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Emodin–phospholipid complex

A potential of herbal drug in the novel drug delivery system

Devendra Singh · M. S. M. Rawat · Ajay Semalty · Mona Semalty

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Abstract Developing the drugs as amphiphilic lipid complexes is a potential approach for improving therapeutic efficacy of the drugs by increasing solubility, reducing drug crystallinity, modifying dissolution behavior (sustained or controlled release), and improving bioavailability. Emodin (1,3,8-trihydroxy-6-methylanthraquinone), an anthranoid derivative, shows several biological effects like antimicrobial, antidiuretic, anti-cancerous, and potent antioxidant but due to poor solubility, the dissolution restrains its valuable importance. To overcome this limitation, the emodin–phospholipid complex was developed and investigated by thermal analysis (differential scanning calorimetry), crystallographic (X-ray diffractography), surface morphology (scanning electron microscopy), spectroscopic methods (FT-IR, ^{1}H-NMR), solubility, and the dissolution (in vitro drug release) study. The phospholipid complex of emodin was found, fluffy and porous with rough surface morphology in the SEM. FT-IR, ^{1}H-NMR, DSC, and X-RPD data confirmed the formation of the complex. The water and *n*-octanol solubility of emodin was improved from 2.25 to 9.97 and 53.45 to 77.62 μg/ml, respectively, in the prepared complex. The improved dissolution was shown by the phospholipid complex. Based on the results of the study, it can be concluded that the phospholipid complex may be considered as promising drug delivery system for improving the overall absorption and bioavailability of the emodin molecule.

D. Singh (✉) · M. S. M. Rawat
Department of Chemistry, HNB Garhwal University, Srinagar (Garhwal) 246 174, Uttarakhand, India
e-mail: rawatdevendr@yahoo.in

A. Semalty · M. Semalty
Department of Pharmaceutical Sciences, HNB Garhwal University, Srinagar (Garhwal) 246 174, Uttarakhand, India

Keywords Emodin · Anti-cancer herbal drug · Phospholipid complex · Chemical interaction · DSC · XR-PD

Introduction

The plant extracts containing anthraquinones (a group of polyphenolics) are being increasingly used for food, cosmetics, and pharmaceuticals due to their wide therapeutic and pharmacological effects and received much attention as potential protectors against a variety of human diseases (particularly, cardiovascular, and cancer) [1–4]. These are found in the roots, barks, or leaves of numerous plants such as Senna, Cascara, Aloe, Frangula, Rhubarb, and many herbal laxatives of the genus rhamnus and family polygonaceae [5–7].

Rheum preparations are well known for their valid medicinal properties. It has been widely used as traditional Indian, Tibetan, and Chinese medicine as a purgative and detoxicant [8]. This genus also has antimicrobial, anti-inflammatory, antitumor, haemostatic, and cholesterol lowering noteworthy activities [8, 9].

Emodin (1,3,8-trihydroxy-6-methylanthraquinone, Fig. 1) an anthraquinone derivative isolated from genus Rheum, has numerous biochemical and pharmacological activities and is known as a potent anti-cancerous agent [2, 9]. Besides its anti-carcinogenic activity [10–12], the component also shows antimicrobial, diuretic, vasorelaxant [13–15], monoamine oxidase [16, 17], and tyrosine kinase inhibitory effects [18, 19]. Moreover, it plays an important role in the brain protection against cerebral injury, GI mortality [4, 20], able to trigger acetylcholine release, and cause muscle contraction by binding with muscarinic receptors [21, 22].

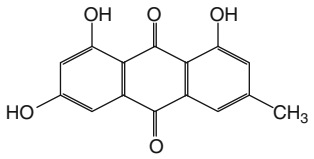
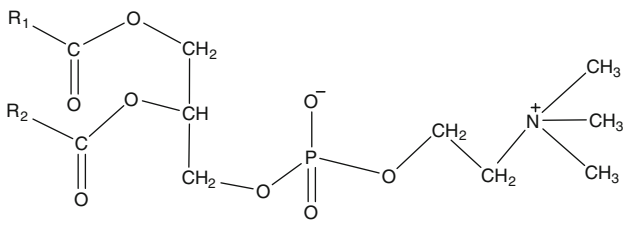


Fig. 1 Emodin

Despite having this wide range of therapeutic activity, the emodin is poorly bioavailable and restricts its use as a potent phyto molecule [23–26]. The absorption and bioavailability is mainly dependent on the rate of dissolution of the drug in the gastrointestinal tract and influenced by the reduced particle size (to increase surface area), solubilization in surfactant systems, formation of more-soluble complexes, drug derivatization, and conversion of solid state by reducing crystallinity of drug substance [27–31].

