Development and Characterization of Aspirin-Phospholipid Complex for Improved Drug Delivery

A. Semalty*, M. Semalty, D. Singh and M.S.M. Rawat
H.N.B. Garhwal University, Srinagar, India.

ABSTRACT: Aspirin (acetylsalicylic acid) is one of the most widely used analgesic. Aspirin is poorly soluble in water and causes gastrointestinal (GI) irritation. To improve the solubility (and hence the bioavailability) and minimize the GI irritation, its complexes with soya-phospholipid-80 % (in 1:1 molar ratio) were prepared in an organic solvent and evaluated for solubility, drug content, scanning electron microscopy (SEM), FT-IR spectra, X ray diffraction, differential scanning calorimetry (DSC) and in vitro dissolution study. Aspirin-phospholipid complex were found to be disc shaped with rough surface in SEM. Drug content in the complex was found to be 95.6 %. DSC thermograms, XRD and FTIR confirmed the formation of phospholipid complex. Solubility of the prepared complex was found to be improved. Aspirin complex and aspirin showed 90.93 % and 69.42 % of drug release at the end of 10 h in dissolution study in pH 1.2 acid buffer. It was concluded that the phospholipid complex of aspirin may be of potential use for improving the solubility of aspirin and hence its bioavailability. The complexes may also reduce GI toxicity of the drug.

KEYWORDS: Pharmacosomes; Phospholipids complex; Aspirin; NSAIDs

Introduction
Aspirin (acetylsalicylic acid, ASA) is one of the most widely used therapeutic substances due to its analgesic, antipyretic and anti-inflammatory properties. Despite the proliferation in development of new non-steroidal anti-inflammatory drugs (NSAIDs), ASA remains one of the most effective ‘over-the-counter’ drugs in the treatment of rheumatic diseases. Furthermore, due to its anti-thrombotic properties, ASA is now prescribed at low doses in the prevention and treatment of cardiovascular diseases, strokes and disorders associated with platelet aggregability (Van and Botting, 1992).

ASA use is commonly associated with gastrointestinal (GI) side-effects ranging from dyspeptic symptoms and ulceration to life-threatening episodes of bleeding (Hollander, 1994). ASA is poorly soluble in the acidic conditions of the stomach, which can delay absorption of high doses for 8 to 24 hours. Modifying the water solubility of aspirin may prove to be beneficial for improving its absorption.

The rate of release of a drug is a function of its intrinsic solubility and influenced by particle size, crystallinity, drug derivatization and formation of more-soluble complexes (Leuner and Dressmann, 2002; Rabinow, 2004; Patravale et al., 2004; Kesisoglon et al. 2007; Nijlen 2003; Nokhodchi, 2005). Various approaches have been investigated to improve the absorption and permeation of biologically active constituents of synthetic and natural origin. These include, development of more soluble pro-drug, solid dispersions, preparing complexes with complexing agents like metals, cyclodextrin and phospholipids. Apart from other methods used for modifying the solubility, the complexation with phospholipids have been found to show improvement in both, absorption as well as permeation of the active constituents (Semalty et al., 2009a; Semalty et al., 2009b).

Phospholipids (like phosphatidylcholine) play a major role in drug delivery due to its amphiphilic nature, that can modify the rate of drug release for the enhancement of drug absorption across biological barriers. Developing of amphiphilic drug-lipid complexes or pharmacosomes may prove to be a potential approach for improving therapeutic efficacy of the drugs by improving the bioavailability (through improvement in solubility in GI fluid and permeation across the biomembranes). Any drug possessing an active hydrogen atom (-COOH, -OH, -NH2, etc.) can be esterified to the lipid, with or without spacer chain. Synthesis of such compound may be guided in such
a way that strongly results in an amphiphilic compound, which will facilitate 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. The salient features of pharmacosomes are, increased entrapment efficiency, easy removal of unentrapped drug from the formulation, no loss of drug due to leakage, no problem of drug incorporation and no influence of encaptured volume and drug-bilayer interaction on entrapment efficiency (Semalty et al., 2009a; Biju et al., 2006). These amphiphilic drug-lipid complexes, are stable and more bioavailable with low interfacial tension between the system and the GI fluid, thereby facilitating membrane, tissue, or cell wall transfer, in the organism.

