. For an instance take, an account of a report published by our group that was an attempt to prepare elrotinib-phospholipid complex was made (47). The complex formation resulted in reduced crystallinity and was confirmed using DSC and PXRD. The disappearance of sharp melting peaks of drug and characteristic lowering of Tg for phospholipid in DSC thermograph suggested the formation of amorphous state. Similarly, the PXRD data for drug phospholipid complex revealed the absence of sharp characteristic crystalline peaks of drug, thereby confirming the amorphization of drug upon complexation.

In Silico Estimation of Drug and Phospholipid Complexation

There are very few reports which showcase *in silico* estimation for phospholipid complex formation to get an insight of the mechanism involved in complex formation. In

an attempt to do so, our lab has reported *in silico* method to identify the molecular stability of the complex by using Molecular Mechanics Energy Relationship (MMER) along with Static Lattice Atomistic Simulations (SLAS). Molecular Mechanics Energy Relationship (MMER), a method for analytico-mathematical representation, provides energy relationships. Energy relationships can indicate about the contribution of valence terms, non-covalent columbic terms, and non-covalent van der Waals interaction between molecules which accounts from ideal bond angles and distances (56). The MMER model for potential energy factor in various molecular complexes can be written as

$$E_{molecular/complex} = V_{\sum} = V_b + V_\theta + V_\varphi + V_{ij} + V_{hb} + V_{el}$$

$V_{\sum}$ is related to total steric energy, V_b corresponds to the bond stretching contributions, V_θ denotes the bond angle contributions, V_φ represents the torsional contribution arising from deviations from optimum dihedral angles, V_{ij} incorporates van der Waals interactions due to non-bonded interatomic distances, V_{hb} symbolizes the hydrogen-bond energy function, and V_{el} stands for electrostatic energy.

In addition, the total potential energy deviation, ΔE_{total}, can be calculated as the difference between the total potential energy of the complex system and the sum of the potential energies of isolated individual molecules, as follows:

$$\Delta E_{total(A/B)} = E_{total}(A/B) - (E_{total}(A) + E_{total}(B))$$

The molecular stability can then be estimated by comparing the total potential energies of the isolated and complexed systems. If the total potential energy of the complex is smaller than the sum of the potential energies of individual molecules in the same conformation, then the complex is said to be more stable and formation is favored (51,57).

CURRENT TRENDS IN PHOSPHOLIPID-DRUG COMPLEXES

As there are several therapeutic moieties which have low bioavailability due to several reasons, the phospholipid complex is among the widely opted route to enhance the oral bioavailability. The statement is well justified by the number of research papers which have been published using the same concept for different applications. The approaches can be briefly divided in three categories depending on the mechanism through which it improves the oral bioavailability.

Solubility Enhancement

In this approach, drug-phospholipid complex is used to improve the solubility of drugs which thereby assists in enhancing its oral bioavailability. For example, pranlukast solubility was enhanced by ~ 20-fold in comparison to its raw crystalline form by preparing a nano suspension (58), whereas, in case of pranlukast phospholipid complex, the solubility was enhanced by ~ 150-fold which leads to 20-fold increase in its bioavailability as compared to its natural crystalline form (59). Another report published by Singh et al., (53) wherein the poor bioavailability of rifampicin due to its poor aqueous solubility was resolved by forming a complex with phosphatidinyl choline. The complex formed was confirmed by FTIR, DSC, and XRD and the formed complex showed 319% of relative bioavailability. This enhancement was mostly due to the enhanced solubility followed by enhanced absorption of rifampicin. Also, it is reported that rifampicin induces its own metabolism which leads to its low bioavailability (60). Thus, the complex enhances its absorption thereby decreasing its metabolism eventually, helping to enhance its oral bioavailability. Another observation done by Qin *et al.*, (61) wherein the low solubility of atorvastatin (which was the limiting factor for its bioavailability) was resolved by forming phospholipid complex. The atorvastatin phospholipid complex (ATR-PLC) was developed to form a submicron emulsion for oral delivery. The oral adsorption of ATR-PLC oral emulsion revealed 2.58-fold increment in plasma concentration with better pharmacodynamics activity in comparison to Lipitor (marketed product). A similar examination conducted by our group in which Erlotinib phospholipid complex was prepared to overcome its solubility related limitation for enhanced bioavailability (47). Erlotinib is an epidermal growth-factor receptor (EGFR) tyrosine kinase inhibitor which is orally active, but due to its low solubility, it is administered at a higher dose which leads to dose-dependent toxicity. Thus, prepared Erlotinib phospholipid complex (ERL-PC) in the form of nano structures revealed higher release of ERL-PC due to amorphization and enhanced solubility. This led to 1.7-fold higher bioavailability and superior efficacy of ERL-PC when compared to free ERL. Hence, explaining the role of phospholipid complex in enhancing the solubility and thereby promoting its bioavailability.