Many pharmaceutical systems (like nanoparticles, microemulsion, matrix system, solid dispersion, liposomes, and drug lipid complexes) are receiving increasing attention in the novel drug delivery system [32–34]. In this way, one potential method to reduce the problems associated with the oral administration of emodin may be the use of phospholipid-based novel drug delivery system. The phospholipid complex of bioactive compound may diffuse the active component in a slow and time dependent manner [35–38]. These phospholipid based systems may be expected to have a positive impact upon the problems of oral drug delivery of phytochemicals, which radically alters the pharmacokinetics, distribution, and metabolism of drugs [39].

The natural or biological membranes are complex systems, built up of several types of compounds of which a double layer of phospholipid vesicles (an important barrier to oral absorption) forms an important constituent and studied extensively due to their interaction with several physiological active compounds [35, 36]. Phospholipids (phosphatidylcholine: Fig. 2, phosphatidylserine, phosphatidylethanolamine, etc.), as a carrier, play a major role in the drug delivery due to their amphiphilic nature that can modify the solubility behavior and the rate of drug release for the enhancement of drug absorption across the biological barriers.



(Where R_1, R_2 = fatty acid chain)

Fig. 2 Phosphatidylcholine

The phospholipids (phosphatidylcholine) are a bifunctional compound, the phosphatidyl moiety being lipophilic and the choline moiety being hydrophilic in nature. Specifically, the choline head of the phosphatidylcholine molecule binds to active compounds while the lipid soluble phosphatidyl portion comprise the body and tail which then envelopes the choline bound material. The phosphatidylcholine besides as a carrier for the phytochemicals is itself a bioactive nutrient with documented clinical efficacy for liver diseases including alcoholic hepatic steatosis, drug-induced liver damage, and hepatitis [37, 38]. Moreover, it is also an excellent emulsifier that enhances the bioavailability of constituents with which it is co-administered. The bioavailability of poorly absorbed drugs can be improved by preparing their phospholipid complexes.

The present study deals with the development of emodin–phospholipid complex, with the aim of improving solubility and modifying the dissolution profile for making the basis for better absorption of emodin through the gastrointestinal tract (GIT), which may improve the overall bioavailability of the molecule. In this study, for the very first time an anthraquinone (emodin) was taken for the development of phospholipid complex. The prepared emodin–phospholipid complex was evaluated for various physico-chemical parameters like drug content, chemical interaction (FT-IR, ^{1}H-NMR), thermal behavior (DSC), crystallinity (X-RPD), surface morphology (SEM), and solubility and dissolution study.

Materials and methods

Materials

Emodin was purchased from Sigma–Aldrich Mumbai (India), and Soya phosphatidylcholine (LIPIOD S-80) was obtained as a gift sample from LIPIOD, Germany. All the other chemical reagents were of analytic grade.

Method of Preparation

The emodin–phospholipid complex (Em-PLc) was prepared by refluxing the emodin and phosphatidylcholine in (1:1) molar ratio. Both the reactants were placed in 100 ml round-bottomed flask containing 20 ml of dichloromethane. The reaction proceeded by refluxing the reaction mixture in a magnetic stirrer at 45–50 °C for 5 h. Thereafter, the volume of resulting solution concentrated to 2–3 ml, and sufficient amount of *n*-hexane was added to get precipitation. The complex was collected, filtered, washed, dried under vacuum, and stored in an air tight container until further use.

Drug content

The complex equivalent to 50 mg of emodin was weighed and added into a volumetric flask with 100 mL of pH 6.8 phosphate buffer saline. The volumetric flask was stirred continuously at room temperature on a magnetic stirrer. At the end of 24 h, suitable dilutions were made and measured spectrophotometrically (double beam UV–Visible spectrophotometer, Lambda 25, Perkin Elmer, USA) at 289 nm.

Infrared spectroscopy

FTIR spectra for the various powders were recorded 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} in KBr pellets.

Nuclear magnetic resonance (NMR)

^{1}H-NMR spectra of free emodin and the complex were obtained from a BRUKER AM-400 spectrophotometer with the solvent $CDCl_3$.

Differential scanning calorimetry (DSC)

DSC curve of emodin, phosphatidylcholine, and the Em-PLc were recorded using a 2910-Modulated Differential Scanning Calorimeter V4.4E (TA Instrument, USA). 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. The investigation was carried out over the temperature range 0–300 °C at a heating rate of 10 °C min^{-1}.

