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Pharmacosomes: the lipid-based new drug delivery system

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Lipid-based drug delivery systems have been investigated in various studies and shown their potential in controlled and targeted drug delivery. Pharmacosomes are amphiphilic phospholipid complexes of drugs bearing active hydrogen that bind to phospholipids. Pharmacosomes impart better biopharmaceutical properties to the drug, resulting in improved bioavailability. Pharmacosomes have been prepared for various non-steroidal anti-inflammatory drugs, proteins, cardiovascular and antineoplastic drugs. Developing the pharmacosomes of the drugs has been found to improve the absorption and minimize the gastrointestinal toxicity. This article reviews the potential of pharmacosomes as a controlled and targeted drug delivery system and highlights the methods of preparation and characterization.

Keywords: bioavailability, non-steroidal anti-inflammatory drugs, pharmacosome, phospholipid complex

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1. Introduction

On oral ingestion, a drug is dissolved into the gastric fluid (hydrophilic environment) initially, followed by permeation across the biological membranes (lipophilic environment) and finally reaching the blood. Many synthetic and herbal drugs are associated with the problem of either poor absorption or poor permeation through the biological membrane, thereby limiting their absorption and overall availability to the body system. Poor absorption may be due to their poor water solubility, whereas poor permeation may be due to the structure of the drug (multiple-ring molecules such as herbal drugs may be too large to be absorbed by simple diffusion) or the poor miscibility with oils and other lipids, thereby severely limiting their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine [1].

Various approaches have been investigated to improve the absorption and permeation of biologically active constituents of synthetic and natural origin. These include the development of more soluble pro-drug, solid dispersions and complexation with agents such as metals, cyclodextrin and phospholipids (PLs). Apart from other methods used for modifying the solubility, the complexation with PLs has been found to show improvement in both absorption as well as permeation of the active constituents [2,3]. Therefore, developing the drugs as lipid complexes (also termed pharmacosomes) may prove to be a potential approach to improving solubility and permeation and to minimizing the gastrointestinal (GI) toxicity. Any drug possessing an active hydrogen atom (-COOH, -OH, $-NH_2$ etc.) can be esterified to the lipid with or without spacer chain. Active hydrogen is a hydrogen atom that has an extra electron in its outer shell that gives it a negative charge. This extra electron is able to react with other compounds. Carboxylic

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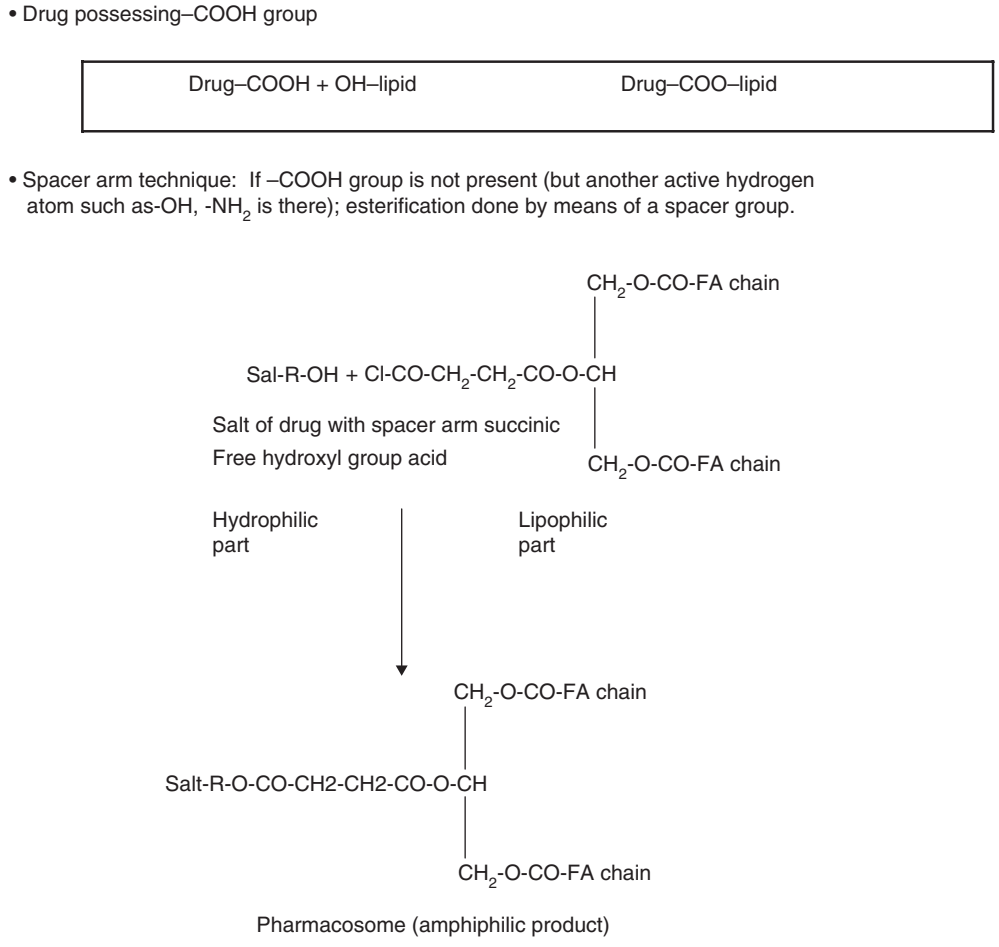

Figure 1. Basic approaches of pharmacosome preparations.

(–COOH) group-bearing drugs can be esterified without any spacer chain (**Figure 1**). On the other hand, if the -COOH group is not present (but another active hydrogen atom such as -OH, $-NH_2$ is there), esterification can be done by means of a spacer group (such as succinic acid as a spacer arm). Synthesis of such complexes may be guided in such a way that it results strongly in an amphiphilic complex, which facilitates membrane, tissue, or cell wall transfer in the organism. Pharmacosomes are defined as colloidal dispersions of drugs covalently bound to lipids, and may exist as ultrafine vesicular, micellar, or hexagonal aggregates, depending on the chemical structure of the drug–lipid complex [4-6].

Pharmacosomes as new drug delivery systems were first reviewed by Vaizoglu and Speiser in 1986 [5]; but this article is a first attempt to cater for the need of know-how and the latest advancements in the field of process and product development of pharmacosomal research. This article provides the basic concepts of preparation and characterization of pharmacosomes, with current technological trends for them. The latest investigations of pharmacosomes of various drugs are reviewed.

2. Advantages of pharmacosomes

Pharmacosomes are zwitterionic, amphiphilic, stoichiometric complexes of polyphenolic compounds with PLs. Unlike other lipid based delivery system, pharmacosomes shows better result in many ways [6].