Moreover, the incorporation of NSAIDs with phospholipids has been also suggested to improve GI safety of these drugs. It has been reported that the diffusion of NSAIDs across lipid membranes and into target cells is accelerated when it is present as a complex with phosphatidylcholine (PC) (Lichtenberger et al., 1995). Therefore, developing the drugs as lipid complexes (pharmacosomes) may prove to be a potential approaches to improve solubility and to minimize the GI toxicity of NSAIDs.

Therefore, this study aims to develop the amphiphilic lipid complexes or pharmacosomes of aspirin to improve solubility and hence its dissolution. The study deals with its characterization for drug content, solubility, crystallinity (XRD), chemical interaction (FTIR), phase transition behaviour (DSC) and in vitro dissolution study.

Materials and Methods

Materials

Aspirin was purchased from E-Merck Mumbai. Soya phosphatidylcholine (LIPOID S-80) was obtained as a gift sample from LIPOID GmbH Germany. All other chemicals were of analytical grade.

Methods

Preparation of Aspirin-PC Complex

Aspirin-PC complex was prepared by associating aspirin with an equimolar concentration of PC (80 % purity grade of soya-phospholipids). The equimolar concentration of PC (in 1:1 molar ratio) and aspirin were placed in a 100 mL round bottom flask and dissolved in dichloromethane. 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 overnight and then subjected to characterization.

Drug content

To determine the drug content in Aspirin-PC complex, complex equivalent to 100mg were weighed and added in 100mL of pH 1.2 hydrogen chloride buffers. The volumetric flask was stirred continuously for 24hr on a magnetic stirrer. Dilutions were made suitably and measured for the drug content at 228.3nm UV spectrophotometrically by Lambda25 Perkin Elmer UV/Visible Spectrophotometer.

Solubility

To determine the change in solubility due to complexation, solubility of aspirin and aspirin-PC complex was determined in pH 1.2 HCl buffer and n-octanol by shake flask method. 50 mg of aspirin (and 50 mg equivalent in case of complex) was taken in a 100 mL conical flask. 50mL of pH 1.2 HCl buffer was added and then stirred for 15 minutes. The suspension was then transferred to 250mL separating funnel with 50mL of n-octanol and was shaken well. Separating funnel was allowed to stand for about 30 minutes. Concentration of the drug was determined from the aqueous layer spectrophotometrically at 228.3nm.

Scanning electron microscopy (SEM)

To detect the surface morphology of the pharmacosomes, SEM of complex was performed at UGC-DAE consortium Indore (India) by Scanning Electron Microscope (JEOL JSM 5600).

Infrared Spectroscopic Analysis

FTIR spectra for the various powders were obtained on a Perkin Elmer FTIR spectrometer (Perkin Elmer Life and Analytical Sciences, MA, USA) in the transmission mode with the wave number region 4,000-500 cm⁻¹. KBr pellets were prepared by gently mixing 1 mg sample powder with 100mg KBr. The results were shown in Fig. 2.

Differential Scanning Calorimetry (DSC)

Thermograms of Aspirin, phosphatidylcholine (80%), and aspirin-PC complex were recorded using a 2910 Modulated differential scanning calorimeter V4.4E. The thermal behavior was studied by heating 2.0±0.2 mg of each individual sample in a covered sample pan under nitrogen gas flow (Fig. 3). The investigations were carried out over the temperature range 25-250° with a heating rate of 10°C/min.
X-ray Powder Diffraction Analysis

The crystalline state of aspirin in the different samples was evaluated with X-ray powder diffraction. Diffraction patterns were obtained on a Bruker Axs- D8 Discover Powder X-ray diffractometer (Germany). The X-ray generator was operated at 40 KV tube voltages and 40 mA of tube current, using the Ka lines of copper as the radiation source. The scanning angle ranged from 1 to 60° of 20 in step scan mode (step width 0.4 °/min). Aspirin, phosphatidylcholine (80%), and aspirin-PC phosphatidylcholine complex were analyzed with X-ray diffractions. The results were shown in Fig. 4.