Increased Metabolic Stability

In this approach, the drug phospholipid complex enhances the bioavailability by providing the metabolic stability which assists in maintaining the desired therapeutic level. To investigate this approach, an investigation was conducted by our group (50), in which gemcitabine (GEM) phospholipid complex was formed, which was confirmed using FTIR, DSC, XRD, ^{1}H NMR, and ^{31}P NMR. As gemcitabine gets metabolized to form 2′-deoxy-2′, 2′-diflurouridine (dFdU) when given I.V. which leads to its poor bioavailability (62). Hence, *in vitro* plasma stability studies for GEM-phospholipid complex (GEM-PC) were performed, and it revealed that the GEM-PC was capable enough to protect GEM from metabolism and this substantiated the *in vivo* pharmacokinetics data which revealed 2-fold enhanced oral bioavailability in comparison to that of free GEM. Thus, the study reveals the capability of phospholipid to protect the metabolic conversion of drug and assisting to increase its bioavailability. A similar study was reported by Elnaggar *et al.,* (63) wherein daidzein, an anticancer drug for breast and prostate cancer, was complex with phosphatidylcholine. This complexation improved its solubility and permeation into the lymphatic system and thereby preventing it from undergoing hepatic metabolism which was the major reason for its low bioavailability. The oral bioavailability was enhanced by 2.38 times with minimum drug-induced intestinal irritation compared to free drug suspension. Thus, low bioavailability of drugs due to metabolism either in plasma or by first pass

metabolism could be well manipulated by forming phospholipid drug complexes.

Permeability Enhancement

This approach is generally utilized for the drugs which are not capable of showing required permeability across the biological membrane leading to their low bioavailability. This approach can be well explained through an investigation done by Ma *et al.*, (64) wherein mangiferin was complexed with lipoid E80 (egg source phosphatidylcholine 80%). Mangiferin has antitumor, antiviral, antidiabetic, anti-inflammatory, and immunomodulatory activities; thus, several approaches to enhance its bioavailability are already made, like mangiferin-HP-β-CD which showed 1.9-fold enhancement in bioavailability (65). But it only focused on increasing its solubility, and no manipulation for its permeability was done. Thus, the mangiferin phospholipid complex manipulated both the solubility and permeation which lead to 2.3-fold enhancement in oral bioavailability. Similar studies to improve the permeability of chlorogenic acid (CGA) (which is a qunic acid and caffeic acid ester) were explored by Li *et al.* (66). In their published report, the CGA was complexed with phosphatidylcholine (CGA-PC) so as to manipulate its permeation ability through GIT. The investigation showed a significant increment in the Tmax and Cmax of CGA and CGA-PC. The oral bioavailability enhancement of up to 3.8-fold was achieved by using this CGA-PC complex when compared to free CGA. Thus, concluding that the phospholipid complex not only manipulates the solubility but also the permeability of the drug which eventually helps in enhancing its oral bioavailability.

OVERCOMING THE LACUNA'S ASSOCIATED WITH DRUG PHOSPHOLIPID COMPLEX

Although phospholipid complex technology has broad potential, there are few limitations associated with this technology. The major issues related to them are their stability (*e.g.*, risk of aggregation and chemical degradation) and the drug leakage which can then lead to irregular pharmacokinetics. Research groups across the globe have explored various novel formulations loaded with phospholipid complex in order to overcome all these challenges associated with this system. Few examples of drug phospholipid complex loaded novel formulations are mentioned in Table III and Fig. 5.

Phospholipid Complex-Based Micro/Nanoemulsion

Micro/nanoemulsion-based systems are considered to be a superior type of micellar system in terms of its solubilization potential and thermodynamic stability (71). Hence, its ability to resolve the solubility and permeability-related issues of several class of drugs are well reported in literature (74). Thus, a combined technology of phospholipid complex and micro emulsion strategy came in to picture to overcome the formulation-related issues like drug leakage from emulsion system, pH-dependent solubility issues of drug and provide efficient bioavailability of drugs. This approach is well

