J Incl Phenom Macrocycl Chem (2010) 67:253–260
DOI 10.1007/s10847-009-9705-8

ORIGINAL ARTICLE

Preparation and characterization of phospholipid complexes of naringenin for effective drug delivery

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

Received: 5 September 2009 / Accepted: 9 November 2009 / Published online: 19 November 2009

Abstract Naringenin is a flavonoid specific to citrus fruits and possesses anti-inflammatory, anticarcinogenic, and antitumour effects. But due to a lower half-life and rapid clearance from the body, frequent administration of the molecule is required. To improve the bioavailability and prolong its duration in body system, its phospholipid complexes were prepared by a simple and reproducible method. Naringenin was complexed with phosphatidylcholine in equimolar ratio, in presence of dichloromethane. The prepared Phytosomes (naringenin–phospholipid complex) were evaluated for various physical parameters like FT-IR spectroscopy, Differential Scanning Calorimetry (DSC), X-ray powder diffractometry (XRPD), Solubility, Scanning Electron Microscopy (SEM) and the in vitro drug release study. These phospholipid complexes of naringenin were found to be irregular and disc shaped with rough surface in SEM. Drug content was found to be 91.7% (w/w). FTIR, ^{1}H NMR, DSC and XRPD data confirmed the formation of phospholipid complex. Water solubility of naringenin improved from 43.83 to 79.31 μg/mL in the prepared complex. Unlike the free naringenin (which showed a total of only 27% drug release at the end of 10 h), naringenin complex showed 99.80% release at the end of 10 h of dissolution study. Thus it can be concluded that the phospholipid complex of naringenin may be of potential use for improving bioavailability.

A. Semalty (✉) · M. Semalty
Department of Pharmaceutical Sciences, H.N.B. Garhwal University, Srinagar (Garhwal), India
e-mail: semaltyajay@gmail.com

D. Singh · M. S. M. Rawat
Department of Chemistry, H.N.B. Garhwal University, Srinagar (Garhwal), India

Keywords Phospholipid complex · Naringenin · DSC · XRPD · NMR · In vitro dissolution

Introduction

Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-pyrone structure. Over 4,000 different flavonoids have been described, and they are categorized into flavonols, flavones, flavanones, isoflavones, flavonols, and anthocyanidins. They occur naturally in fruits and vegetables, mainly as flavonoid glycosides, and are thus important constituents of the human diet.

Naringenin (4′,5,7-trihydroxyflavanone) is an aglycone of naringin (a flavonoid glycoside, specific to citrus fruits) and the predominant flavanone (up to 10% of the dry weight) in grapefruit (*Citrus paradisi*). The glycosidic form of naringenin (naringin) is responsible for the bitterness of grapefruit juices [1]. The main sources of naringenin are citrus fruits, plants and tomato (*Lycopersicum esculentum*) [2, 3]. In citrus fruits, naringenin is principally present in glycosidic forms such as naringenin-7-neohesperidoside (naringin) and naringenin-7-rutinoside (narirutin), whereas in tomato, where naringenin is one of the most abundant polyphenols, is present in the skin as aglycone. The naringenin concentration of tomato is reported to range from 0.8 to 4.2 mg/100 g whole red tomato [3, 4].

This bioflavonoid possesses anti-inflammatory, anticarcinogenic, and antitumour effects [5–7]. It is also found to exert anti-estrogenic activity [8–10]. Furthermore, naringenin seems to affect different oxidative processes associated with chronic degenerative diseases. It partially deactivates the Fenton reaction and restores glutathione-dependent protection against lipid peroxidation in

α-tocopherol- deficient liver microsomes [11, 12]. It produces inhibitory activity in malonaldehyde production and inhibits cytochrome P450 enzymes [13–15].

Despite this wide range of therapeutic activity, its unfavourable pharmacokinetics associated with a lower half-life and rapid clearance from the body restricts its use as a potent phyto molecule. It was reported that the elimination half-lives of naringenin from orange juice and grapefruit juice (when taken orally) were 1.3 and 2.2 h, respectively [16]. Therefore, to maintain steady plasma concentration so as to exert the desired therapeutic activity, frequent administration of the molecule is required, which necessitates the need for development of a dosage form that can maintain the concentration of naringenin in blood for a longer period.

Phospholipids play a major role in drug delivery technology. It is an important carrier for those drug molecules which require sustained/controlled release in vivo due to faster elimination from the body. Developing the drugs as lipid complexes may prove to be a potential approaches to improve solubility and to minimize the GI toxicity of drugs. These amphiphilic drug–lipid complexes, are stable and more bioavailable drug delivery systems with low interfacial tension between the system and the GI fluid thereby facilitating membrane, tissue, or cell wall transfer, in the organism [17].