X-ray powder diffraction (X-RPD)

The crystalline state of emodin in the different samples was evaluated using X-ray powder diffraction. Diffraction patterns were obtained on a Brucker Axs- D8 Discover Powder X-ray Diffractometer, Germany. The X-ray generator was operated at 40 kV tube voltages and 40 mA tube current, using the K α lines of copper as the radiation source. The scanning angle ranged from 5 to 50° of 2θ in the step scan mode (step width 0.019° min^{-1}).

Scanning electron microscopy (SEM)

SEM imaging of the complex was performed using a scanning electron microscope (JEOL JSM 5600).

Apparent solubility study

Apparent solubility was determined by adding excess of emodin and emodin–complex to 5 ml of water or *n*-octanol in sealed glass containers at room temperature (25–30 °C). The liquid was agitated for 24 h then centrifuged for 20 min at 1,000 rpm to remove excessive emodin. The supernatant was filtered through membrane filter then 1 ml filtrate was mixed with 9 ml of distilled water/*n*-octanol to prepare dilutions, and the samples were measured at wavelength of 289 nm spectrophotometrically.

Dissolution study (in vitro drug release)

The dissolution studies were carried out in a USP XXIII, six station dissolution test apparatus, type II (VEEGO Model No. 6 DR, India) at 100 rpm and at 37 °C. An accurately weighed amount of the complex equivalent to 50 mg of emodin was put into 900 mL of pH 6.8 phosphate buffer. Samples (3 mL each) of dissolution fluid were withdrawn at different time intervals and replaced with an equal volume of fresh medium to maintain sink conditions. Withdrawn samples were filtered (through a 0.45 µm membrane filter), diluted suitably, and then analyzed spectrophotometrically.

Results and discussion

In the present experiment, the prepared complex (Em-PLc) showed a good encapsulation efficiency of emodin and found 89.9% (w/w) as estimated by UV spectrophotometry. Complexation provided good encapsulation efficiency, which could make the delivery of emodin clinically feasible.

FT-IR (infrared absorption)

FTIR studies were done to detect the possible interaction between the emodin and phosphatidylcholine in the phospholipid complex (Fig. 3). In IR spectra, the characteristic C–H stretching band of long fatty acid chain at 2918 and 2850 cm^{-1}, carbonyl stretching band at 1738 cm^{-1} in the fatty acid ester, P=O stretching band at 1236 cm^{-1}, P–O–C stretching band at 1091 cm^{-1}, and $N^{+}(CH_3)_3$ stretching at 970 cm^{-1} were observed in phosphatidylcholine. In the case of emodin the O–H stretching at 3389.42 cm^{-1}, C=O stretching at 1618.18 cm^{-1}, C=C vibrations in the benzene ring near at 1500 cm^{-1}, C–C stretching in the ring at 875.29, 1034.15 cm^{-1}, and O–H bending at 1369.70 cm^{-1} were characterized.

The FTIR of the complex shows the significant changes in the spectrum, and the absorption peak of hydroxyl (O–H) stretching of emodin has remarkable broadening, and the keto (C=O) group frequency has been shifted to higher wave number in the complex. On the other hand, the P=O

absorption band of phosphatidylcholine shifted to lower wave number with higher shifting of P–O–C stretching vibrations.

The spectrum of the physical mixture is quite different from the spectrum of the complex and seems to be only a summation of both the constituents. Therefore, the spectroscopic changes show that the shifting of hydroxyl and keto group frequencies of emodin indicated the interaction of emodin to the polar end of the phosphatidylcholine.

^{1}H-NMR (Nuclear Magnetic Resonance)

In the phosphatidylcholine molecule, the protons of methyl group attached to N–atom [–$N^+(CH_3)_3$] were indicated by the signals at 3.277 δ (ppm). The methylene group proton near N–atom (–CH_2–N^+) at shift value 3.705 δ (ppm) and the value of methylene proton attached to (–P–O–CH_2–) at 4.25 δ (ppm). The value near about at 2.304 δ (ppm) showed the presence of methylene proton attached to –C(=O)C. The signals of chemical shift value near at 0.859 δ (ppm) was due to the methyl proton of aliphatic side chain, while the value of 1.229 δ (ppm) suggested the presence of methylene proton of aliphatic side chain of the molecule.

In ^{1}H-NMR spectrum of emodin (Fig. 4a), the two singlets for highly deshielded protons at δ 11.88 and 11.96 ppm assignable to intra-molecular hydroxy protons at the 8th and 1st positions, respectively, and δ value at 11.329 may be assigned to 3rd hydroxyl proton. The proton of aromatic region occurs at δ 6.4–7.3 ppm, and the methyl singlet was recorded at δ 2.30 ppm.