Table III. Novel Formulation Approaches Involving Drug Phospholipid Complex

Drug	Phospholipid	Dosage form	Remarks	Ref
Micro/nanoemulsion				
Puerarin	Lecithin	W/O micro emulsion	Improved bioavailability	(62)
Dabigatran etexilate	Lipoid S75	Nanoemulsion	Sorts out pH dependent solubility issues	(63)
Urso deoxycholic acid	Phosphotidylcholine (60% *w*/w)	Submicro emulsion	Improved the hydrophilicity and lipophilic property	(67)
Self emulsifying drug delivery systems (SEDDS)				
Matrine	Soya phosphotidyl choline	SNEDDS	Improved lipophilicity for incorpaoration in to SEDDS	(64)
Baicalin	Phospholipids (Lipoid S-75)	Self-emulsifying micro emulsion	Enhanced oral bioavailability	(65)
Etoposide	Phospholipid	SEDDS	Enhanced oral bioavailability	(68)
Morin	Soya phospholipids	SNEDDS	Enhanced oral bioavailability	(69)
Scutellarin	Lipoid S75	Supersaturated SEDDS	Improved solubility and antitumor activity	(70)
Micelle				
20(S)-protopanaxadiol	Soyabean phospholipids	Micelle	Enhanced oral bioavailability	(66)
Apigenin	L-α-phosphotidylcholine	Micelle	Improved oral bioavailability	(71)
Bahuoside I	Phospholipids	Micelle	Enhanced oral bioavailability	(72)
Repaglinde	L-α-phosphotidylcholine	Micelle	Promoted anti diabetic effect	(73)
Other novel formulations				
Baohuoside	Phospholipids	Nanoscale complex	Improved oral bioavailability	(74)
Salvianolic acid	Soyabean phospholipid	Phospholipid complex in to nanoparticles	Improved oral bioavailability	(75)
Baicalein	Soyabean phospholipid	Matrix dispersion	Improved oral bioavailability	(76)
Rosuvastatin calcium	Phospholipon 90G	Nanolipospheres	Improved intestinal lymph targeting	(77)