Thus naringenin–phospholipid complex were prepared and evaluated for various physical parameters (FTIR, DSC, XRD, Solubility, SEM, etc.) and the in vitro dissolution study of naringenin–phospholipid complex in comparison with pure naringenin.

Materials and methods

Materials

Naringenin was purchased from Sigma–Aldrich Mumbai. Soya phosphatidylcholine (LIPOID S-80) was obtained as a gift sample from LIPOID GmbH Germany. All other chemicals were of analytical grade.

Methods

Naringenin–PC complex was prepared by taking naringenin with an equimolar concentration of phosphatidylcholine (PC). The equimolar concentration of PC and naringenin were placed in a 100 mL round bottom flask and refluxed in dichloromethane for 3 h. On concentrating the solution to 5–10 mL, 30 mL of *n*-hexane was added to get the complex as a precipitate followed by filtration. The precipitate was collected and placed in vacuum desiccators.

Drug content

To determine the drug content in the complex, complex equivalent to 100 mg were weighed and added in 100 mL of methanol taken in a 100 mL volumetric flask. The volumetric flask was stirred continuously for 24hr on a magnetic stirrer. Dilutions were made suitably and measured for the drug content UV spectrophotometrically by Lambda25 Perkin Elmer UV/Visible Spectrophotometer.

Solubility

To determine the change in solubility due to complexation, solubility of drug and the complex was determined in buffer/water and *n*-octanol by shake flask method. 50 mg of drug (and 50 mg equivalent in case of complex) was taken in a 100 mL conical flask. 50 mL of distilled water was added and then stirred for 15 min. The suspension was then transferred to 250 mL separating funnel with 50 mL of *n*-octanol and was shaken well for 2 h. Then the separating funnel was allowed to stand for about 30 min. Concentration of the drug was determined from the aqueous layer spectrophotometrically.

Scanning electron microscopy (SEM)

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

Fourier transform infrared spectroscopy (FT-IR)

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 100 mg KBr.

Differential scanning calorimetry (DSC)

Thermograms of naringenin, phosphatidylcholine (80%) and the naringenin–PC complex were recorded using a differential scanning calorimeter (2910 Modulated DSC V4.4E, TA Instruments, US). 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 investigations were carried out over the temperature range 25–250 °C with a heating rate of 10 °C min^{-1}.

X-ray powder diffractometry (XRPD)

The crystalline state of drugs 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 2θ in step scan mode (step width 1°/min). Drug, phosphatidylcholine (80%), and drug–PC complex were analyzed with X-ray diffractions.

Nuclear magnetic resonance (NMR)

Proton NMR of drug, phospholipids and their complexes were recorded on Bruker 400 MHz NMR (Germany), using TMS as internal standard.

Dissolution study

In vitro dissolution studies for drug complex as well as plain drug were performed in triplicate in a USP XXIII six station dissolution test apparatus (Veego Model No. 6DR, India) at 100 rpm and at 37 °C. An accurately weighed amount of the complex equivalent to 100 mg of drug was put into 900 mL distilled water (media). Samples (3 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.

Statistical analysis

Results were expressed as mean values and standard deviations (±SD).

Results and discussion

In the present experiment naringenin–phospholipid complex were prepared by a simple and reproducible method.

Content (percent loading) of naringenin in the complex, as estimated by UV spectrophotometry (at 288 nm in methanol), was found to be 91.7% (w/w). Phyto–phospholipid complex of herbal drugs showed a high percentage of drug loading. Like phospholipid complexes (pharmacosomes) of synthetic drugs, complexation is giving a good percent loading of the drug which makes the delivery of drug clinically feasible.

Solubility of the naringenin complex was found to be much higher (in water and *n*-octanol) than the naringenin.

Table 1 Solubility study of naringenin and its complex

Drug	Aqueous solubility (μg/mL)[a]	*n*-Octanol solubility (μg/mL)[a]
Naringenin	43.83 ± 0.039	440.163 ± 2.641
Naringenin–PC complex	79.31 ± 0.718	468.13 ± 7.590

[a] Data expressed as mean values and standard deviations (±SD); $n = 3$

Table 1 provides the solubility data. Naringenin is practically insoluble in water. But the complex of naringenin with the phospholipid increased the solubility of naringenin in water as well as *n*-octanol. This increase of solubility of the complex may be explained by the solubilization of the drug 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. So the complex showed an amphiphilic nature, which in turn may show the improved bioavailability of the drug.