In the case of ^{1}H-NMR of emodin–phospholipid complex (Fig. 4b), the value of the phospholipid at δ 3.27 and 3.705 shifted to downfield at 3.293 and 3.734 δ (ppm), respectively, which was due to methyl proton of [–$N^+(CH_3)_3$] and methylene proton of (–CH_2–N^+). The methylene proton of (–P–O–CH_2–) at 4.25 δ (ppm) also shifted to 4.34 δ (ppm). Similarly, the signals of compound (emodin) at δ 11.88 and 11.96 ppm assignable to hydroxy protons shifted to δ 11.942 and 12.021 (ppm). The hydroxyl proton at C_3 position did not show any signal in the complex. The overall observation

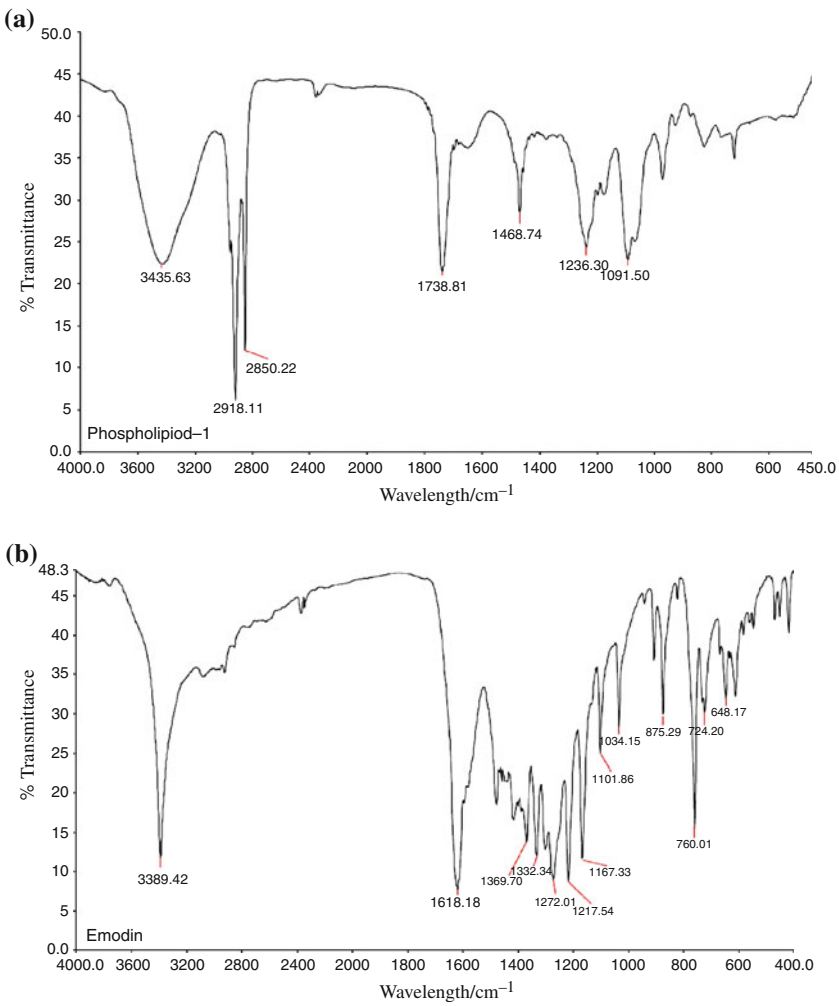


Fig. 3 Infrared emission spectra over the wavelength range 400–4000 cm^{-1}: **a** phospholipid, **b** emodin, **c** emodin–phospholipid complex, **d** physical mixture