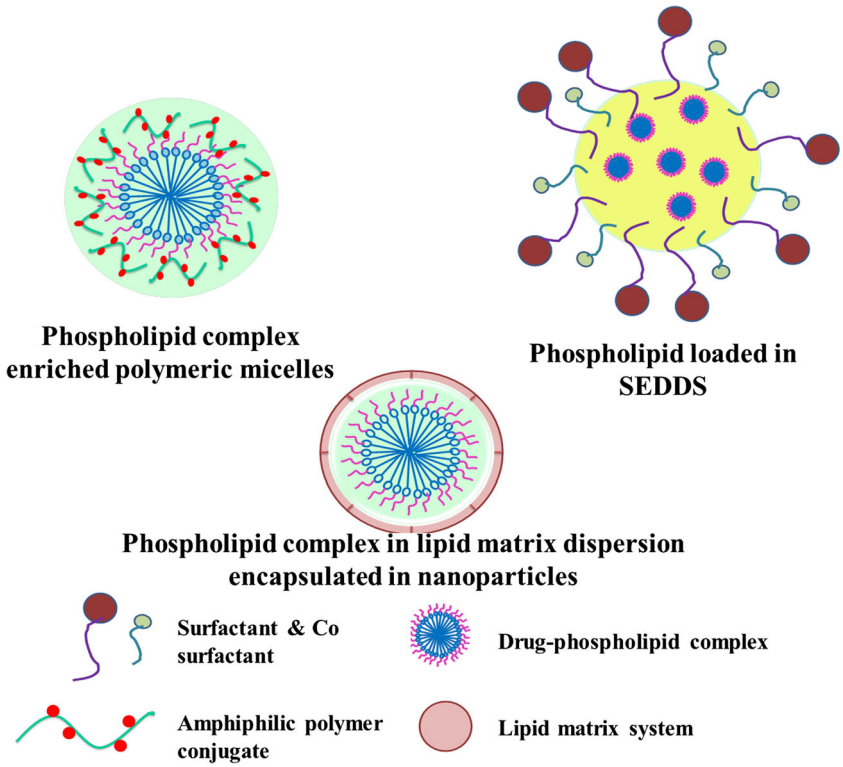

Fig. 5. Phospholipid complexes loaded novel formulations

elaborated in a study conducted using puerarin. Puerarin, a phytochemical, has solubility and bioavailability issues (67,77). In order to overcome these issues, Wu *et al.* (68) formulated puerarin phospholipid complex-based W/O emulsion utilizing both advantages of phospholipid complex and multiple emulsion system. They claimed that phospholipid complex increases the absorption of puerarin by preventing Pgp efflux while the microemulsion acts as an intestinal permeability enhancer. Pharmacokinetics data revealed that puerarin phospholipid complex-based microemulsion shows improved $AUC_{0\rightarrow\infty}$ and C_{max} compared to simple multiple emulsion and puerarin phospholipid complex alone. Another benefit of drug-phospholipid complex-based nanoemulsion is that the problems of pH-dependent solubility issues are resolved. Dabigatran etexilate has limitation of pH-dependent solubility, which is soluble in low pH and insoluble at neutral pH. Simple nanoemulsion-based formulation results in drug leakage through the oil phase in gastric fluids followed by precipitation once it reaches to intestinal phase. In order to overcome this, lipophilicity of drug is increased by phospholipid complex to prevent the drug leakage through the nanoemulsion in gastric fluids and thereby increases the dissolution efficiency in intestinal fluids (69).

Phospholipid Complex-Based SEDDS

In an investigation conducted (LBDD) system generally are composed of surfactant, lipid, and co-solvents. In this field, Pouton *et al.* (70,72) introduced a lipid formulation classification system (LFCS) which classifies the LBDD into four classes. According to this classification, self-emulsifying drug delivery system (SEDDS) falls under class II as they require a hydrophobic surfactant along with their oil component to attain greater stability, whereas self-microemulsifying (SMEDDS) or self-nanoemulsifying drug delivery systems (SNEDDS) composed of additional hydrophilic surfactants classify them to be under class III system.

The major difference between SMEDDS and SNEDDS includes the composition, droplet size in dispersion, stability of dispersion, and formulation technique. An extensive compilation of reported studies carried out using SEDDS/SMEDDS/SNEDDS is well organized by Cerpnjak *et al.* (73). However, loading of hydrophilic molecules in these systems continues to be a tedious task due to its hydrophobic oil phase. In such case, the drug can be complexed with phospholipids in order to increase their lipophicility and thereby improving its incorporation efficiency. This synergistic utilization of drug-phospholipid complex and SEDDS assists in improving the bioavailability of drugs with suitable solubility but limited permeability (BCS class III). Likewise, consider an investigation done by Ruan *et al.*, (75) wherein SNEDDSs containing matrine–phospholipid complex were prepared. Matrine is hydrophilic compound and has poor solubility in oil phase; hence, to increase its lipophilicity for efficient incorporation in SEDDS, phospholipid complex was developed. In similar report, Wu *et al.* (76) developed phospholipid complex-based SEDDS of class IV drug, Baicalin. The combined effect of phospholipid complex and SMEDDS leads to increase the oral bioavailability of baicalin. Although authors claimed that it is difficult to form complex with BCS class IV drugs due to absence of interacting functional groups, some BCS class IV compounds showing phenolic hydroxyl groups can illustrate the required interaction thereby can form the complex (76). However, this field of application still remains to be unexplored to an in-depth reasons for such observation.

Phospholipid Complex-Based Micelles

As for several drugs, the aqueous solubility is considered to be the limiting factor for their absorption; hence, approaches like addition of solubilizer or auxiliary solvent, salt formation, pro-drug formation, solid dispersion and cyclodextrin inclusions have been employed. Apart from these strategies, mixed micelles are currently being assumed as a vital method to resolve the bioavailability issue. Thus, in recent approaches, the drug phospholipid complexes along with other micelles are used to form the mixed micelles system to improve the drug delivery of poorly soluble drugs. This could be explained by understanding the report submitted by Xia *et al.*, (78) in which 20(S)-protopanaxadiol (PPD) phospholipid complex enhriched micelles are prepared to overcome the low solubility and poor bioavailability of drug. The solubility of PPD in micelles increased 64 times more than free PPD. Apart from this, the PPD-phospholipid complex-based micelles showed improved absorption and increased bioavailability. A similar report includes micelles of tpgs modified with apigenin phospholipid complex. In their findings, authors claimed that apigenin was completely protected in micellar system which lead to its improved oral bioavailability and decreased metabolism in GIT (79).

Other Nanoformulations

There are several other nano-approaches that can be employed in synergy with drug phospholipid complex to efficiently deliver the drug. A novel concept reported by Jin *et al.*, (80) wherein emphasis on size of baohuoside I-phospholipid complex formed was given. The complexes formed were subjected through multiple cycles of homogenization for reducing the size. The baohuoside I-phospholipid complex of lower size (range of 81 ± 10 nm) obtained after homogenization revealed better permeation/absorption across Caco-2 monolayer than larger size range complexes. Thus, mentioning the fact that the overall size of the formed complex can also be cosidered as a parameter to manipulate the drug bioavailability. Other nano formulation approch of incorporating the drug-phospholipid complex in to nanolipospheres was introduced by Beg *et al.* (81). Authors reported rosuvastatin phospholipid complex-loaded nanolipospheres. The authors revealed that synergititc effect of phopsholipid complex and lipid nanospheres in improving the physicochemical attributes of rosuvastatin. Also, improvement in its pharmacodynamic performance was observed. Thus, the phospholipid complex-loaded novel formulations opens a new doorway to increase the physicochemical attributes and resolve the bioavailability issues of drugs.

