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Phosphatidylcholine Complex in Improving Oral Drug Delivery of Epicatechin: Preparation and Characterization

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Abstract: Epicatechin, a polyphenolic flavonoid, shows antioxidant, anti-bacterial, anticarcinogenic and antitumour activity. Like other flavonoids, epicatechin is poorly absorbed across the gastrointestinal tract because it has multiple ring molecules that are too large to be absorbed by simple diffusion. It shows poor miscibility with oils and other lipids which limit its ability to pass across the lipid rich biomembranes of small intestine. Therefore, in an attempt to improve the problem of poor absorption, solubility and dissolution of epicatechin its phospholipid complexes were prepared. The prepared epicatechin-phospholipid complex was characterized for various physico-chemical parameters like drug loading, infrared absorption (FTIR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), aqueous/n-octanol solubility and dissolution study. In the SEM, the complex was observed as nonporous irregular particles with rough surface morphology. FTIR and DSC data confirmed the formation of phospholipid complex. The water and n-octanol solubility of epicatechin was improved from 326.32 to 427.12 μg/ml and 378.53 to 502.67 μg/ml, respectively in the complex. The dissolution was also improved significantly in the phospholipid complex. It was concluded that the phospholipid complex may be used for improving solubility, dissolution and hence the bioavailability of epicatechin molecule.

Key words: Epicatechin, Phosphatidylcholine, FTIR, Differential Scanning Calorimetry, Scanning Electron Microscopy, Solubility behavior and Controlled release.

Introduction

Poor aqueous solubility (and hence the dissolution) or permeability (across the biomembranes) of drugs are the major factors which govern bioavailability of drugs. During the course of drug discovery and development of dosage form the solubility and permeability are the key issue and for the same the drugs are classified in different classes on the same basis in Biopharmaceutical Classification System (BCS) [1,2]. The phyto-constituents may have the solubility (dissolution in aqueous media/gastro intestinal fluid)) rate limited or the permeation rate limited absorption from the oral route. For improving solubility various techniques like solvent deposition, micronization, solid dispersion, supercritical fluid process, use of surfactants, use of salt forms, complexation etc. have been investigated [2-5]. Out

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of these, the complexation technique has been studied exhaustively to improve the solubility and the dissolution of poor water soluble drugs. In particular the lipid complexation improves the solubility as well as the permeability, due to the formation of an amphiphilic drug-lipid complex. Moreover, by virtue of their ability to form a protective coat over the mucosa, the lipid complexes have also been reported to reduce the gastric and hepatic toxicities of drug molecule [6-8]. The lipid complexes are prepared with phosphatidylcholine (PC, fig. 1b). PC is an integral part of the cell membrane and exists in zwitterionic form. PC is not only a passive carrier for drug delivery but is itself a natural component with well investigated and reported clinical efficacy for various liver diseases. 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 improving the permeation of drugs across the biomembranes [9, 10].

Polyphenoles, in particular the flavonoids are the largest group of bioactive phytoconstituents found very abundantly in nature. But the bioavailability differs greatly from one polyphenol to another. Either these are very less soluble in aqueous media (or the gastrointestinal fluid) or show very low permeability across the biomembranes. These limitations ultimately lead to poor dissolution, permeation and bioavailability of drugs upon oral ingestion [10].

Green and black tea is the richest source of polyphenols (specially flavones) which show a broad spectrum of bioactivity including the prophylactic action against the cancer [11]. Among these polyphenoles, epicatechin (Fig 1.b) has been reported to exhibit a wide range of biological activities such as antioxidant, anti-bacterial, anticarcinogenic and antitumour [12-16]. It produces protective effect against peroxynitrite mediated renal damage [17]. It is also a potent inhibitor of DNA polymerase and angiogenesis [18]. Besides this, the extracts of *Margyricarpus setosus* in which catechin and epicatechin are the main principles, has been reported to show the anti-HIV activity [19]. Despite this wide range of therapeutic activity, unfavorable pharmacokinetics (lower bioavailability) of epicatechin associated with a lower half-life and rapid clearance from the body restricts its use as a potent phyto molecule [20-22]. Like other flavonoids, epicatechin is poorly absorbed across the GIT because it has multiple ring molecules that are too large to be absorbed by simple diffusion. It has poor miscibility with oils and other lipids which limit their ability to pass across the lipid rich outer membranes of enterocytes of small intestine. Epicatechin (Fig. 1.a) consists of two benzene rings (A and B) and a pyran ring (called C – ring), which might be the cause for poor oral absorption