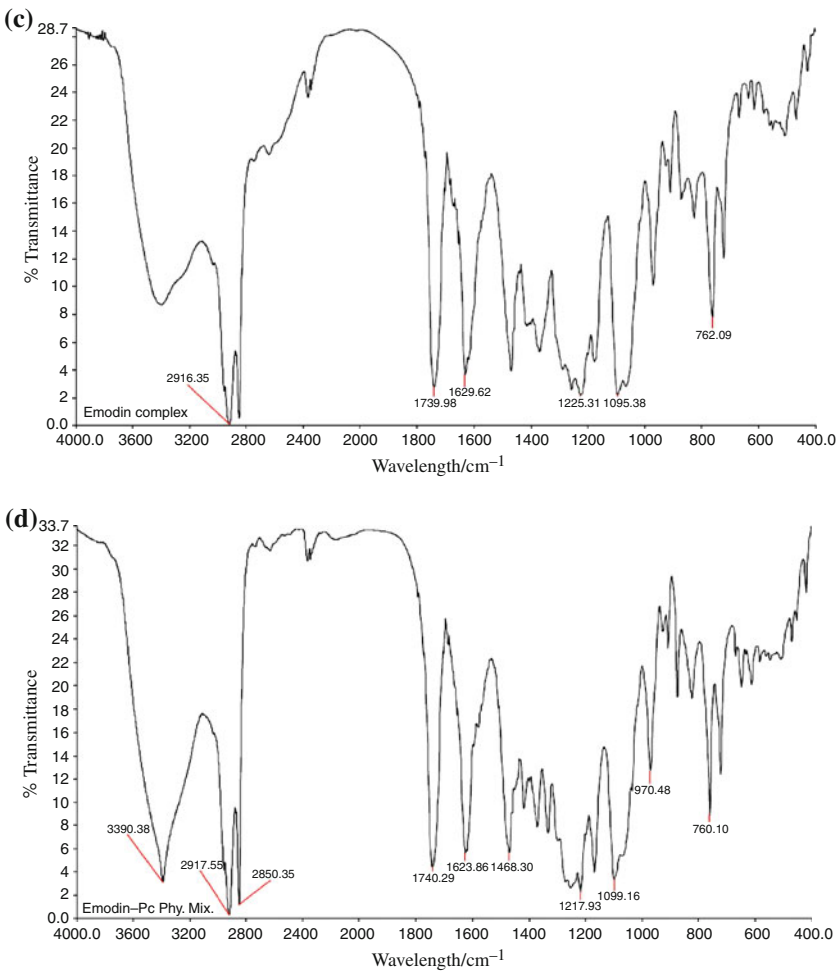


Fig. 3 continued

indicated that emodin phenolic groups have participated in the complex formation with the polar head of the phosphatidylcholine molecule.

Thermal analysis (differential scanning calorimetry)

Differential scanning calorimetry (DSC) is a fast and reliable method to detect drug-excipient compatibility to provide maximum information regarding the possible interactions. An interaction is concluded by the elimination of endothermic peaks, appearance of new peaks, change in peak shape and its onset, peak temperature/melting point, and relative peak area or enthalpy. From the Fig. 5, the crystals of emodin show the endothermic peak at about 260.98 °C ($\Delta H_f = 167.4$ J/g), corresponding to the product melting and the phosphatidylcholine exhibited a typical endothermic peak at about 76.74 °C ($\Delta H_f = 31.51$ J/g). The emodin phospholipid complex showed complete disappearance of the endothermic peaks of the individual component and exhibited a broad new peak at about 70.22 °C ($\Delta H_f = 63.58$ J/g) in the DSC curve and supported the interaction of emodin molecule with the phosphatidylcholine. The study also supported the previous study [40–42].

X-ray powder diffractometry (X-RPD)

Figure 6 shows the X-ray diffraction patterns of the emodin, phospholipid, and the complex. In the X-ray diffractogram, emodin showed intense diffraction peaks of crystallinity at a diffraction angle of 2θ and suggested that the drug is present as a crystalline material. The phospholipid showed a single diffraction peak. A total drug amorphization was induced by the complex formation where X-ray diffraction pattern of the emodin–phospholipid complex was characterized only by large diffraction peaks in which it is no longer possible to distinguish the characteristic peaks of the drug. The results confirmed that emodin is no longer present as a crystalline material, and its phospholipid complex exist in the amorphous state. Thus, XRD data supports the DSC studies which indicated the reduced crystallinity of drug in the prepared complex by exhibiting lower values of