A drug is reacted with the PL in equimolar concentration; this ensures the high loading of drug. The high entrapment efficiency of the drug in the complex may be attributed to the approach of complexation, which is based on the esterification of drugs. Unlike liposomes, there is no need to follow the tedious, time-consuming step for removing the free, unentrapped drug from the formulation.

The drug is covalently linked with PLs in pharmacosome, therefore the loss resulting from leakage of drug does not take place. However, loss may occur by hydrolysis. Entrapped volume and drug–bilayer interactions do not influence entrapment efficiency. These factors, on the other hand, have great influence on entrapment efficiency in the case of liposomes.

The physicochemical stability of the pharmacosome depends on the physicochemical properties of the drug–lipid

Table 1. Difference between liposomes and pharmacosomes.

Properties	Liposomes	Pharmacosomes
Principle	Incorporation of drug in the aqueous or lipid phase of a mixture of lipid where the physicochemical properties of the carrier and release of drug will be functions of different lipid used	Covalent binding of a drug to a lipid where the resulting compound is the carrier and the active compound at the same time. The physicochemical properties depend on the drug as well as the lipid
Loss of drug	Through leakage	No leakage, as drug is covalently bound, but loss of drug by hydrolysis is possible
Manufacturing	Cast film method Extrusion/sonication Injection method Reverse phase evaporation	Self-dispersion through moderate mixing and Sonication, SCF
Separation of free drug	Gel filtration Dialysis Ultrafiltration Ultracentrifugation	Not necessary because the drug is bound with PtdCho
Volume of inclusion	Decisive incorporation of drug molecules	Irrelevant, as the drug is electrostatically bound
Surface charge	Achieved through lipid combination	Depends on the physicochemical structure of the drug–lipid complex
Membrane fluidity	Depends on lipid combination and presence of cholesterol. Fluidity influences the rate of drug release and physical stability of the system	Depends on the phase transition temperature of the drug–lipid complex. No effect on release rate because the drug is covalently bound
Release of drug	Diffusion through the bilayer, desorption from the surface or release through degradation of the liposomes	Hydrolysis (including enzymatic)
Physical stability	Relatively good; aggregation through double-valenced cation	Depends on the physicochemical properties the drug–lipid complex

PtdCho: Phosphatidylcholine; SCF: Supercritical fluid.

complex, such as phase transition temperature, solubility, melting point, lipid composition, and so on. The lipid composition in liposomes decides its membrane fluidity, which in turn influences the rate of drug release and physical stability of the system. However, in pharmacosomes, membrane fluidity depends on the phase transition temperature of the drug–lipid complex, but it does not affect release rate because the drug is covalently bound. The drug is released from pharmacosome by hydrolysis (including enzymatic). The drug release from the complex also depends on the state of crystallinity of the drug in the complex. The more the drug is molecularly dispersed or amorphous in nature in the complex, the higher the drug release.

The pharmacosomes, being amphiphilic in nature, allow better dissolution in the GI fluid and better absorption through the lipophilic membrane system or tissue. This improves the bioavailability of drugs that have either very low lipid solubility or very low water solubility. So both kinds of drug can be complexed for improved biopharmaceutical properties.

The rate of degradation of the complex into active drug molecule following absorption depends, to a great extent, on the size and functional groups of the drug molecule, the chain length of the lipids, and the spacer. These can be varied relatively precisely for optimized *in vivo* pharmacokinetics.

Among the various lipid-based delivery systems, liposomes have been the most exhaustively investigated. Structurally, liposomes are concentric bilayer vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer composed mainly of natural or synthetic PLs [7]. Liposomes are lyotropic liquid crystals composed of relatively biocompatible and biodegradable materials and consist of an aqueous core entrapped by one or more bilayers of natural and/or synthetic lipids [8]. In spite of a similarity of components of delivery systems, liposomes have some fundamental differences from pharmacosomes. The main distinguishing feature of pharmacosomes is the covalent bonding between the drug and the lipid. This bonding not only imparts the stability, but also improves the efficacy of pharmacosomes. **Table 1** shows the comparative features of liposome and pharmacosomes.

3. Components of pharmacosomes

For a delivery system three components must be there (namely, drugs, solvent and carrier (lipids)).

3.1 Drugs

Any drug possessing an active hydrogen atom (-COOH, -OH, $-NH_2$ etc.) can be esterified to the lipid, with or without