CONCLUSION AND FUTURE PROSPECTIVE

Despite the fact that lipid-based systems can resolve several drug delivery issues, most of them have some limitations. Such limitations could be countered by employing drug-phospholipid complexes. Examining the studies discussed in the literature, it can be presumed that this technology can assist in improving bioavailability by enhancing solubility, permeability, and by improving metabolic stability in GIT. Being comprised with such abilities, this nominates it to be a suitable delivery vehicle for drugs which has limited solubility (BCS class II), limited permeability (BCS class III), and/or both (BCS class IV). Additionally, this technology enjoys the benefits of being cost effective, simple to formulate, and easy to scale up over other lipid-based drug delivery systems. Considering the reports, it was evident that, to form a stable drug-lipid complex, a thorough knowledge on pre-formulation aspect is mandatory as this governs the overall stability of the system. Also, *in silico* analysis can collectively assist in predicting the stability of complex thereby assisting in selecting the stable drug-phospholipid combination. In recent years, phospholipid complex-loaded novel formulations like SNEDDS, micelles, and lipospheres *etc.* have played a crucial role in synergizing the overall efficacy of the system. Such combinational system provides a stable platform which ensures improved hydrophilic drug loading, reduced drug leakage, and enhanced stability of the system. Conclusively, from the current reports encompassing the crucial know how about drug-phospholipid complex, it is evident that this system can still be developed and used in numerous ways to improve the overall bioavailability of drugs.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no conflict of interest.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002;45(12):2615–23.
2. Kuche K, Maheshwari R, Tambe V, Mak K-K, Jogi H, Raval N, et al. Carbon nanotubes (CNTs) based advanced dermal therapeutics: current trends and future potential. Nanoscale. 2018;10(19):8911–37.
3. Yan C, Gu J, Lv Y, Shi W, Jing H. Improved intestinal absorption of water-soluble drugs by acetylation of G2 PAMAM dendrimer nanocomplexes in rat. Drug Deliv Transl Res. 2017;7(3):408–15.
4. Kirtane AR, Narayan P, Liu G, Panyam J. Polymer-surfactant nanoparticles for improving oral bioavailability of doxorubicin. J Pharm Investig. 2017;47(1):65–73.
5. Pandey SC, Kaur R, Gangadharapp H, Sachin J. Self-emulsifying drug delivery system: a review. RJPT. 2018;1(4):313–23.
6. Vilas PC, Gujarathi NA, Rane BR, Pawar SP. A Review on self microemulsifying drug delivery system. Pharma Sci Monit. 2013;4(1):3628–48.
7. Andonova V, Peneva P. Characterization methods for solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). Curr Pharm Des. 2017;23(43):6630–42.
8. Semalty A, Semalty M, Rawat BS, Singh D, Rawat M. Pharmacosomes: the lipid-based new drug delivery system. Expert Opin Drug Deliv. 2009;6(6):599–612.
9. J-n Y, Zhu Y, Wang L, Peng M, S-s T, Cao X, et al. Enhancement of oral bioavailability of the poorly water-