The problem of poor absorption of epicatechin can be overcome by preparing its phospholipid complexes. These complexes may improve their absorption by imparting an environment of improved lipophilicity. Thus epicatechin–phospholipid complex was evaluated for various physico-chemical investigations like drug content, chemical interaction (by FTIR), thermal behavior (by DSC), surface morphology (by SEM) and *in-vitro* dissolution study in comparison with pure epicatechin.

Materials and methods

Materials

(-)-Epicatechin was purchased from Sigma Aldrich, Mumbai (India), with 90 % purity. Soya

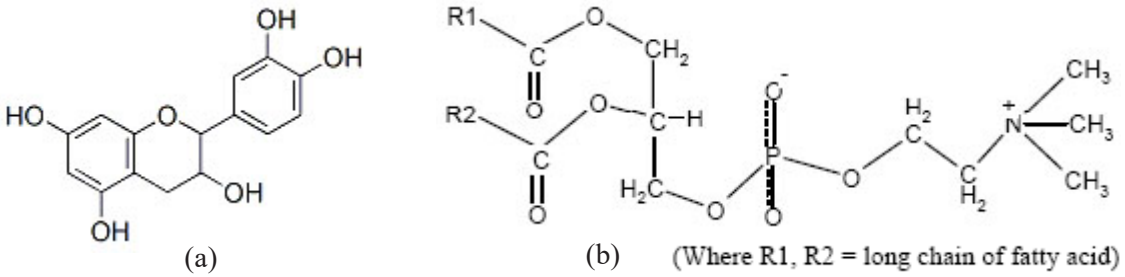

Fig.1 (a) Epicatechin (b) Phosphatidylcholine

phosphatidylcholine (LIPOID S-80) was obtained as a gift sample from LIPOID, Germany. All other chemical reagents were of analytical grade.

Method of preparation

To prepare the epicatechin-PC complex equimolar concentration of phosphatidylcholine (PC) and epicatechin was taken in a 100 mL round bottom flask and refluxed in dichloromethane for 3-4 hours. When the solution was concentrated to about 10 ml, n-hexane (30 ml) was added to get the complex. The complex was collected and stored in vacuum desiccators.

Drug content

Epicatechin-PC complex equivalent to 50 mg of epicatechin was weighed. To the weighed complex 100 mL of pH 6.8 phosphate buffer was added in a volumetric flask. After the continuous stirring on a magnetic stirrer (Remi, 5MLH, India) for 24 h at room temperature samples were taken, filtered, diluted suitably and then analyzed spectrophotometrically (Lambda 25, Perkin Elmer, USA) at 278 nm to determine the drug content.

Infrared spectroscopy (FTIR)

The IR spectra were recorded on a Perkin Elmer FT-IR, RX-1 spectrophotometer in KBr pellets.

Differential scanning calorimetry (DSC)

DSC study was performed for the samples of epicatechin, phosphatidylcholine and the prepared complex using a 2910 Modulated Differential Scanning Calorimeter V4.4E (TA Instrument, USA). The investigations were carried out over the temperature range 0-300°C (@ 10°C min^{-1}).

Scanning electron microscopy (SEM)

To assess the surface morphology of the prepared complex as compared to its components Scanning Electron Microscopy (SEM) of the complex was performed using JEOL JSM 5600.

Solubility Study

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[23,24].

Dissolution study (*in-vitro* drug release)

The dissolution studies were carried out in a USP XXIII, six station dissolution test apparatus, type II (6DR, VEEGO, India) at 100 rpm and at 37°C using 900 mL of pH 6.8 phosphate buffer. The complex equivalent to 50 mg of epicatechin was taken for the study and its comparison was done with the dissolution of plain epicatechin (50 mg). Samples of dissolution fluid were withdrawn at different intervals and replaced with the equal volume of fresh media. Withdrawn samples were filtered (through a 0.45 µm membrane filter), diluted suitably and then analysed spectrophotometrically at 278 nm.