Scanning Electron Micrographs of the complex are shown in Fig. 1. Unlike the needle shaped structure of naringenin, its phospholipid complex were found to be of irregular shaped with rough surface morphology. Complexes were found to be as free flowing particles. The average particle size of phospholipid complex was found to be 100 μm.

The formation of the complex can be confirmed by the FT-IR spectroscopy comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. FTIR spectra showed the changes in peaks in complexes and positions from that of naringenin (Fig. 2a) and PC (Fig. 2b). FT-IR spectra of complex (Fig. 2c) were significantly different from that of components and that of physical mixtures (Fig. 2d). Naringenin showed the characteristic IR (KBr) peaks at 3285.79, 3117.36, 3035.80, 1629.76, 1602.09, 1519.88, 1498.10, 1463.39 cm^{-1}. PC showed a broad peak at 3,437.05; sharp peaks at 2,917.93 and 2,849.91; and several sharp peaks below 1,800 cm^{-1}. IR spectra of naringenin–phospholipid complex showed disappearance of peaks of phenolic –OH of (of naringenin), which indicates that the phospholipid has interacted with naringenin through these phenolic –OH groups in the process of complexation. On the other hand the physical mixture of PC and naringenin showed broad peak like PC at 3,301.98 cm^{-1} while it shows all the sharp peaks at about the same positions as that of naringenin.

DSC studies

In order to substantiate the association of naringenin with PC, DSC analysis was performed on naringenin, PC, and the

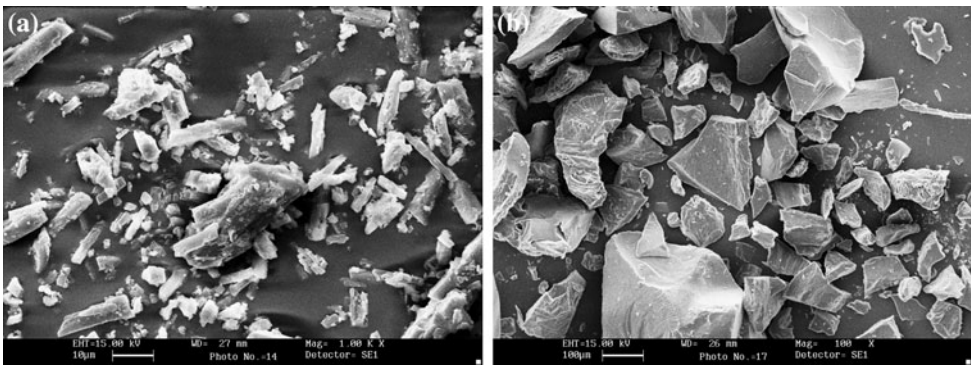

Fig. 1 SEM of naringenin (**a**) and naringenin–phospholipid complex (**b**)

naringenin–PC complex. The results of the DSC test confirmed the association of naringenin and PC in the complex as both peaks representing naringenin and PC changed position (Fig. 3). Phospholipids (Fig. 3b) showed two major peaks at 83.21 and 107.90 °C and a small peak at 64.45 °C. The first one peak of phospholipids is mild peak (at 64.45 °C), which is probably due to the hot movement of phospholipids polar head group. The second (83.21 °C) peak is very sharp and it appears due to phase transition from gel to liquid crystalline state. The non-polar hydrocarbon tail of phospholipids may be melted during this phase, yielding a sharp peak. This melting might have occurred in two phases which subsequently gave another peak (107.90 °C) which is relatively less sharp. Naringenin (Fig. 3a) showed a sharp endothermic peak at 253.08 °C. On the other hand naringenin–PC complex (Fig. 3c) showed two peaks at 51.23 and 62.21 °C, which is different from the peaks of the individual components of the complex. Moreover the onset temperature is 48.18 °C only. It is evident that the original peaks of naringenin and phospholipids disappear from the thermogram of complex and the phase transition temperature is lower than that of phospholipids.

These DSC data are well supported by the results of DSC thermograms of the phospholipid complexes of some phytoconstituents like silybin, puerarin and curcumin [18–21]. In all these studies the thermogram of the complex also exhibited a single peak which was different from the peak of phytoconstituents and phospholipids.

This interaction may be due to hydrophobic interaction and/or hydrogen bonding. The –OH groups of the phenol rings of naringenin may be involved in hydrogen bonding whereas the aromatic rings may be involved in hydrophobic interaction. As a result, the major sharp peaks of phospholipids disappear and lower the phase transition temperature.