soluble drug silybin by sodium cholate/phospholipid-mixed micelles. Acta Pharmacol Sin. 2010;31(6):759.
10. Gnananath K, Nataraj KS, Rao BG. Phospholipid complex technique for superior bioavailability of Phytoconstituents. Adv Pharm Bull. 2017;7(1):35–42.
11. Singh RP, Gangadharappa H, Mruthunjaya K. Phospholipids: unique carriers for drug delivery systems. J Drug Deliv Sci Technol. 2017;39:166–79.
12. Marsh D. General features of phospholipid phase transitions. Chem Phys Lipids. 1991;57(2–3):109–20.
13. Seimiya T, Ohki S. Ionic structure of phospholipid membranes, and binding of calcium ions. Biochim Biophys Acta (BBA)-Biomembr. 1973;298(3):546–61.
14. Sosenko IR, Innis SM, Frank L. Intralipid increases lung polyunsaturated fatty acids and protects newborn rats from oxygen toxicity. Pediatr Res. 1991;30(5):413–7.
15. de Boer JF, Kuipers F, Groen AK. Cholesterol transport revisited: A new Turbo mechanism to drive cholesterol excretion. Trends Endocrin Met. 2017;29(2):123–33.
16. Mills PC, Chen Y, Hills YC, Hills BA. Comparison of surfactant lipids between pleural and pulmonary lining fluids. Pulm Pharmacol Ther. 2006;19(4):292–6.
17. Cevc G. Phospholipids handbook: CRC press; 1993.
18. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, et al. A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci. 2015;10(2):81–98.
19. Intermolecular JI. Surface forces. San Diego: Academic press; 1992.
20. McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature. 2005;438(7068):590–6.
21. Phillips R, Theriot J, Kondev J, Garcia H. Physical biology of the cell: Garland. Science. 2012.
22. Sharma S, Roy RK. Phytosomes: an emerging technology. Int J Pharm Res Dev. 2010;2(5):1–7.
23. Kapoor B, Gupta R, Singh SK, Gulati M, Singh S. Prodrugs, phospholipids and vesicular delivery-an effective triumvirate of pharmacosomes. Adv Colloid Interf Sci. 2018;253:35–65.
24. Carey MC, Small DM. The characteristics of mixed micellar solutions with particular reference to bile. Am J Med. 1970;49(5):590–608.
25. Gangwar M, Singh R, Goel R, Nath G. Recent advances in various emerging vescicular systems: an overview. Asian Pac J Trop Biomed. 2012;2(2):S1176–S88.
26. Bhingare U, Khadabadi S, Shinde N. Pharmacosomes: A novel drug delivery system. Int J. 2014;3(1):14–20.
27. Gavhane YN, Yadav AV. Loss of orally administered drugs in GI tract. Saudi Pharm J. 2012;20(4):331–44.
28. Jena SK, Singh C, Dora CP, Suresh S. Development of tamoxifen-phospholipid complex: novel approach for improving solubility and bioavailability. Int J Pharm. 2014;473(1–2):1–9.
29. van Hoogevest P. Review–an update on the use of oral phospholipid excipients. Eur J Pharm Sci. 2017;108:1–12.
30. Chaudhri O, Small C, Bloom S. Gastrointestinal hormones regulating appetite. Phil Trans R Soc London B: Biol Sci. 2006;361(1471):1187–209.
31. Marieb EN, Hoehn K. Human anatomy and physiology. 8e éd ed. San Francisco: Benjamin Cummings; 2010.
32. Kossena GA, Charman WN, Wilson CG, O'Mahony B, Lindsay B, Hempenstall JM, et al. Low dose lipid formulations: effects on gastric emptying and biliary secretion. Pharm Res. 2007;24(11):2084–96.
33. Higgins J, Fielding C. Lipoprotein lipase. Mechanism of formation of triglyceride-rich remnant particles from very low density lipoproteins and chylomicrons. Biochemistry. 1975;14(11):2288–93.
34. Harde H, Das M, Jain S. Solid lipid nanoparticles: an oral bioavailability enhancer vehicle. Expert Opin Drug Deliv. 2011;8(11):1407–24.
35. Nestel P, Havel R, Bezman A. Sites of initial removal of chylomicron triglyceride fatty acids from the blood. J Clin Invest. 1962;41(10):1915–21.
36. Semalty A, Semalty M, Singh D, Rawat M. Phyto-phospholipid complex of catechin in value added herbal drug delivery. J Incl Phenom Macrocycl Chem. 2012;73(1–4):377–86.
37. Alexander A, Ajazuddin S, Verma T, Swarna MJ, Patel S. Mechanism responsible for mucoadhesion of mucoadhesive drug delivery system: a review. Int J Appl Biol Pharm Technol. 2011;2(1):434–45.