Results and discussion

In the present study the prepared complex (Epi-Pc) showed a good encapsulation efficiency of epicatechin and found to be 98 % as estimated UV spectrophotometrically. Complexation provided good encapsulation efficiency, which could make the delivery of epicatechin clinically feasible.

Chemical interaction

The possible interaction between epicatechin and PC in the phospholipid complex was studied by IR spectroscopy. IR spectra of epicatechin, phosphatidylcholine, complex and physical mixtures of both the constituents are shown in fig 2. PC showed characteristic peaks at 3435 cm^{-1} (Hydroxyl stretching); 2918 cm^{-1} and 2850 cm^{-1} (C-H stretching of long fatty acid chain); 1738 cm^{-1} (carbonyl stretching of the fatty acid ester); 1236 cm^{-1} (P=O stretching band); 1091 cm^{-1} (P-O-C stretching); and 970 cm^{-1} ($N^{-+}(CH_3)_3$ stretching). Epicatechin showed the main characteristic bands at 3458.84 cm^{-1} (hydroxyl (O-H) stretching) and at 1618 cm^{-1} (C=O stretching). In the range of 1300-1500 cm^{-1} vibrations of benzene ring were observed.

The FTIR of the complex showed the significant changes in the spectrum. The absorption peaks of hydroxyl (O-H) group at 3458 cm^{-1} was disappeared and remarkably broadened, whereas, the keto group frequency shifted to higher wave number in the complex. The P=O and P-O-C absorption band of the phosphatidylcholine also remarkably broadened with shifting towards lower wave numbers. The spectrum of the