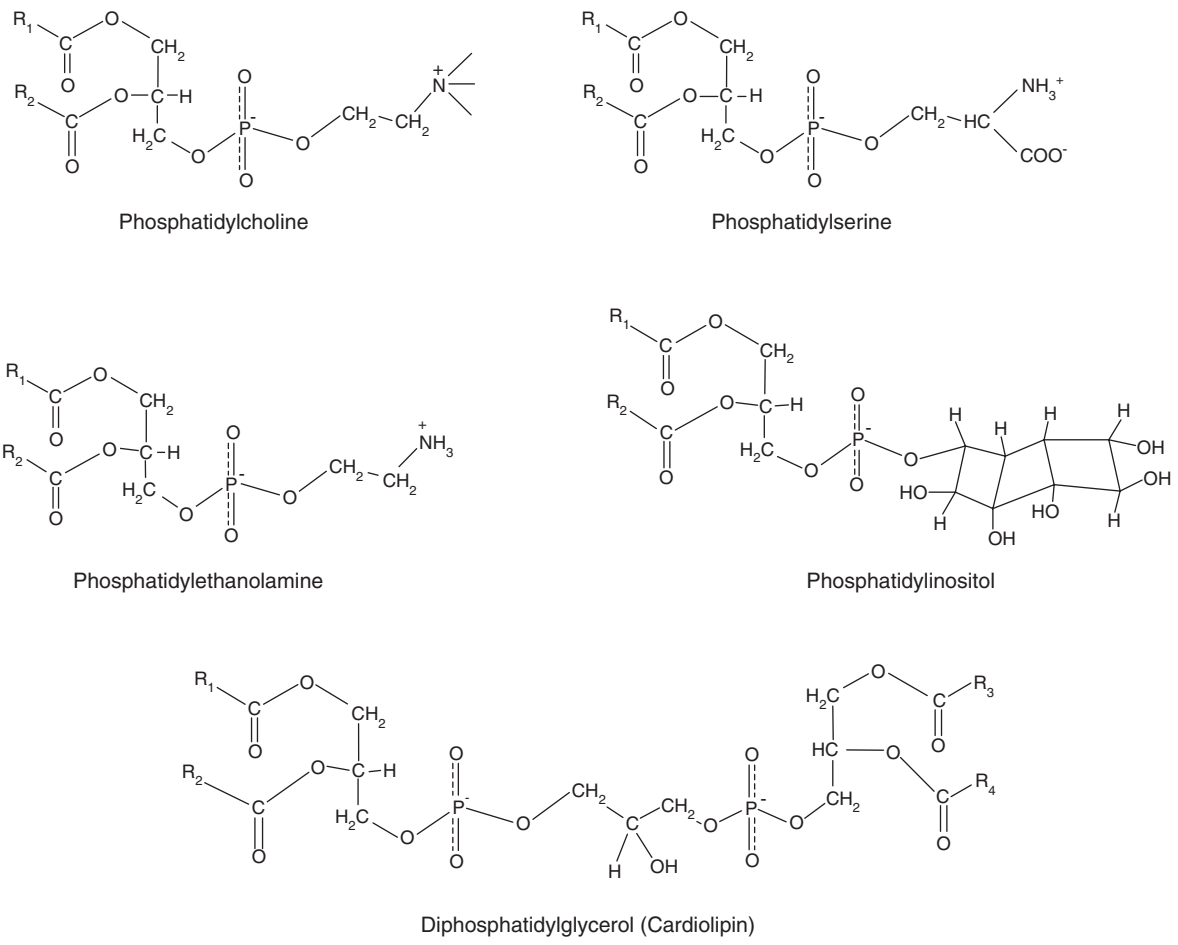

Figure 2. Chemical structures of some phospholipids.

spacer chain, resulting in amphiphilic complexes. These synthesized amphiphilic complexes (pharmacosome) facilitate membrane, tissue, or cell wall transfer in the organism [6].

3.2 Solvent

An analytical grade organic solvent is required for the preparation of pharmacosomes. It must be of high purity and volatile in nature. The PLs and the drug must be dissolved in the selected solvent either simply by its addition or by refluxing. The selection of solvent depends on polarity of the drug and the lipid. A solvent with intermediate polarity (between the polarity of PLs and drug) is selected for pharmacosome preparation.

3.3 Lipid

Lipid or lecithin or phosphatidylcholine (PtdCho) is the principal molecular building block of cell membranes. It is miscible both in water and in an oil/lipid environment and absorbed well orally. Phospholipids (**Figure 2**) are small lipid molecules in which the glycerol is bonded only to two fatty acids, instead of three as in triglycerides, with the remaining site occupied by a phosphate group [9]. Commercial grade PtdCho is a natural mixture of neutral and polar lipids. Phosphatidylcholine, which is a polar lipid, is present in commercial lecithin in concentrations of 20 – 90%. Most commercial lecithin products contain ~ 20% phosphatidylcholine Lecithins containing phosphatidylcholine are produced from vegetable, animal and microbial sources (mainly from vegetable sources) (**Table 2**). Lecithin is also available as a dietary supplement in two forms: as granular lecithin (oil-free refined lecithin with calcium phosphate as a flow agent); and as capsules containing a dispersion in oil. Composition by weight of unrefined and refined soy lecithin is given in **Table 3**.

Phosphatidylcholine may have hepatoprotective activity and its role in the maintenance of cell membrane integrity, which is vital to all of the basic biological processes. It also has a marked fluidizing effect on cellular membranes. Decreased cell membrane fluidization and breakdown of cell membrane integrity, as well as impairment of cell membrane repair mechanisms, are associated with several disorders, including liver disease, neurological diseases, various cancers and cell death. It is absorbed from the small intestine, mainly in the duodenum and upper jejunum. Following

Table 2. Natural sources of lecithin.

Sources	Unrefined lecithin (%)	Refined lecithin (%)	Types of fatty acid and their percentage
From vegetable			
Soybean	1.48 – 3.08	1.8	Saturated fatty acids, such as palmitic and stearic acid (19 – 24%). Monounsaturated fatty acid oleic acid (9 – 11%), linoleic acid (56 – 60%), α-linolenic acid (6 – 9%)
Peanuts	1.11	–	
Wheat	0.61	2.82	
From animal			
Eggs	0.39	7 – 10	Saturated fatty acids, palmitic and stearic (41 – 46%), Monounsaturated fatty acid, oleic acid (35 – 38%), linoleic acid (15 – 18%) and α-linolenic (0 – 1%)
Dehydrated (powdered) egg yolk	–	14 – 20	
Calf liver	0.85	–	
Oatmeal	0.65	–	
Human spinal cord	6 – 10	–	
Human brain	4 – 6	–	

Table 3. Composition by weight of unrefined and refined soy lecithin.

Oil-free compound	Unrefined lecithin (%)	Refined lecithin (%)
Phosphatidylcholine	17.5	23
Phosphatidylethanolamine	15.0	20
Phosphatidylinositol	10.0	14
Other phospholipids	14 – 18	–
Unrefined soy oil	31 – 34	0 – 3
Glycolipids	13 – 16	13 – 16
Neutral lipids (mostly triglycerides)	2 – 4	–

some digestion by the pancreatic enzyme phospholipase, phosphatidylcholine is transported in the blood in various lipoprotein particles, including very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). It is then distributed to the various tissues of the body. Some phosphatidylcholine is incorporated into cell membranes. Phosphatidylcholine is also metabolized to choline, fatty acids and glycerol.

Unlike other carriers used in new drugs delivery systems (NDDS), PLs are natural carriers that also have their own therapeutic benefits. Clinical studies have shown that choline is essential for normal liver function and acts as an effective hepatoprotective. *In vitro* studies have shown that these PLs increase hepatic collagenase activity and may thus help prevent fibrosis and cirrhosis by encouraging collagen breakdown [10,11]. In addition, phosphatidylcholine has demonstrated other protective effects in non-alcoholic fatty liver disorders and protection against various other toxic substances, such as hepatitis A and hepatitis B. Thus, it helps in the protection of liver function [12]. In one study chronic active hepatitis C patients were treated with 3 g daily of phosphatidylcholine in double-blind fashion [13]. The patients taking phosphatidylcholine showed significantly reduced symptoms compared with controls. From this followed the hypothesis that PtdCho increases cellular membrane fluidity and repairs the membranes of liver cells. The supplemental choline and phosphatidylcholine were found to reduce the muscular hyperactivity of tardive dyskinesia (a neurological disorder, characterized by defective cholinergic nerve activity) by ~ 50% [14,15].

Alzheimer's disease is characterized by a diminished ability to synthesize and/or utilize the neurotransmitter acetylcholine, particularly in the areas of the brain related to memory. Thus, a supplement of PtdCho may help in the treatment of Alzheimer's disease [16]. Recently it has been suggested that phosphatidylcholine might eventually have some therapeutic role in some cancers as well. There is ample evidence that liver cancer is promoted in various animals by choline-deficient diets, and it has been shown that excess choline can protect against liver cancer in a mouse model [17]. Phosphatidylcholine has been used to lower serum cholesterol levels, based on the premise that lecithin cholesterol acyltransferase activity has an important role in the removal of cholesterol from tissues. A few studies have shown reduction in serum cholesterol with phosphatidylcholine intake [18].