Dissolution Study

In vitro dissolution studies for aspirin complex as well as plain aspirin were performed in triplicate in a USP XXIII six station dissolution test apparatus (Veego Model No.6DR) at 100 rpm and at 37 °C. An accurately weighed amount of the complex equivalent to 100 mg of aspirin was put into 900 ml pH 1.2 HCl buffer. Samples (three ml each) of dissolution fluid were withdrawn at different intervals and replaced with the equal volume of fresh media, to maintain sink conditions. Withdrawn samples were filtered (through a 0.45 mm membrane filter) and diluted suitably and then analyzed spectrophotometrically at 228.3nm.

Statistical analysis

Results are expressed as mean values and standard deviations (± SD) and the significance of the difference observed was analyzed by the Student’s t test. In all tests, a probability value of P<0.05 was considered statistically significant.

Results and Discussion

In the present experiment aspirin–phospholipid complex (pharmacosomes) were prepared by a simple and reproducible method.

Content of aspirin in phospholipid complex, as estimated by UV spectrophotometry, was 95.6% (w/w). Pharmacosomes showed a high percentage of drug loading, which is peculiar to them. Complexation is giving a good percent loading of the drug that makes the delivery of drug clinically feasible.

Solubility of the aspirin complex was found to be much higher (in water and n octanol) than the aspirin. Table 1 provides the solubility data. The increase in solubility of aspirin in the complex can be explained by the solubilization resulted from the formation of micelle in the medium and by the amorphous characteristics of the complex. As an amphiphilic surfactant, phospholipids could increase the solubility of the drug by the action of wetting and dispersion. Unlike non-polar nature of aspirin, the complex showed an amphiphilic nature, which in turn may prove to be responsible for improved bioavailability of the drug.

Table 1 Solubility study of aspirin and its complex.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Solubility in aqueous layer (in µg/ mL)*</th>
<th>Solubility in n-Octanol layer (in µg/ mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>8.45 ± 1.20</td>
<td>24.54 ± 2.46</td>
</tr>
<tr>
<td>Aspirin-PC complex</td>
<td>15.98 ± 1.98</td>
<td>31.44 ± 1.86</td>
</tr>
</tbody>
</table>

* Data expressed as mean values and standard deviations (± SD); n=3
Scanning Electron Micrographs of the complex are shown in Fig. 1. Pharmacosomes were found to be of disc shaped with rough surface morphology. Complexes were found to be as free flowing particles. As the phospholipids are natural component their different purity grades may have different effects in shape and surface morphology. The surface was found to be sticky in the complexes prepared with low purity grades (40 %) of phospholipids. On the other hand the surface of the complexes prepared with the high purity grades of phospholipids (80 %) show rough, non-sticky and free flowing nature as in the present study (Semalty et al., 2009b).

The formation of the complex can be confirmed by the IR spectroscopy comparing the spectrum of the complex with the spectra of the individual components and their mechanical mixtures (Semalty et al., 2009a). FT-IR spectra for various powders were obtained on a Perkin Elmer FTIR spectrometer in the transmission mode with the wave number region 4,000-500 cm⁻¹. FTIR spectra showed the changes in peaks in complexes and positions from that of aspirin and PC. FT-IR spectra of complex were significantly different from that of components and that of physical mixtures (Fig. 2).

Aspirin showed the characteristic IR (KBr) peaks of O-CO- stretching at 1756.01, -COO at 1697.75, -OH bending at 1454.70 and -OH bending at 916 cm⁻¹. The O-CO- stretching vibrations at 1697.75 cm⁻¹ in aspirin has been shifted to higher wave number side in the complex with medium absorption band. –OH (out of plane) bending at 916.62 cm⁻¹ in aspirin has merged with broadband at 1057.71 cm⁻¹. –OH (in plane) bending at 1371.18 cm⁻¹ is missing in the complex. The –CN stretching in the phosphatidylcholine at 1238 cm⁻¹ (strong band) has shifted to 1240 cm⁻¹ (broadband) in the complex. Thus the FTIR spectra indicate the interaction of PC with the aspirin’s –COOH group.

The differential scanning calorimetry is a tool used to measure the temperature and energy variation involved in the phase transitions, which reflects the degree of crystallinity and stability of the solid state of pharmaceutical compounds (Sang et al., 2003). The peak size and shape of the DSC curves are useful in determining the crystallinity of the drug and the carrier. In order to
substantiate the association of aspirin with PC. DSC analysis was performed on aspirin, PC, and the aspirin-PC complex. The results of the DSC test confirmed the association of aspirin and PC in the complex as both peaks representing aspirin acid and PC changed position (Fig. 3).