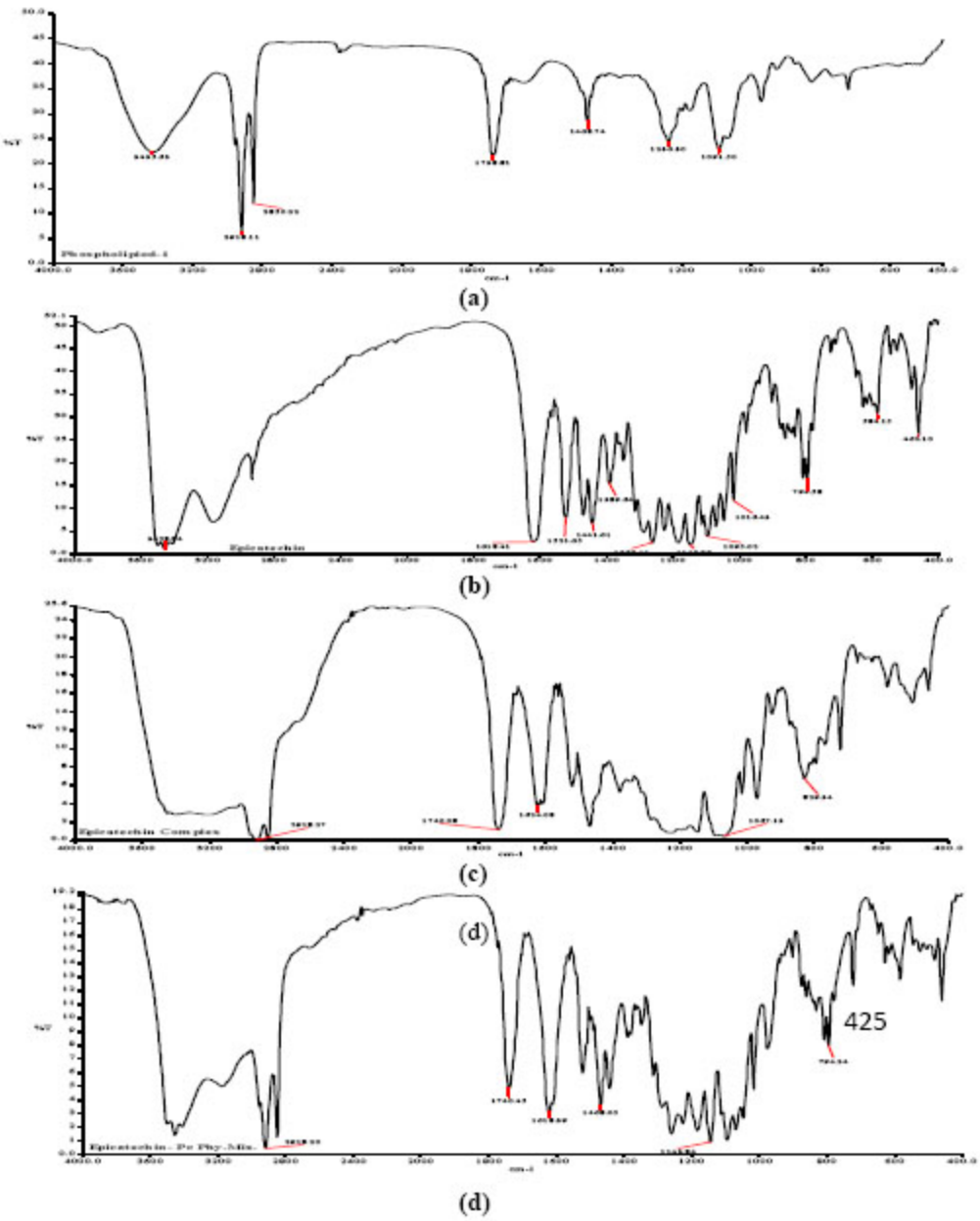

Fig.2 IR spectra: (a) Phospholipid, (b) Epicatechin
(c) Epicatechin-phospholipid complex and (d) Physical mixture

physical mixture was quite different from the spectrum of the complex and showed the same characteristic peaks of epicatechin and PC without any significant change and this indicated no interaction in between them. Therefore, the spectroscopic changes confirmed the formation of the complex due to the interaction of molecule to polar end of the phospholipid and this interaction was indicated by the disappearance/ shifting of hydroxyl and keto group frequencies of epicatechin from their original place [25,26].

Thermal analysis

DSC is a fast and reliable method which provides the maximum information regarding the possible interactions and detects drug-excipient compatibility. The complex formation is indicated by any significant change (elimination of

endothermic peaks, the appearance of new peaks, change in peak, shape and its onset, peak temperature, melting point and relative peak area or enthalpy) in characteristic peak of the drug and or the complexation agent in DSC.

The DSC curve of epicatechin exhibited a sharp endothermic peak at 246.38°C (ΔH_f= 125.7 J/g), caused by the melting of epicatechin (Fig. 3). The phosphatidylcholine exhibited a broad endothermic peak at 76.74°C (ΔH_f = 31.51 J/g).