38. Behra A, Giri TK, Tripathi DK, Ajazuddin AA. An exhaustive review on recent advancement in pharmaceutical bioadhesive used for systemic drug delivery through oral mucosa for achieving maximum pharmacological response and effect. Int J Pharmacol. 2012;8(5):283–305.
39. Afanas'eva YG, Fakhretdinova E, Spirikhin L, Nasibullin R. Mechanism of interaction of certain flavonoids with phosphatidylcholine of cellular membranes. Pharm Chem J. 2007;41(7):354–6.
40. Li N, Ye Y, Yang M, Jiang X, Ma J. Pharmacokinetics of baicalin-phospholipid complex in rat plasma and brain tissues after intranasal and intravenous administration. Die Pharmazie-an international. J Pharm Sci. 2011;66(5):374–7.
41. Yue P-F, Yuan H-L, Li X-Y, Yang M, Zhu W-F. Process optimization, characterization and evaluation *in vivo* of oxymatrine–phospholipid complex. Int J Pharm. 2010;387(1–2):139–46.
42. Maryana W, Rachmawati H, Mudhakir D. Formation of Phytosome containing Silymarin using thin layer-hydration technique aimed for Oral delivery. Mater Today Proc. 2016;3(3):855–66.
43. Qin X, Yang Y, T-t F, Gong T, X-n Z, Huang Y. Preparation, characterization and *in vivo* evaluation of bergenin-phospholipid complex. Acta Pharmacol Sin. 2010;31(1):127–36.
44. Guo B, Liu H, Li Y, Zhao J, Yang D, Wang X, et al. Application of phospholipid complex technique to improve the dissolution and pharmacokinetic of probucol by solvent-evaporation and co-grinding methods. Int J Pharm. 2014;474(1–2):50–6.
45. Sikarwar MS, Sharma S, Jain AK, Parial S. Preparation, characterization and evaluation of marsupsin–phospholipid complex. AAPS PharmSciTech. 2008;9(1):129–37.
46. Li Y, Yang D-J, Chen S-L, Chen S-B, AS-C C. Comparative physicochemical characterization of phospholipids complex of puerarin formulated by conventional and supercritical methods. Pharm Res. 2008;25(3):563–77.
47. Dora CP, Kushwah V, Katiyar SS, Kumar P, Pillay V, Suresh S, et al. Improved oral bioavailability and therapeutic efficacy of erlotinib through molecular complexation with phospholipid. Int J Pharm. 2017;534(1–2):1–13.
48. Cui F, Shi K, Zhang L, Tao A, Kawashima Y. Biodegradable nanoparticles loaded with insulin–phospholipid complex for oral delivery: preparation, *in vitro* characterization and *in vivo* evaluation. J Control Release. 2006;114(2):242–50.
49. Murugan V, Mukherjee K, Maiti K, Mukherjee PK. Enhanced oral bioavailability and antioxidant profile of ellagic acid by phospholipids. J Agric Food Chem. 2009;57(11):4559–65.
50. Dora CP, Kushwah V, Katiyar SS, Kumar P, Pillay V, Suresh S, et al. Improved metabolic stability and therapeutic efficacy of a novel molecular gemcitabine phospholipid complex. Int J Pharm. 2017;530(1–2):113–27.
51. Kumar P, Choonara YE, Pillay V. In silico affinity profiling of neuroactive polyphenols for post-traumatic calpain inactivation: a molecular docking and atomistic simulation sensitivity analysis. Molecules. 2014;20(1):135–68.
52. Larkin P. Infrared and Raman spectroscopy: principles and spectral interpretation. Amsterdam: Elsevier; 2017.
53. 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(1–2):220–7.
54. Rajan S, Kang S-Y, Gutowsky HS, Oldfield E. Phosphorus nuclear magnetic resonance study of membrane structure. Interactions of lipids with protein, polypeptide, and cholesterol. J Biol Chem. 1981;256(3):1160–6.
55. Jin Y, Wunderlich B. Single-run heat capacity measurement by DSC: principle, experimental and data analysis. Thermochim Acta. 1993;226:155–61.
56. Chandrasekaran B, Abed SN, Al-Attraqchi O, Kuche K, Tekade RK. Computer-aided prediction of pharmacokinetic (ADMET) properties. In: Dosage Form Design Parameters. Amsterdam: Elsevier; 2018. p. 731–55.
57. Ngwuluka NC, Choonara YE, Kumar P, du Toit LC, Khan RA, Pillay V. A novel pH-responsive interpolyelectrolyte hydrogel