X-Ray powder diffraction study

The XRPD of naringenin–phospholipid complex (Fig. 4) revealed a broad peak similar to PC. It suggested that the naringenin in phospholipid complex was either in amorphous form or molecularly dispersed.

These results were well supported by the previous studies done with the phospholipid complexes of puerarin, insulin and diclofenac [22–25]. The disappearance of naringenin's crystalline diffraction peaks confirmed the formation of phospholipid complex. Unlike liposomes, bonding between drug and the phospholipids in development of pharmcosomes (drug–phospholipid complex), might have resulted into the significant change of its X-ray diffraction.

NMR

Proton NMR of Naringenin: δ 5.1 (d, 1H, H-2); δ 2.6 (d, 1H, H-3 equatorial); δ 2.9 (q, 1H, H-4, H-3 axial); δ 15.8 (d, 2H, H-6); δ 6.72 (d, 1H, H-8); δ 7.1 (d, 2H, H-2′ and H-6′); δ 6.74 (d, 2H, H-3′ and H-5′); δ 9.1 (s, 1H, 4′-OH); δ 10.2 (s, 1H, 7-OH); δ 11.9 (s, 1H, 5-OH).

In ^{1}H NMR proton peaks at δ 9.1 for H-4′ and δ 10.2 for H-7′-OH are missing in the naringenin–PC complex (Fig. 5), which indicates that PC has made interaction with the negative oxygen of labile –OH group. The downfield shift of H-5 proton in naringenin and naringenin–PC complex at δ 11.9 indicates its intramolecular hydrogen bonding with the C-4 ketonic function.

Dissolution study

The Naringenin–phospholipid complex showed better dissolution profile than the naringenin (Fig. 6). Unlike the free naringenin (which showed a total of only 27% drug release at the end of 10 h), naringenin complex showed 99.80% release at the end of 10 h of dissolution study in distilled water.