4. Methods of preparation

Pharmacosomes can be prepared by various methods. Fundamentally, a drug (bearing an active hydrogen group) when reacted with PL in the presence of a suitable organic solvent yields pharmacosomes. The drug and the PtdCho are reacted in a 1:1 or 1:2 molar ratio. Some methods described in various studies are discussed below.

4.1 Conventional solvent evaporation technique

To prepare the pharmacosomes of diclofenac sodium, it was acidified first so that active hydrogen might be available for complexation. Diclofenac acid was prepared by acidification of an aqueous solution of diclofenac sodium, extraction into chloroform, and subsequent recrystallization. Diclofenac–DPPtdCho (dipalmitoylphosphatidylcholine) complex was prepared by associating diclofenac acid with an equimolar concentration of DPPtdCho. A suspension of the complex in water was used as the diclofenac–DPPtdCho complex [19].

Semalty *et al.* prepared Diclofenac–PtdCho complex by reacting diclofenac acid with an equimolar concentration of PtdCho by a conventional solvent evaporation technique. The equimolar concentration of PtdCho (LIPOID S80) and diclofenac acid were placed in a 100 ml round bottom flask and dissolved in dichloromethane and refluxed. The solvent was evaporated off under vacuum at 40°C in a rotary vacuum evaporator (Perfit Model No. 5600 Buchi type). The dried residues were collected and placed in vacuum desiccators [20].

4.2 Supercritical fluid process

The conventional formulation methods used for the preparation of drug PL complex, most frequently solvent evaporation, are always time-consuming and involve multistage processing. In addition, the dissolution of pharmacosomes does not improve ideally [21]. Parameters related to solid morphology, including the particle size, the crystal habit and crystal pattern, influence the dissolution rate of a compound and thus can affect their bioavailability significantly [22]. So the supercritical fluid (SCF) process may be used to enhance the dissolution of pharmacosomes and to simplify the experimental procedures for preparing complexes. In recent years supercritical antisolvent precipitation (SAS), which is one of the SCF technologies, has become a promising technique that can be used to produce micrometer and submicrometer particles with controlled size and size distribution. The process is characterized by very mild conditions of temperature, and smaller particles can be obtained depending on the drug and process conditions when compared with the common industrial comminution techniques such as jet milling, liquid antisolvent precipitation and crystallization [23,24]. Particle size is particularly related to the dissolution of drugs and thus can significantly affect their bioavailability; so the SAS process may increase the dissolution of pharmacosomes.

Two different SCF technologies, gas antisolvent (GAS) and solution-enhanced dispersion by supercritical fluid (SEDS), belonging to the SAS process, were used by Li *et al.* for preparation of PL complexes of puerarin [21]. In the GAS process, mass transfer typically occurs by the mechanism of convection and molecular diffusion, leading to relatively small supersaturation for many solutes. Although, theoretically, very slow expansion in the GAS process should produce a homogeneous supersaturated solution, such expansion is very difficult to control. In addition, it is impossible to achieve high supersaturation levels in the GAS because of the faster process of nucleation. In the SEDS process, premixing is created between a fresh liquid solution and supercritical CO_2 (SC-CO_2), which produces high supersaturation and occurs predominantly within the nozzle mixing chamber. This process features a highly turbulent flow of solvent and CO_2, leading to a very fast mixing or dispersion. Thus, mass transfer is not limited by molecular diffusion or convective phenomena. By using this technique, it is possible to control the size, shape and morphology of the material of interest.

In the SEDS method, the CO_2 was passed through a heat exchanger to ensure that it was supercritical before entering the nozzle with a diameter of 0.1 mm, which consisted of two concentric tubes and a small premixing chamber. The mixing solution of puerarin and PLs or the single component solution and the supercritical antisolvent were continuously added by two pumps, one for delivering liquid CO_2 and the other for drug solution. Both of them were controlled by an Isco Series D pump power controller to the precipitation vessel in co-current mode through the nozzle. The temperature of the vessel was maintained in an Athena heater. The high velocity of the SC-CO_2 stream thoroughly mixed and dispersed the solvent stream and extracted solvent, leaving dry powder in the vessel. The particles formed were collected at the end of the experimental runs. The SC-CO_2 left the high-pressure vessel and flowed to the backpressure regulator, which controlled the pressure discharge in the system. The contents of the residual organic solvents within the microparticles were reduced by a washing step for 90 min or more to remove any residual solvent to avoid the recondensation of the liquid inside the chamber. The vessel was then slowly depressurized for 60 min and the powder removed. The conditions optimized for preparing puerarin or its complex were as follows: temperature 35°C, pressure 10 mPa, flow rate of CO_2 45 ml/min, flow rate ratio of drug-to-CO_2 solution 1%, the mass ratio of drug-to-PLs used for preparing puerarin PL complexes (PPC) in all methods retaining 1:1.2, and concentration of puerarin 100 mg/ml. Handling and storage conditions for SCF products were identical to the conventional processed PPC.

In the GAS precipitation for preparing PPC, Li *et al.* used almost the same equipment as used with SEDS, except that the pump controller in charge of delivering drug solution was not turned on [21]. The drug solution was not imported into the precipitation vessel by this pump, but was poured into the vessel before the experiment started. In the batch GAS step configuration, the precipitation unit attached to the Isco syringe pump was initially loaded with 100 ml of puerarin or 10 ml of PL solution. Then CO_2 was added until the final pressure was reached. The rate of CO_2 addition was 107 ml/min. The volume of the precipitation vessel was 250 ml. The vessel was filled with SC-CO_2 at the desired pressure (10 mPa) and temperature (38°C) and left for 3 h without any agitation. A pure constant carbon dioxide flow rate of 25 ml/min was then maintained in order to remove completely the residual solvent. After this washing step, which lasted for ~ 90 min, the autoclave was depressurized

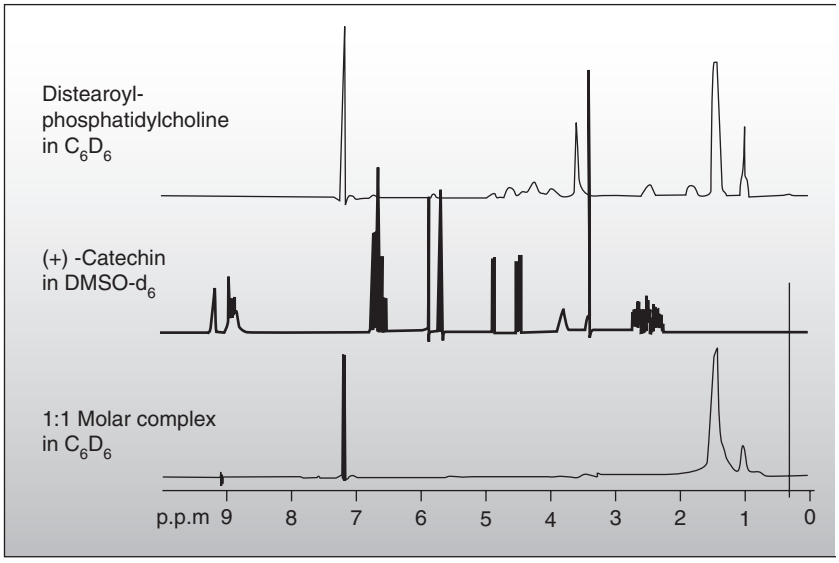

Figure 3. 1H-NMR spectrum of (+)-catechin–distearoylphosphatidylcholine complex in comparison with those of its constituents.

for 30 min at the experimental temperature. At the end of experiment, solid product was scraped out with a spatula from the filter located at the bottom of the vessel and subjected to handling and storage conditions identical to the unprocessed materials.