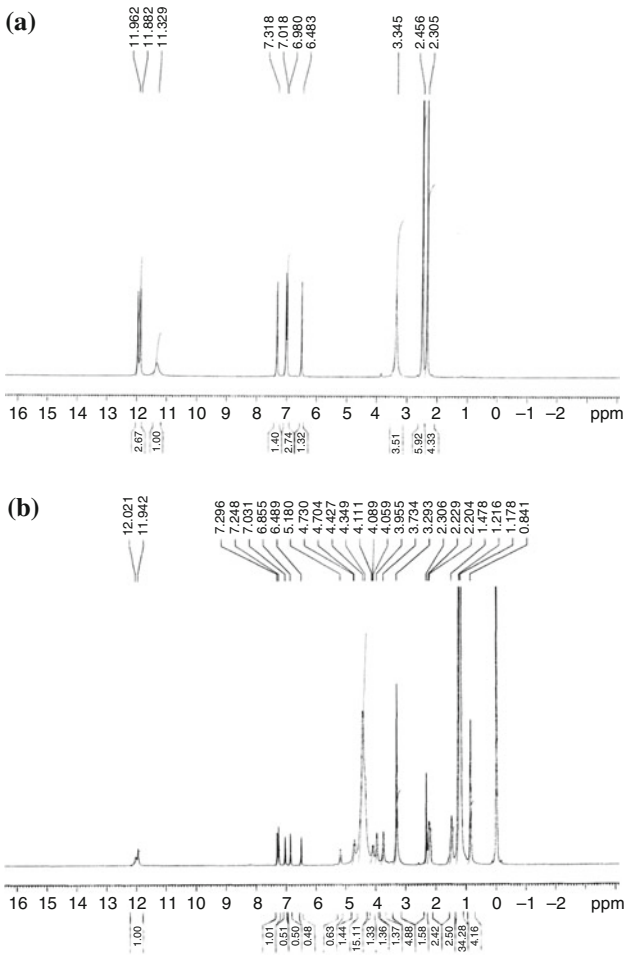


Fig. 4 ^{1}H-NMR spectra: **a** emodin, **b** emodin–phospholipid complex

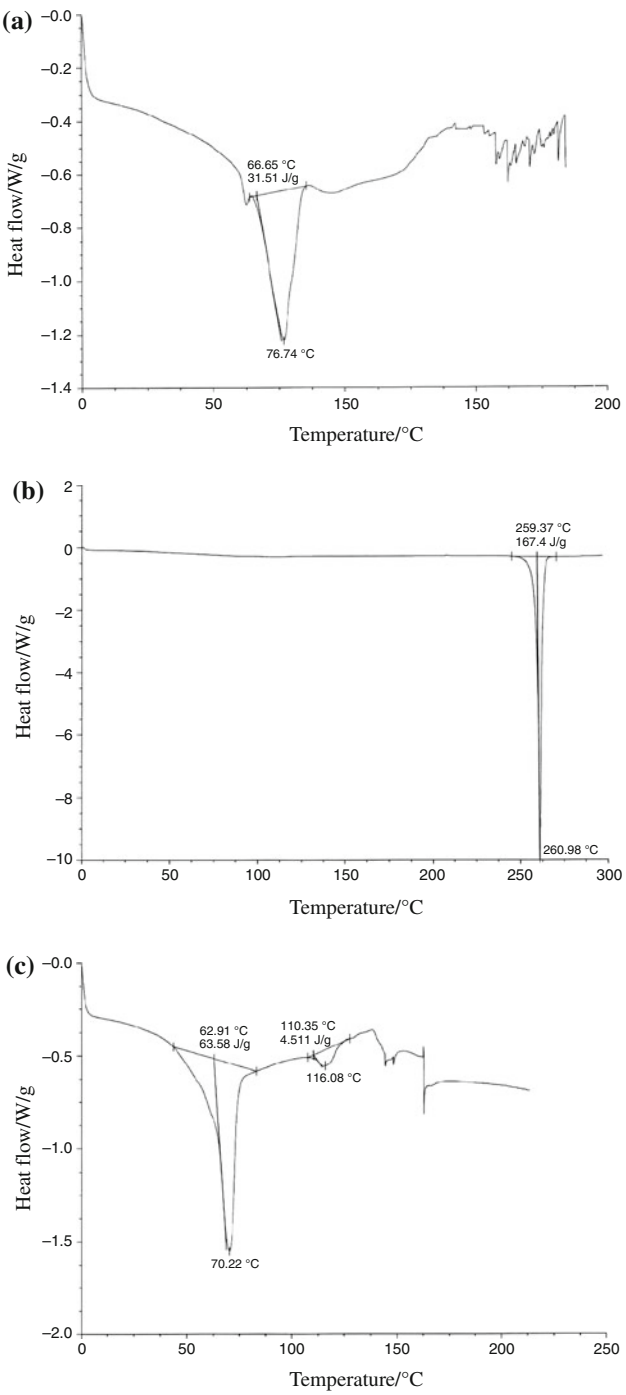


Fig. 5 DSC curves: **a** phospholipid, **b** emodin, **c** emodin–phospholipid complex; Sample mass 2.0 mg, heating rate 10 °C min^{-1}, nitrogen atmosphere

enthalpy and melting points. This study supports well the previous studies [37, 43–45].

Surface morphology (SEM)

The scanning electron micrographs of emodin and the complex are given in Fig. 7. The emodin was characterized as needle-like crystals of smaller size and regular shape with an apparently smooth surface. In contrast, a clear change in the morphology and the shape of the particles was observed in the phospholipid complex and showed fluffy, porous, and rough surface, revealing an apparent interaction in the solid state which might have resulted to the enhanced dissolution rate as compared with pure drug.