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. In some studies done

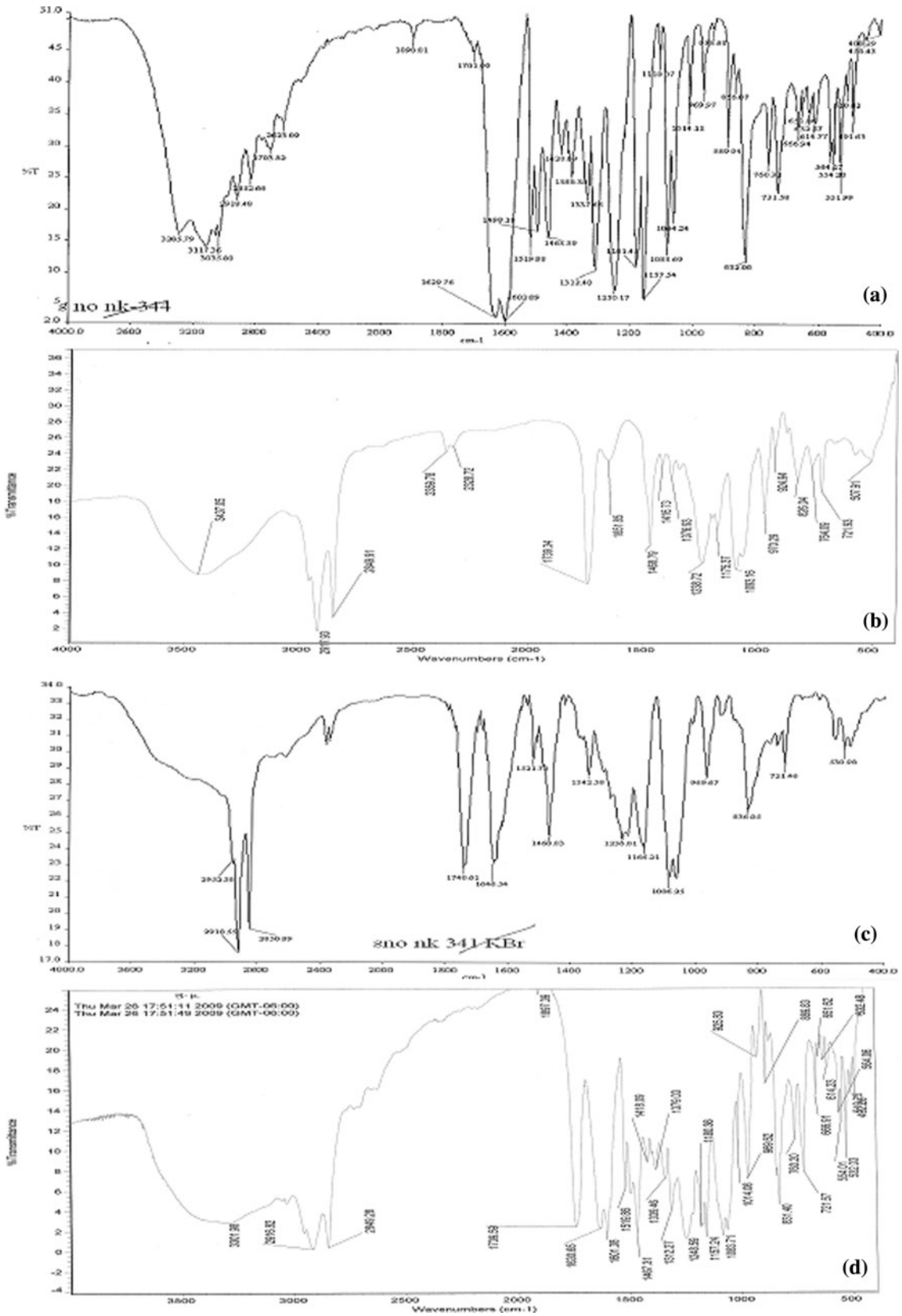

Fig. 2 FTIR spectra of naringenin (**a**); phosphatidylcholine or PC (**b**); naringenin–phosphatidylcholine complex (**c**); and physical mixture of naringenin with PC (**d**)