4.3 Anhydrous co-solvent lyophilization method

Shi *et al*. prepared a new insulin–PL complex by an anhydrous co-solvent lyophilization method. In this method insulin powder and PLs were co-dissolved in 1 ml dimethyl sulfoxide (DMSO) containing 5% glacial acetic acid accompanied by gentle agitation until formation of a clear mixture [25]. The resultant homogeneous solution was then freeze-dried overnight at a condenser temperature of -40°C and under a vacuum of 10 Pa. The resultant complex was flushed with nitrogen and stored at 4°C.

5. Characterization of pharmacosomes

5.1 Spectroscopic evaluations

To confirm the formation of a complex or to study the reciprocal interaction between the drugs and the PLs, the following spectroscopic methods are used [4].

5.1.1 Fourier transform infrared spectroscopy

The formation of the complex can also be confirmed by infrared spectroscopy comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. The infrared spectrum of the complex generally shows a broad peak for the hydroxyl group (which shows that some interaction has occurred at the hydroxyl (-OH) group) in place of the sharp peak (which indicates the presence of free hydroxyl group) of the -OH group in the drug.

Fourier transform infrared spectroscopy (FTIR) spectroscopy is also a useful tool for the evaluation of stability of the pharmacosome when microdispersed in water or when incorporated in very simple cosmetic gels [4]. From a practical point of view, stability can be confirmed by comparing the spectrum of the complex in solid form (phytosomes) with the spectrum of its microdispersion in water after lyophilization, at different time intervals. In the case of simple formulations, it is necessary to subtract the spectrum of the excipients (blank) from the spectrum of the cosmetic form at different times, comparing the remaining spectrum of the complex itself.

5.1.2 ^{13}C-NMR

In the ^{13}C-NMR spectrum of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine, particularly when recorded in C_6D_6 (solvent – deuterated benzene) at room temperature, all the flavonoid carbons are practically invisible. The signals corresponding to the glycerol and choline portion of the lipid (between 60 and 80 p.p.m.) are broadened and some are shifted, whereas most of the resonances of fatty acid chains retain their original sharp line shape. After heating to 60°C, all the signals belonging to the flavonoid moiety reappear, although they are still very broad and partially overlapping.

5.1.3 ^{1}H-NMR

The NMR spectrum of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine (**Figure 3**) were studied by Bombardelli and Spelta [4]. In nonpolar solvents, there is a marked change of the ^{1}H-NMR signal originating from the atoms involved in the formation of the complex, without any summation of the signal peculiar to the individual molecules. The signals of the protons belonging to the flavonoid moiety are so broadened that they cannot be revealed. In the PL,

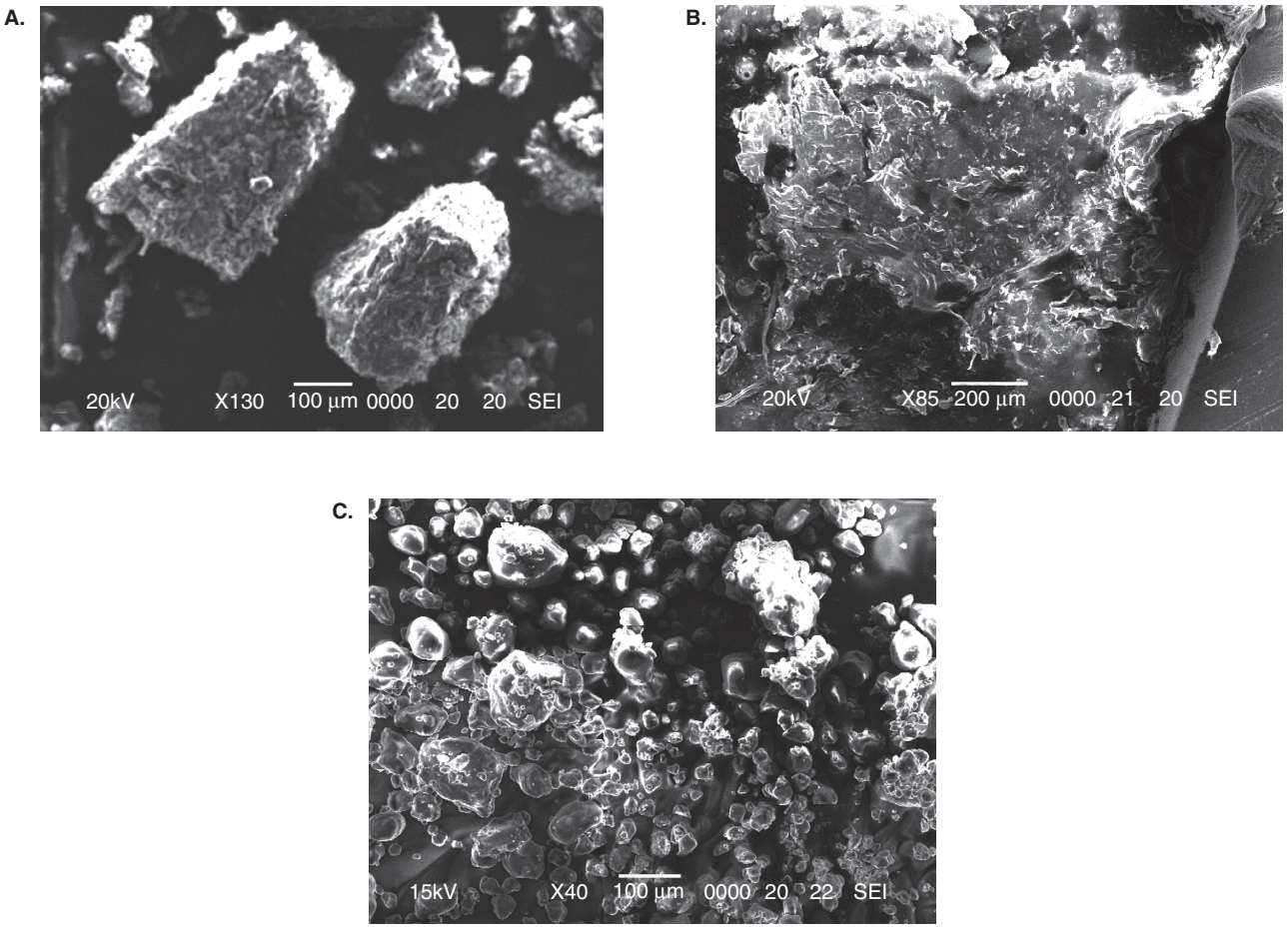

Figure 4. Scanning electron microscope (SEM) photographs of phospholipid complexes prepared from (A) diclofenac and (B) aspirin. C. SEM photographs of soya phosphatidylcholine.