![DSC thermograms](image)

**Fig. 3** DSC thermograms of (a) aspirin; (b) PC-80 and (c) phospholipid complex of aspirin.
PC showed two major peaks at 83.21°C and 107.90°C and a small peak at 64.45°C, while aspirin showed a sharp endothermic peak at 142.6°C. On the other hand, complex showed two unique peaks (at 50.8°C and at 63.8°C), which were different from the peaks of the individual components of the complex. The DSC thermograms of the phospholipid complexes of some phytoconstituents like silybin, puerarin, curcumin and naringenin also revealed similar results (Yanyu et al., 2006; Li et al., 2007; Maiti et al., 2007; Semalty et al., 2009c). In all these studies, the thermogram of the complex also exhibited a single peak which was different from the peak of phytoconstituent and phospholipids. It is evident that the original peaks of phytoconstituent and phospholipids disappear from the thermogram of complex and the phase transition temperature is lower than that of phospholipids. The acemetacin and indomethacin also showed significant changes in the DSC thermogram when these arylacetic acid derivatives were complexed with the 1,2-dipalmitoylphosphatidylcholine (Lúcio et al., 2008).

To check whether the changes in the aspirin crystal morphology correspond to a polymorphic transition and to study the solid state of aspirin phospholipid complex, 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 Fig. 4.

The XRPD of aspirin complex revealed a broad peak similar to PC indicating that the aspirin was in amorphous form in phospholipid complex. The disappearance of aspirin crystalline diffraction peaks confirmed the formation of phospholipid complex. These results are well supported by our previous studies done with the phospholipid complexes of diclofenac and naringenin (Semalty et al., 2009b; Semalty et al., 2009c).

The phospholipid complexes of puerarin, insulin and salmon calcitonin also supported the results obtained (Li et al., 2007; Shi et al., 2006; Cui et al., 2006; Yoo et al., 2003). Unlike liposomes, bonding between drug and the phospholipids in development of pharmacosomes (drug-phospholipid complex), might have resulted into the significant change of its X-ray diffraction.

![Fig. 4](image)

**Fig. 4** High resolution X-ray powder diffraction (XRPD) study of Aspirin complex (APC-80) and its components.
The aspirin–phospholipid complex showed better dissolution profile than the aspirin (Fig. 5). Unlike the free aspirin (which showed a total of only 69.42 % drug release at the end of 10 h) aspirin complex showed 90.93 % at the end of 10 h of dissolution study in pH 1.2 acid (HCl) buffer. The solid dissolution is a complex operation influenced by a great number of factors, not only the particle size. Differences in crystal habit, surface area, surface energies, particle size and wettability may all play a role in affecting the dissolution rate of powder (Jarmer et al., 2005). Phospholipids being an amphiphilic surfactant, increased the solubility of the drug by the action of wetting and dispersion. And that’s why the dissolution profile of the complex was found to be improved.

Fig. 5 Dissolution study of aspirin from the phospholipid complex.
(■ aspirin; ● aspirin phospholipid complex).

Conclusions
In the present study aspirin–phospholipid complex (pharmacosomes) were prepared by a simple and reproducible method. The physicochemical investigations showed that aspirin formed a complex with phospholipids with better solubility and dissolution profile. The phospholipid complex of aspirin may be of potential use for improving bioavailability. As the phospholipid complexes have also been reported to reduce the GI toxicity of the drugs, the phospholipid complex of aspirin may also be useful or minimizing the GI toxicity of aspirin, which may be validated further through in vivo studies.

The pharmacosomes may be developed for other NSAIDs with poor bioavailability and GI side effects. Moreover the pharmacosomes of phytoconstituents (with poor water and/or lipid solubility) may also be developed for improving their bioavailability.

Acknowledgments
Authors acknowledge the grant provided by the Department of Science & Technology, Govt. of India for the research work. Authors are thankful to LIPOID GmbH Germany for providing the gift sample of phosphatidylcholine for this work. Facilities provided by the UGC-DAE Consortium for Scientific Research, Indore (M.P.) and Department of Chemistry, University of Delhi are thankfully acknowledged.

References