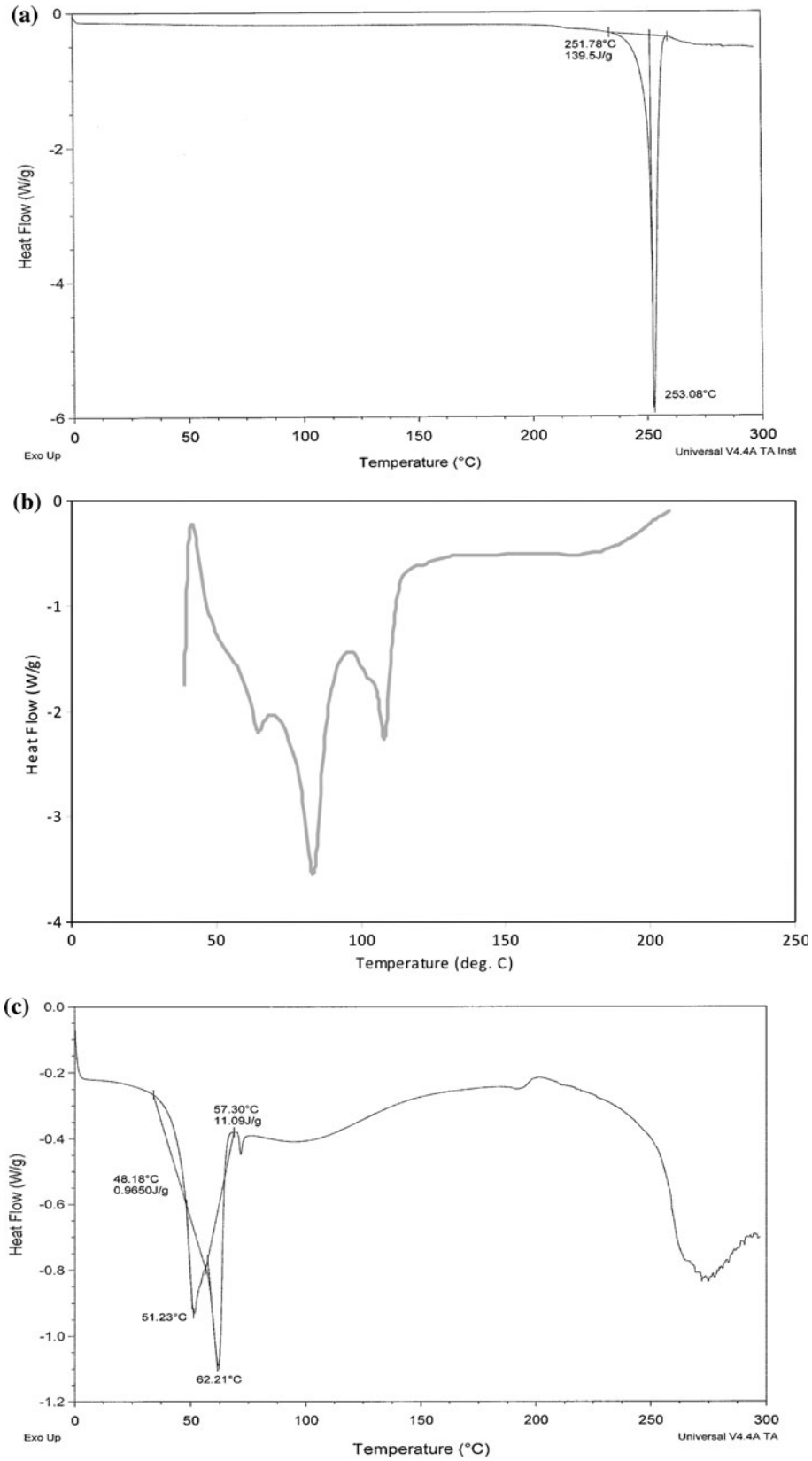

Fig. 3 DSC thermograms of naringenin (**a**); PC (**b**) and naringenin–PC complex (**c**)

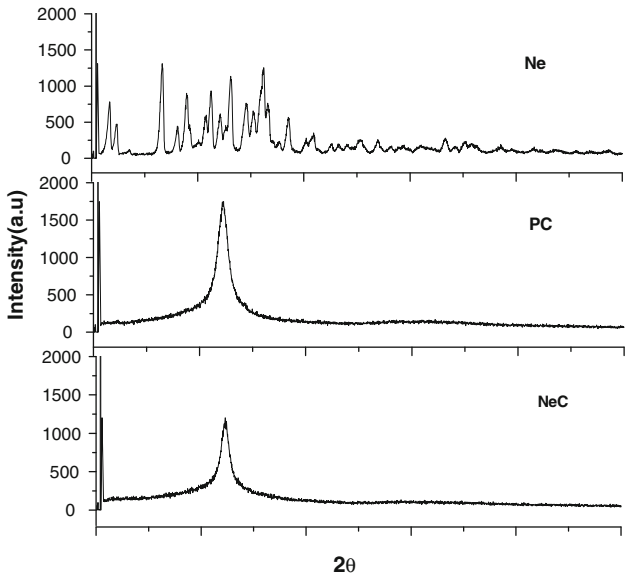

Fig. 4 High resolution X-ray diffraction (HRXRD) study of naringenin (Ne) and its complex (NeC) and its components

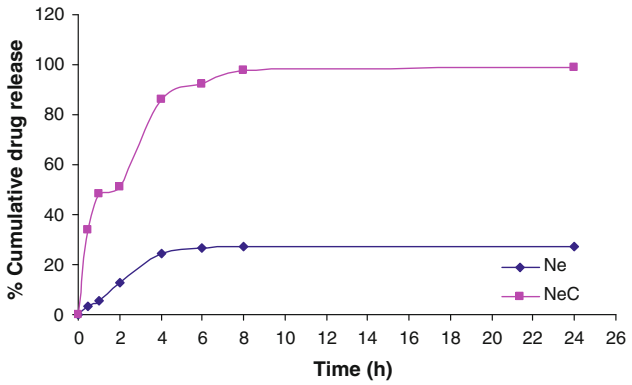

Fig. 6 Dissolution study of naringenin (Ne) and naringenin–phospholipid complex (NeC)

with silybin, the in vitro drug release from the complexes was found to be pH dependent and with the increase of the pH of media the dissolution amount of drug was increased [18, 20].