there is a broadening of all the signals while the singlet corresponding to the N-$(CH_3)_3$ (trimethylammonium group) of choline undergoes an uplift shift. Heating the sample to 60°C results in the appearance of some new broad lines, which correspond mainly to the resonance of flavonoid moiety.

5.2 Surface morphology

Surface morphology of the pharmacosome can be studied and observed with scanning electron microscopy (SEM) or transmission electron microscopy (TEM). **Figure 4** shows the pharmacosomes of diclofenac and aspirin and their comparison with the SEM of soya-PL. Both the complexes are disc or irregular shape with rough surface morphology. The rough surface of complexes is significantly different from the SEM of soya-PL. The particle size of the PL complexes as analyzed by the SEM ranged from 10 to 150 µm. The shape and size of the prepared pharmacosomes may be affected by the process variables such as speed of rotation, vacuum applied or the method used. The purity grade of PL also plays an important role in governing the shape, size and stability of the pharmacosomes. Using low purity grades of lipids for PL complexes' preparation yields a greasy product, which in turn results in PL complexes of large aggregates and sticky nature. On the other hand, very high purity grades (> 90%) are prone to oxidative degradation, hence adversely affecting the stability of PL complexes. In general, PLs of ~ 80% purity have been used in PL complexes' preparation in most studies.

5.3 Solubility studies

Solubility of the drugs the PL, their physical mixture and the complex (pharmacosome) can be determined and compared. The complex (pharmacosome) shows enhanced aqueous and *n*-octanol solubility. This indicates an improvement in the partitioning coefficient and hence the bioavailability. The apparent partition coefficients may be measured by the shake-flask method [26]. In this method, the two phases are mutually saturated before use. Equal volumes of buffer solution with a different pH (from 2.0 to 7.4) and 1-octanol containing PL complex are mixed in the screw-capped penicillin bottles and equilibrated under constant shaking at 37°C for 24 h. The aqueous phase is then separated and the concentration of drug in this phase is determined by HPLC

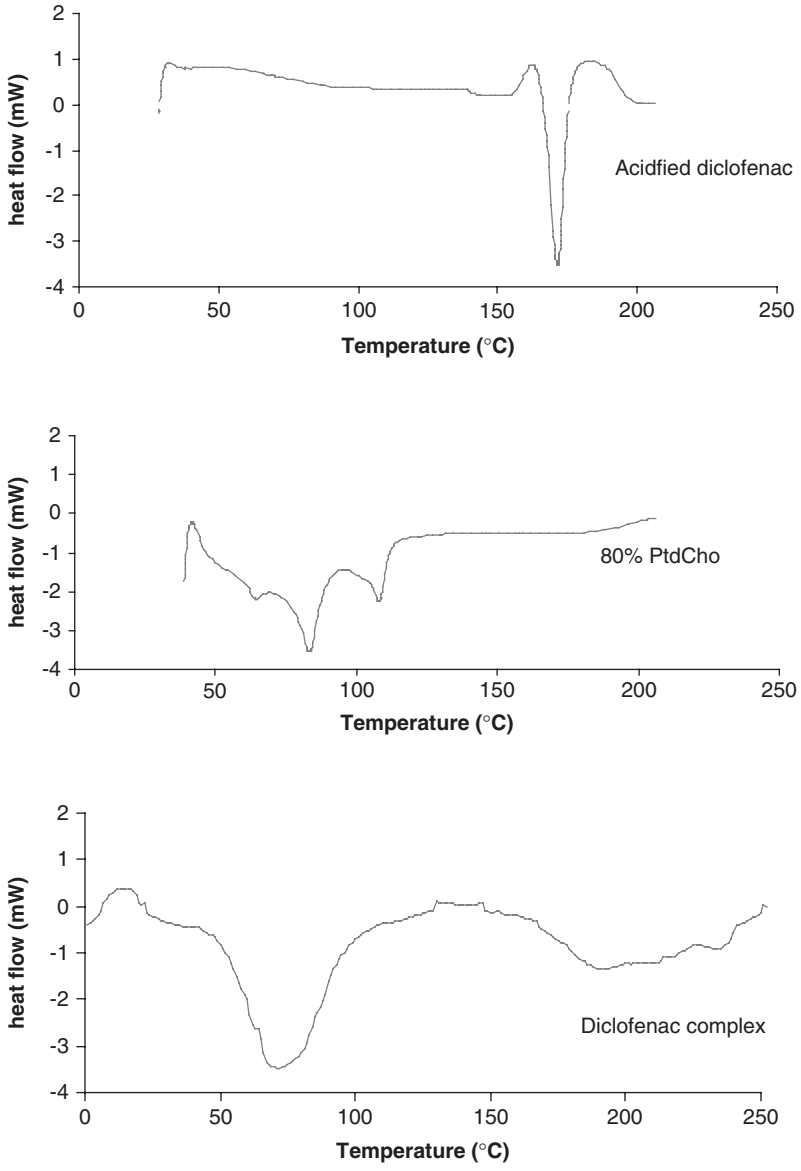

Figure 5. Differential scanning calorimetry thermograms showing peak transition onset temperature of acidified diclofenac, PtdCho and diclofenac–PtdCho complex.

or UV spectrophotometry. The apparent partition coefficients (P_{app}) may be calculated using the follow equation:

$$P_{app} = \frac{C_{octanol}}{C_{aqe}} = \frac{C_{aqi} - C_{aqe}}{C_{aqe}}$$

where $C_{octanol}$ is the equilibrium concentration of drug complexed with PL in 1-octanol (micrograms per milliliter). C_{aqi} and C_{aqe} represent the initial and equilibrium concentrations of drug complexed with PLs in buffer solution (micrograms per milliliter), respectively.

5.4 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a fast and reliable method to screen drug-excipient compatibility and provides maximum information about the possible interactions. In DSC, an interaction is concluded by elimination of endothermic peak(s), appearance of new peak(s), and change in peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy.