Solubility study

Table 1 provides the solubility data. The emodin complex showed good aqueous solubility in hydrophilic as well as lipophilic medium (in water and *n*-octanol) and found to be higher than the free emodin. Emodin is poorly miscible in aqueous media, but the complexation with the phospholipid increased the solubility of emodin in water and *n*-octanol significantly. This increase in solubility of the complex may be explained by the amorphous characteristics of the complex (due to reduced molecular crystallinity of the

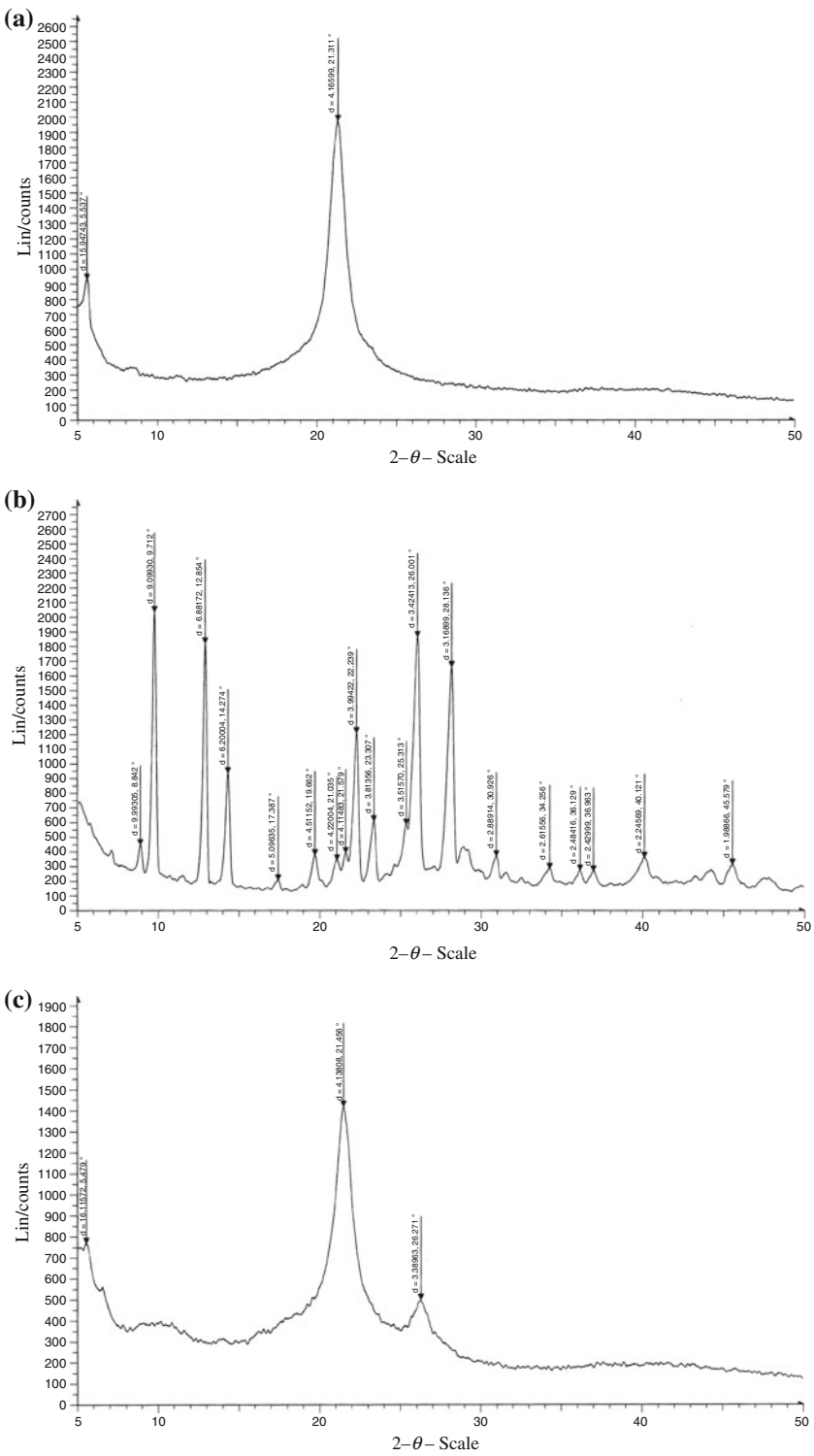


Fig. 6 X-ray diffraction patterns: **a** phospholipid, **b** emodin, **c** emodin–phospholipid complex; scanning angle 5–50° of 2θ, step width 0.019° min^{-1}

drug) and amphiphilic nature of the complex [46, 47]. The complex showed an amphiphilic nature, which in turn may result in improved absorption across the GIT (gastrointestinal tract) for improved availability of the emodin in the systemic circulation.