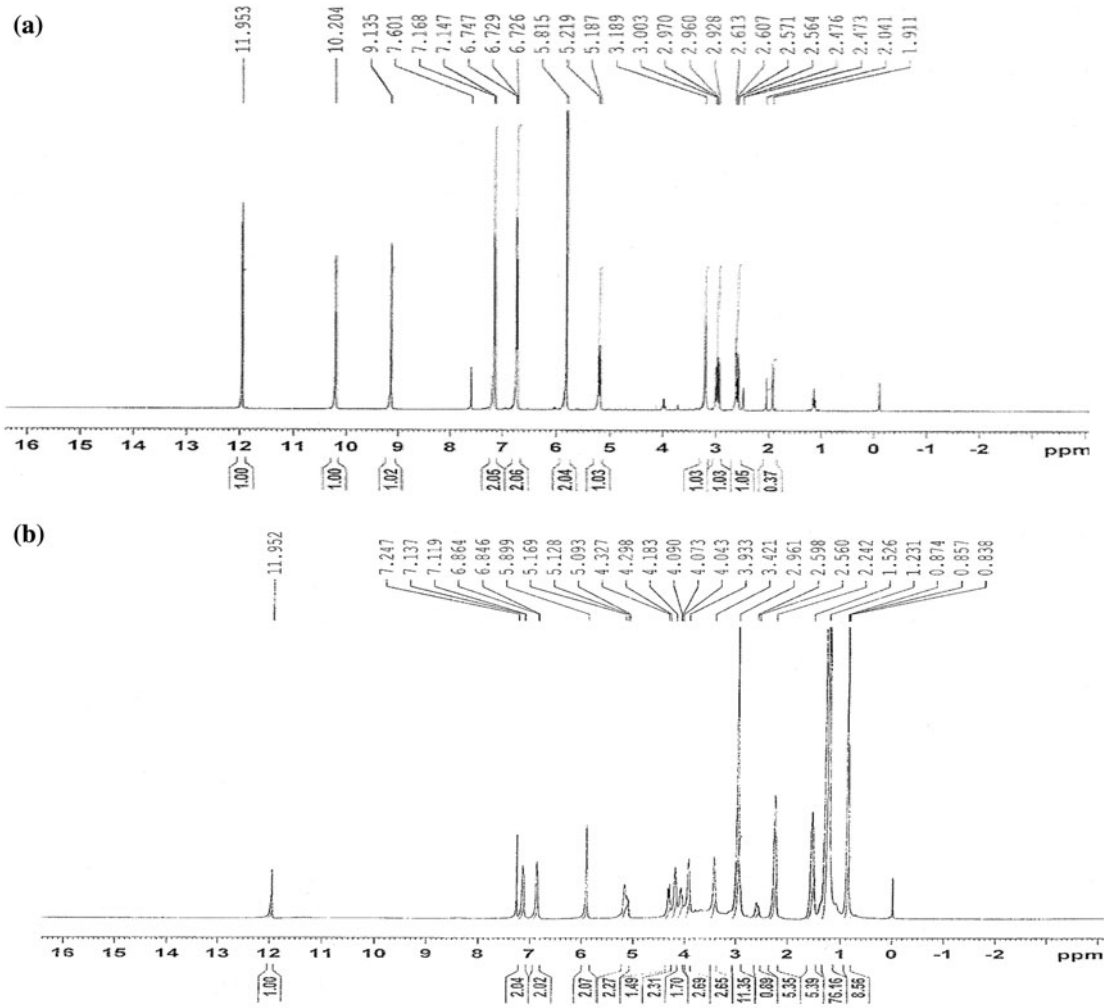

Fig. 5 ^{1}H NMR of **a** naringenin (in DMSO) and **b** naringenin–phospholipid complex (in $CDCl_3$)

Conclusions

In the present study naringenin–phospholipid complex were prepared by a simple and reproducible method and evaluated for various physicochemical parameters. The physicochemical investigations showed that naringenin formed a stoichiometric complex with phosphatidylcholine with better solubility and dissolution profile. The ^{1}H NMR, DSC and XRPD studies confirmed the formation of the complex. The dissolution profile of the complex was found to be improved. Thus it can be concluded that the phospholipid complex of naringenin may be of potential use for improving its bioavailability.

Acknowledgments Authors acknowledge the grant provided by the Department of Science and Technology, Govt. of India for the research work. Authors are also thankful to LIPOID GmbH Germany for providing the gift sample of phosphatidylcholine for the research 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