To show the association of diclofenac acid with PtdCho, DSC analysis was performed on diclofenac acid, PtdCho, and the diclofenac–PtdCho complex by Semalty *et al.* [20]. The results of the DSC test confirmed the association of diclofenac acid and PtdCho in the complex as both peaks representing diclofenac acid and PtdCho changed position (**Figure 5**). PtdCho showed two major peaks at 83.21 and 107.90°C and a small peak at 64.45°C, whereas acidified diclofenac showed a sharp endothermic peak at 171.31°C.

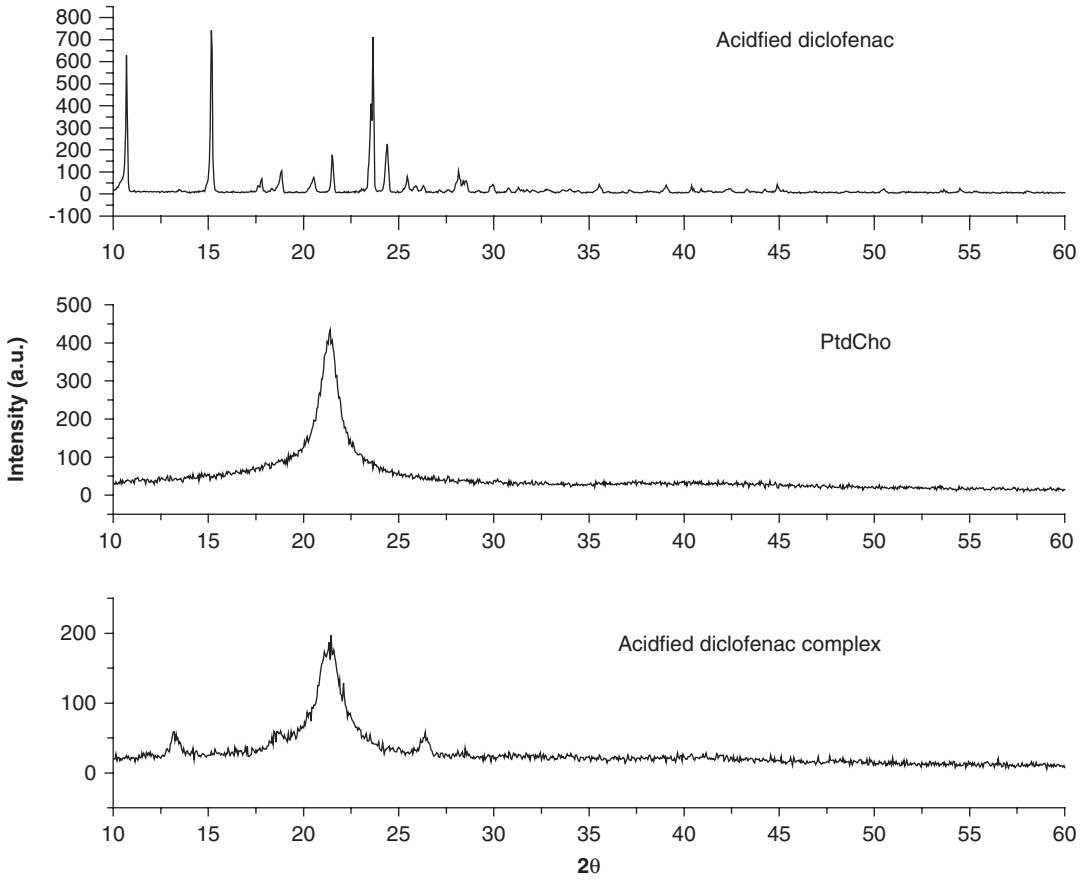

Figure 6. High-resolution X-ray diffraction study of diclofenac complex and its components.

On the other hand, the complex showed a broad peak (at 68.34°C), which is different from the peaks of the individual components of the complex.

5.5 X-ray powder diffraction

To check whether the changes in the diclofenac crystal morphology correspond to a polymorphic transition and to study the solid state of diclofenac PL complex, X-ray powder diffraction (XRPD) analysis was conducted. From these patterns, the degree of crystallinity could be evaluated using the relative integrated intensity of reflection peaks in the given range of reflecting angle, 2θ. The value of 2θ means the diffraction angle of ray beams, which is shown in the abscissa of **Figure 6**. The integrated intensity is given by the area under the curves of the XRPD patterns, and it stands for the characteristics of the specimen.

Semalty *et al.* [20] prepared the diclofenac PL complexes and found that the XRPD of diclofenac complex was devoid of any crystalline peaks that were present in the diclofenac. (**Figure 5**), so the disappearance of diclofenac crystalline diffraction peaks confirmed the formation of PL complex. This suggests that diclofenac in the PLs complex is either molecularly dispersed or in the amorphous form.

Unlike liposomes, chemical bonding between drug and the PLs in the development of pharmacosomes might have resulted in the significant change of its X-ray diffraction. Similar results were reported by other studies done with the PL complexes of insulin, salmon calcitonin and aspirin [25,27,28].

5.6 *In vitro* and *in vivo* evaluations

Models of *in vitro* and *in vivo* evaluations were selected on the basis of expected therapeutic activity of the biologically active phytoconstituents present in phytosomes. For example, *in vitro* antihepatotoxic activity can be assessed by antioxidant and free radical scavenging activity of phytosomes. To observe the pH-dependent dissolution profile of the complex, *in vitro* dissolution studies can be done with media of different pH (pH 1.2 HCl and pH 6.8 phosphate buffer saline) in a standard dissolution test apparatus. For assessing hepatoprotective or antihepatotoxic activity *in vivo*, the effect of prepared pharmacosomes on animals against thioacetamide, paracetamol or alcohol-induced hepatotoxicity can be observed.

6. Potential applications of pharmacosomes

The development of pharmacosomes has improved the therapeutic performance of various drugs, that is, pindolol

maleate, bupranolol hydrochloride, taxol, acyclovir, and so on [5,29,30]. The PL complexes of proteins such as insulin and salmon calcitonin have been developed successfully and have been reported to improve their bioavailability [27,31].

Shi *et al.* prepared a new insulin–PL complex by an anhydrous co-solvent lyophilization method. Compared with native insulin, the physicochemical properties of insulin changed significantly after it was complexed with PLs. It was concluded that the characteristics, especially the improved lipophilicity, would contribute to the improved oral absorption of insulin [25].

The phyto–PL complexes have been investigated for cardiovascular, antiskin ageing, anti-inflammatory and hepatoprotective activity. The improved bioavailability of these complexes showed that they can be used as an effective herbal drug delivery system [32].