Dissolution study

The emodin–phospholipid complex showed improved dissolution profile than the emodin (Fig. 8). Unlike the free emodin which showed a total of only 38.02% drug release

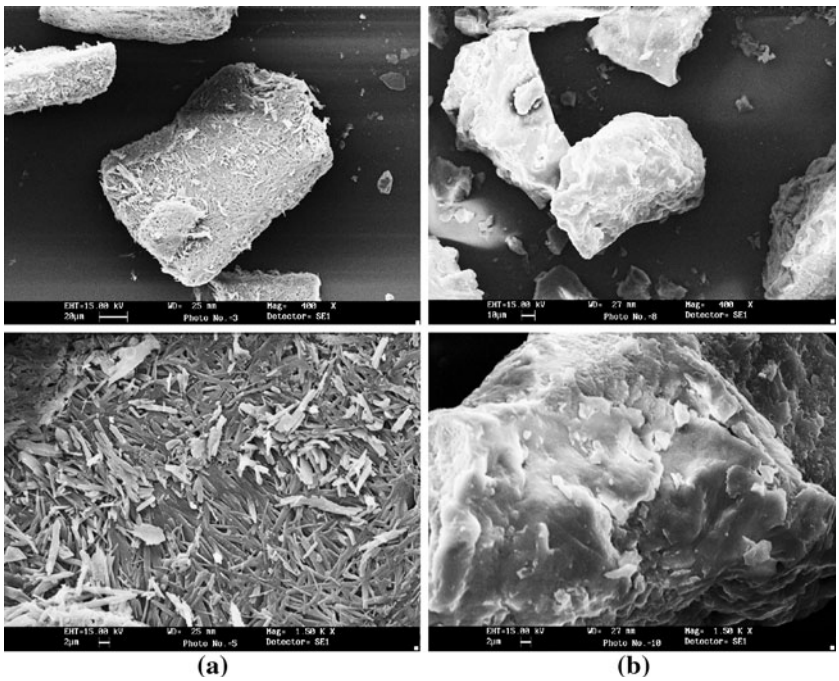


Fig. 7 SEM micrographs: magnification 400X and 1.50 KX **a** emodin, **b** emodin–phospholipid complex

Table 1 Solubility study of emodin and emodin–phospholipid complex

Sample	Aqueous solubility/ μg ml^{-1}	*n*-octanol solubility/ μg ml^{-1}
Emodin	2.25 ± 0.0007	53.45 ± 0.0009
Emodin complex	9.97 ± 0.0005	77.62 ± 0.072

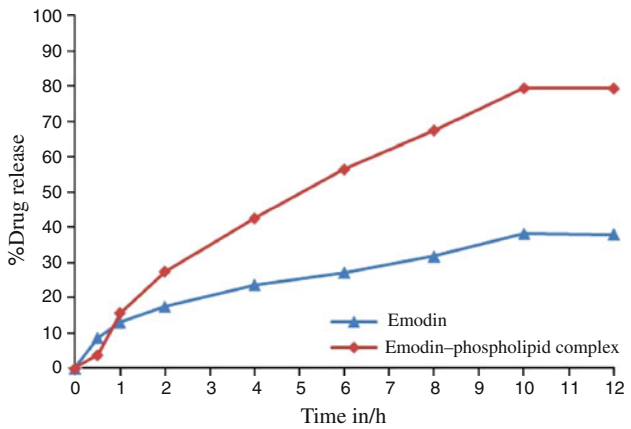


Fig. 8 Dissolution study of emodin and emodin–phospholipid complex

at the end of 12 h, emodin–complex showed 79.4% release at the end of 12 h of the dissolution study. As the dissolution rate is greatly affected by the crystal morphology and wettability [48], the improved dissolution rate of emodin from the phospholipid complex may be explained by the improved solubility and disrupted crystalline phase (amorphous form) in the phospholipid complex. The rate of degradation of the complex into active drug molecule following absorption also depends on the size and functional groups of drug molecule and the chain length of the lipids.

Conclusions

The physicochemical investigations showed that emodin (an anthraquinone) formed an amphiphilic complex (confirmed by FTIR, ^{1}H-NMR, XRD, and DSC) with phosphatidylcholine. With reduced drug crystallinity, the phospholipid complex showed enhanced solubility and dissolution rate. On the basis of the results of the study, it can be concluded that the phospholipid complex may be considered as promising drug delivery system for improved absorption across the gastrointestinal tract for improving the bioavailability of the molecule. However, the bioavailability of the emodin from the complex will have to be further validated with in vivo studies.

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