1. Ortuño, A., Garcia-Puig, D., Fuster, M.D., Pérez, M.L., Sabater, F., Porras, I., Garcia-Lidon, A., Del Rio, A.J.: Flavanone and nootkatone levels in different varieties of grapefruit and pummelo. J. Agric. Food Chem. **43**, 1–5 (1995). doi:10.1021/jf00049a001
2. Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M.: Quantitation of flavonoids constituents in citrus fruits. J. Agric. Food Chem. **47**, 3565–3571 (1999). doi:10.1021/jf990153+
3. Davies, J.N., Graeme, E.H.: The constituents of tomato fruit: the influence of environment, nutrition, and genotype. Crit. Rev. Food Sci. Nutr. **15**, 205–280 (1981)
4. Paganga, G., Miller, N., Rice-Evans, C.: The polyphenolic content of fruit and vegetables and their antioxidant activities: what does a serving constitute? Free Radic. Res. **30**, 153–162 (1999). doi:10.1080/10715769900300161
5. Middleton, E., Kandaswami, C.: The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne, J.B. (ed.) The flavonoids: advances in research since 1986, pp. 619–652. Chapman and Hall, London (1994)
6. Benavente-Garcia, O., Castillo, J., Marin, F.R., Ortuño, A., Del Rio, J.A.: Uses and properties of citrus flavonoids. J. Agric. Food Chem. **45**, 4505–4515 (1997). doi:10.1021/jf970373s
7. Montanari, A., Chen, J., Widmer, W.: Citrus flavonoids: a review of past biological activity against disease. Discovery of new flavonoids from Dancy tangerine cold pressed peel oil solids and leaves. In: Manthey, J., Buslig, B. (eds.) Flavonoids in the living system, pp. 103–116. Plenum, New York (1998)
8. Ruh, M.F., Zacharewsky, T., Connor, K., Howell, J., Chen, I., Safe, S.: Naringenin: a weakly estrogenic bioflavonoid that exhibits antiestrogenic activity. Biochem. Phamacol. **50**, 1485–1493 (1995). doi:10.1016/0006-2952(95)02061-6
9. Miksicek, R.J.: Commonly occurring plant flavonoids have estrogenic activity. Mol. Pharmacol. **44**, 37–43 (1993)
10. Kuiper, J.M., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., Van der Saag, P.T., Van der Burg, B., Gustafsson, J.A.: Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinolology **139**, 4252–4263 (1998). doi:10.1210/en.139.10.4252
11. Cheng, F., Breen, K.: On the ability of four flavonoids, baicilein, luteolin, naringenin and quercetin, to suppress the fenton reaction of the iron–ATP complex. Biometals **13**, 77–83 (2000). doi:10.1023/A:1009229429250
12. Van Acker, F.A.A., Schouten, O., Haenen, G.R., Van der Vijgh, W.J.F., Bast, A.: Flavonoids can replace α-tocopherol as an antioxidant. FEBS Lett. **473**, 145–148 (2000). doi:10.1016/S0014-5793(00)01517-9
13. Lee, K.G., Shibamoto, T., Takeoka, G.R., Lee, S.E., Kim, J.H., Park, B.S.: Inhibitory effects of plant-derived flavonoids and phenolic acids on malonaldehyde formation from ethyl arachidonate. J. Agric. Food Chem. **51**, 7203–7207 (2003). doi:10.1021/jf0345447
14. Saija, A., Scalese, M., Lanza, M., Marzullo, D., Bonina, F., Castelli, F.: Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Radic. Biol. Med. **19**, 481–486 (1995). doi:10.1016/0891-5849(94)00240-K
15. Ueng, Y.F., Chang, Y.L., Oda, Y., Park, S.S., Liao, J.F., Lin, M.F., Chen, C.F.: In vitro and in vivo effects of naringin on cytochrome P450-dependent monooxygenase in mouse liver. Life Sci. **65**, 2591–2602 (1999). doi:10.1016/S0024-3205(99)00528-7
16. Erlund, I., Meririnne, E., Alfthan, G., Aro, A.: Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. J. Nutr. **131**, 235–241 (2001)
17. Semalty, A., Semalty, M., Rawat, B.S., Singh, D., Rawat, M.S.M.: Pharmacosomes: the lipid based novel drug delivery system. Expert Opin. Drug Deliv. **6**, 599–612 (2009). doi:10.1517/17425240902967607
18. Yanyu, X., Yunmei, S., Zhipeng, C., Quineng, P.: The preparation of silybin–phospholipid complex and the study on its pharmacokinetics in rats. Int. J. Pharm. **307**, 77–82 (2006). doi:10.1016/j.ijpharm.2005.10.001
19. Li, Y., Yang, D.J., Chen, S.L., Chen, S.B., Chan, A.S.C.: Comparative physicochemical characterization of phospholipids complex of puerarin formulated by conventional and supercritical methods. Pharm. Res. **25**, 563–577 (2007). doi:10.1007/s11095-007-9418-x
20. Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P., Mukherjee, P.K.: Curcumin–phospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats. Int. J. Pharm. **330**, 155–163 (2007). doi:10.1016/j.ijpharm.2006.09.025
21. Kumar, M., Ahuja, M., Sharma, S.K.: Hepatoprotective study of curcumin–soya lecithin complex. Sci. Pharm. **76**, 761–774 (2008). doi:10.3797/scipharm.0808-09
22. Shi, K., Cui, F., Yu, Y., Zhang, L., Tao, A., Cun, D.: Preparation and characterization of a novel insulin phospholipid complex. Asian J. Pharm. Sci. **1**(3–4), 168–174 (2006)
23. 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 **114**, 242–250 (2006). doi:10.1016/j.jconrel.2006.05.013
24. Yoo, H.S., Park, T.G.: Biodegradable nanoparticles containing protein–fatty acid complex for oral delivery of salmon calcitonin. J. Pharm. Sci. **93**, 488–495 (2003)
25. Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M.: Development and physicochemical evaluation of pharmacosomes of diclofenac. Acta Pharma **59**, 335–344 (2009). doi:10.2478/v10007-009-0023-x