In two different studies, the PL complexes of curcumin and naringenin were developed. In the first study phytosome of curcumin was developed to overcome the limitation of absorption and to investigate the protective effect of curcumin–PL complex on carbon tetrachloride-induced acute liver damage in rats. The complex showed enhanced aqueous or *n*-octanol solubility. The antioxidant activity of the complex was significantly higher than pure curcumin in all dose levels tested [33]. In the other study, phytosome of naringenin was developed and produced better antioxidant activity than the free compound with a prolonged duration of action, which may be helpful in reducing the fast elimination of the molecule from body [34]. A quercetin–PL complex was also developed by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride [35].

In another study, Yanyu *et al.* prepared silybin–PL complex and studied their pharmacokinetics in rats. In the study, the bioavailability of silybin was increased remarkably after oral administration of prepared silybin–PL complex [36]. This may be due to a significant improvement in the lipophilic property of the silybin–PL complex, thus leading to an improvement in the biological effect of silybin.

Zhang *et al.* optimized the preparation of 3′,5′-dioctanoyl-5-fluoro-2′-deoxyuridine pharmacosomes (DO-FUdR-PS) by using central composite design [37]. Under the optimized conditions, the mean particle size, entrapment efficiency and drug loading of DO-FUdR-PS were 97.49 and 31.44%, respectively, which agreed well with the predicted values. It was concluded that central composite design was successful in optimizing the preparation of DO-FUdR-PS. The developed 3′,5′-dioctanoyl-5-fluoro-2′-deoxyuridine pharmacosomes were shown to be effective in the brain delivery of the drug.

Phospholipid complexes of non-steroidal anti-inflammatory drugs (NSAIDs) have also been shown to improve GI safety of these drugs. It has been reported that NSAIDs associated with zwitterionic PLs may reduce GI toxicity [38]. The surface of the mucus contains an adsorbed layer of surface-active PLs that covers the surface epithelium. These surface-active PLs have been suggested to protect the GI tissues by providing a hydrophobic layer between the epithelium and the luminal contents [39,40]. The PL layer also increases mucosal resistance to luminal acidity by repelling the diffusion of hydrogen ions [41]. Phosphatidylcholines (with the dipalmitoyl species) are the most potent surface-active PLs among the lipids that appear to be covalently and non-covalently associated with mucus glycoprotein [38,42,43]. It has been suggested that ionic binding between DPPtdCho and an NSAID shields the NSAID from pH-dependent changes; hence the complex remains lipophilic even as the intragastric pH approaches neutrality [44]. Semalty *et al.* prepared the pharmacosomes of diclofenac and aspirin by a simple and reproducible method. In both the studies the pharmacosomes showed better solubility profile and *in vitro* drug release than the uncomplexed drug [20,28]. Khazaeinia and Jamali studied the comparison of gastrointestinal permeability induced by diclofenac PL complex with diclofenac acid and its sodium salt [19,45]. It was concluded that the diclofenac–DPPtdCho complex demonstrated the reduced upper gastroduodenal toxicity. Lichtenberger *et al.* prepared aspirin complex with the zwitterionic PL DPPtdCho for intragastric administration and evaluated it for biological and therapeutic activity of aspirin [46]. It was concluded that the GI absorption of free aspirin and that of the aspirin/DPPtdCho complex were similar in all three rodent models but the complex had significantly greater antipyretic, anti-inflammatory and analgesic efficacy than aspirin alone. Dose–response analysis using the fever model demonstrated that potency of aspirin to reduce fever was increased 5- to 10-fold when the aspirin was intragastrically administered in the lipid-associated state. Therefore, the zwitterionic PLs enhance aspirin's therapeutic activity, as demonstrated in rodent model systems. Garcia *et al.* prepared dioleylphosphatidylcholine (DOPC) complex of ketoprofen (KP) in a molar ratio of 1:3, obtained by dissolution of the components in chloroform followed by drying under a N_2 atmosphere [47]. The complex improved solubility of the drug. The permeation of the drug across the skin was enhanced in the complex when assayed by *in vitro* percutaneous absorption by using a flow-through diffusion cell.

Yue *et al.* prepared PL complex of ursodeoxycholic acid (UDCA–PLC) to enhance oral bioavailability of UDCA, and studied the physicochemical properties of the complex [48]. The X-ray diffraction and DSC studies showed that UDCA and PLs in the UDCA–PLC were combined by a non-covalent bond, not forming a new compound, and *n*-octanol/water partition coefficient of UDCA–PLC was effectively enhanced. The bioavailability of UDCA in rats was significantly different ($p < 0.05$) compared with those of UDCA tablet after administration. Parry *et al.* studied the high-affinity small molecule PL complex of siramesine [49]. Siramesine is a sigma-2 receptor agonist that has recently been shown to inhibit

growth of cancer cells. The PL complex of siramesine showed improved anticancer activity of the drug.

Hyaluronic acid (HA) is an acidic linear mucopoly-saccharide, having regularly alternating units of *N*-acetylglucosamine and D-glucuronic acid linked by 1,4- or 1,3-β-glycosidic bonds. Hyaluronic acid is distributed widely in connective tissues, vitreous body and joint fluid of vertebrates. It possesses an important function of water keeping [50]. It has viscoelastic and lubricating properties, thus uses in cosmetic, healthcare, medical and pharmaceutical applications. It can play a systemic role by increasing the concentration of endogenous HA from dermis to epidermis, therefore the delaying of ageing is possible [51,52]. However, gastrointestinal absorption of exogenous HA is poor when it is administered orally, owing to high molecular mass and poor liposolubility of HA [53]. Huang *et al*. prepared HA complex with PLs and give it the name Haplex. It was concluded that the physicochemical properties of Haplex were different from HA or PL or their mixture [54]. After Haplex was administered to rats orally, the serum concentration of HA was increased when compared with the mixture or HA control groups from 4 to 10 h.

7. Expert opinion

The bioavailability of poorly absorbed drugs can be improved by preparing their PL complexes, provided their absorption is dissolution or permeation rate limited. The PLs are ideal natural carrier with there own therapeutic values. Preparing the zwitterionic, amphiphilic compounds may improve the bioavailability of a wide spectrum of drugs such as insulin, salmon calcitonin, NSAIDs, and so on. With the development of pharmacosomes of NSAIDs, their diffusion across lipid membranes and into target cells is accelerated. The pharmacosomes may even reduce GI toxicity of NSAIDs. Moreover, the similar PL complexes can also improve the biopharmaceutical properties of biologically active phytoconstituents such as flavones, glycosides, xanthones, and so on. Similar to other vesicular systems, pharmacosomes play an important role in the selective targeting and the controlled delivery of various drugs. With the advent of modern techniques such as supercritical fluid and lyophilization methods, even better results can be obtained by pharmacosomes.

It is generally observed that bioavailability of most of the herbal or synthetic drugs is limited by the poor biopharmaceutical properties only. Therefore, the pharmacosomes can play the role of simple, safe, effective and stable drug delivery systems that can be prepared by simple and reproducible methods for better therapeutic performance.

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