complex for the oral delivery of levodopa. Part I. IPEC modeling and synthesis. J Biomed Mater Res A. 2015;103(3):1077–84.
58. Wang L, Hao Y, Liu N, Ma M, Yin Z, Zhang X. Enhance the dissolution rate and oral bioavailability of pranlukast by preparing nanosuspensions with high-pressure homogenizing method. Drug Dev Ind Pharm. 2012;38(11):1381–9.
59. Hao Y, Wang L, Li J, Liu N, Feng J, Zhao M, et al. Enhancement of solubility, transport across Madin-Darby canine kidney monolayers and oral absorption of pranlukast through preparation of a pranlukast-phospholipid complex. J Biomed Nanotechnol. 2015;11(3):469–77.
60. Schuetz EG, Schinkel AH, Relling MV, Schuetz JD. P-glycoprotein: a major determinant of rifampicin-inducible expression of cytochrome P4503A in mice and humans. Proc Natl Acad Sci. 1996;93(9):4001–5.
61. 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(3):1238–47.
62. Immordino ML, Brusa P, Rocco F, Arpicco S, Ceruti M, Cattel L. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing lipophilic gemcitabine prodrugs. J Control Release. 2004;100(3):331–46.
63. Elnaggar YS, Shehata EM, Galal S, Abdallah OY. Self-emulsifying preconcentrates of daidzein–phospholipid complex: design, *in vitro* and *in vivo* appraisal. Nanomedicine. 2017;12(8):893–910.
64. Ma H, Chen H, Sun L, Tong L, Zhang T. Improving permeability and oral absorption of mangiferin by phospholipid complexation. Fitoterapia. 2014;93:54–61.
65. Zhou H, Han Y-m, Zheng Y-m, Xu X-y, Fu S-q, Wang L-I, et al. Preparative procedure of inclusion compound of mangiferin-HP-β-CD. J Chongqing Inst Technol (Nat Sci). 2009;9:011.
66. Li Y, Ren X, Lio C, Sun W, Lai K, Liu Y, et al. A chlorogenic acid-phospholipid complex ameliorates post-myocardial infarction inflammatory response mediated by mitochondrial reactive oxygen species in SAMP8 mice. Pharmacol Res. 2018;130:110–22.
67. Maheshwari R, Kuche KN, Advankar A, Soni N, Raval N, Sharma PA, et al. Natural Ingredients/Botanical Extracts for the Nutraceutical Industry. In: Flavors for Nutraceutical and Functional Foods. Boca Raton: CRC Press; 2018. p. 95–142.
68. Wu J-Y, Li Y-J, Han M, Hu X-B, Yang L, Wang J-M, et al. A microemulsion of puerarin–phospholipid complex for improving bioavailability: preparation, *in vitro* and *in vivo* evaluations. Drug Dev Ind Pharm. 2018;44(8):1336–41.
69. Ge L, He X, Zhang Y, Zhang Y, Chai F, Jiang L, et al. A dabigatran etexilate phospholipid complex nanoemulsion system for further oral bioavailability by reducing drug-leakage in the gastrointestinal tract. Nanomedicine. 2018;14(4):1455–64.
70. Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Adv Drug Deliv Rev. 2008;60(6):625–37.
71. Constantinides PP. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharm Res. 1995;12(11):1561–72.
72. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006;29(3–4):278–87.
73. Čerpnjak K, Zvonar A, Gašperlin M, Vrečer F. Lipid-based systems as promising approach for enhancing the bioavailability of poorly water-soluble drugs. Acta Pharma. 2013;63(4):427–45.
74. Walstra P. Principles of emulsion formation. Chem Eng Sci. 1993;48(2):333–49.
75. Ruan J, Liu J, Zhu D, Gong T, Yang F, Hao X, et al. Preparation and evaluation of self-nanoemulsified drug delivery systems (SNEDDSs) of matrine based on drug–phospholipid complex technique. Int J Pharm. 2010;386(1–2):282–90.
76. Wu H, Long X, Yuan F, Chen L, Pan S, Liu Y, et al. Combined use of phospholipid complexes and self-emulsifying microemulsions for improving the oral absorption of a BCS class IV compound, baicalin. Acta Pharm Sin B. 2014;4(3):217–26.
77. Wei D, Zhang X. Solubility of puerarin in the binary system of methanol and acetic acid solvent mixtures. Fluid Phase Equilib. 2013;339:67–71.
78. Xia H-j, Zhang Z-h, Jin X, Hu Q, Chen X-y, Jia X-b. A novel drug–phospholipid complex enriched with micelles: preparation and evaluation *in vitro* and *in vivo*. Int J Nanomedicine. 2013;8:545.
79. Munyendo WL, Zhang Z, Abbad S, Waddad AY, Lv H, Baraza LD, et al. Micelles of TPGS modified apigenin phospholipid complex for oral administration: preparation, *in vitro* and *in vivo* evaluation. J Biomed Nanotechnol. 2013;9(12):2034–47.
80. Jin X, Zhang Z-h, Sun E, Qian Q, X-b T, X-b J. Preparation of a nanoscale baohuoside I-phospholipid complex and determination of its absorption: *in vivo* and *in vitro* evaluations. Int J Nanomedicine. 2012;7:4907.
81. Beg S, Raza K, Kumar R, Chadha R, Katare O, Singh B. Improved intestinal lymphatic drug targeting via phospholipid complex-loaded nanolipospheres of rosuvastatin calcium. RSC Adv. 2016;6(10):8173–87.