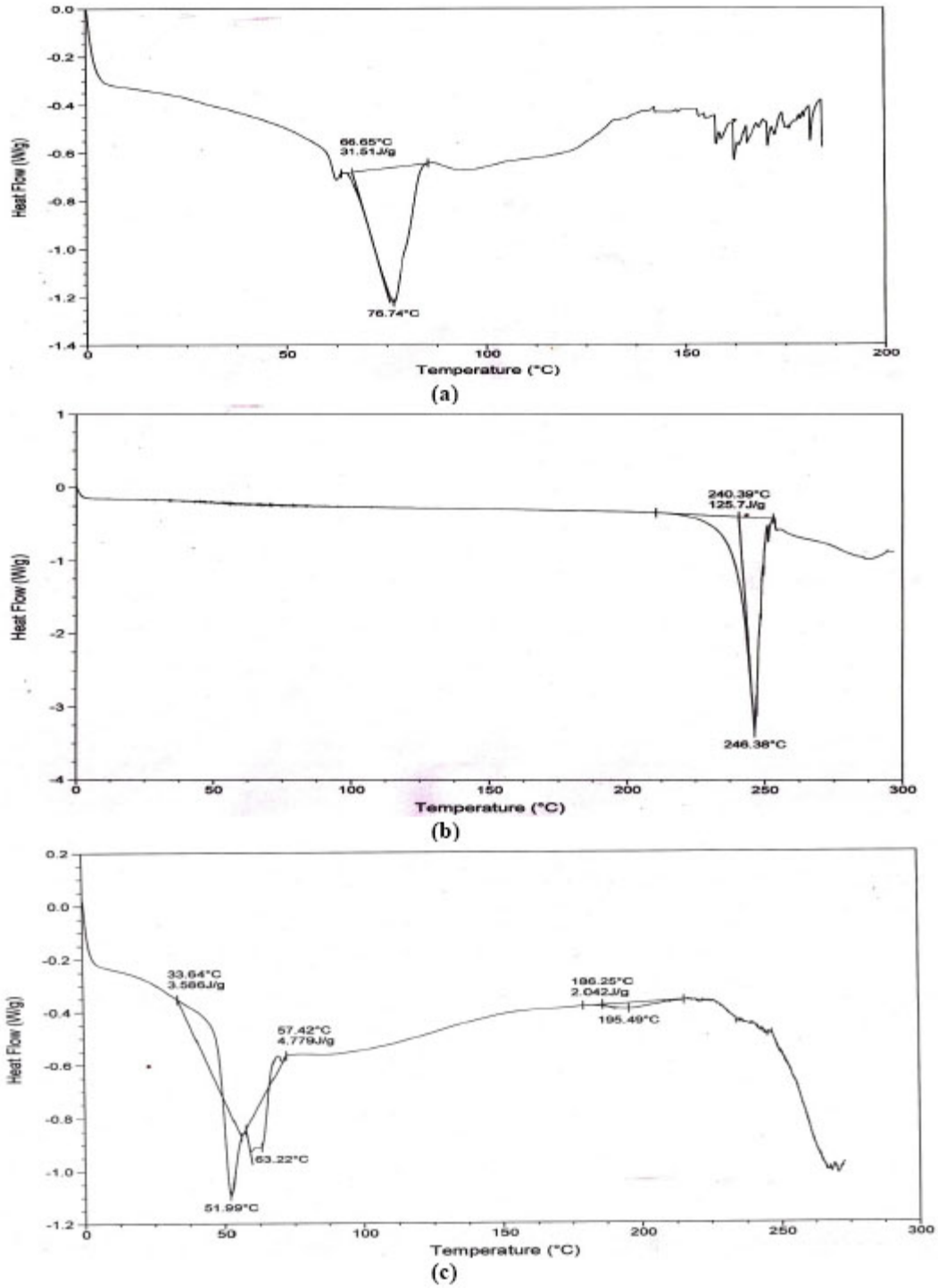

Fig.3 DSC Thermogram: (a) Phospholipid, (b) Epicatechin (c) Epicatechin-phospholipid complex

Whereas in the phospholipid complex, a quite sharp new peak at 51.99°C (ΔH_f = 3.586 J/g) and a mild peak at 63.22°C (ΔH_f = 4.779) was observed. None of the components gave any thermal signals or the melting peak of individuals and therefore the complex formation was confirmed due to the indicated interactions between the epicatechin and the phosphatidylcholine. Hence the complete disappearance of the endothermic peak of the drug in the prepared complexes indicated the formation of a true inclusion complex. Other studies also well supported the results [27-31].

Surface morphology

The SEM micrographs of epicatechin and the complex are shown in fig 4. The pure epicatechin was characterized by crystals of smaller size and regular shape with an apparently smooth surface. In contrast, the complex crystals were nonporous irregular particles with rough surface morphology which might have contributed to the improved solubility and dissolution rate of epicatechin from the complex.

Solubility Study

The epicatechin is poorly soluble in water. The poor solubility leads to its poor absorption and permeation across the intestinal epithelial cells of the gastrointestinal (GI) tract resulting in low bioavailability of the molecule. Aqueous solubility of epicatechin improved significantly from 326.32 to 427.12 µg/mL in the complex (Table 1). It was also evident that the n octanol solubility also increased significantly from 378.53 to 502.67 µg/mL. As the n-octanol solubility indicates the permeability properties of the drug it might be concluded that the complex might cross the biomembranes more effectively with improved bioavailability.

Typically rough surface morphology (as confirmed by SEM) and changes brought about due to complexation (as confirmed by FTIR and DSC) might have been responsible for the improvement in solubility. This increase in the solubility of the complex may be explained by its amorphous

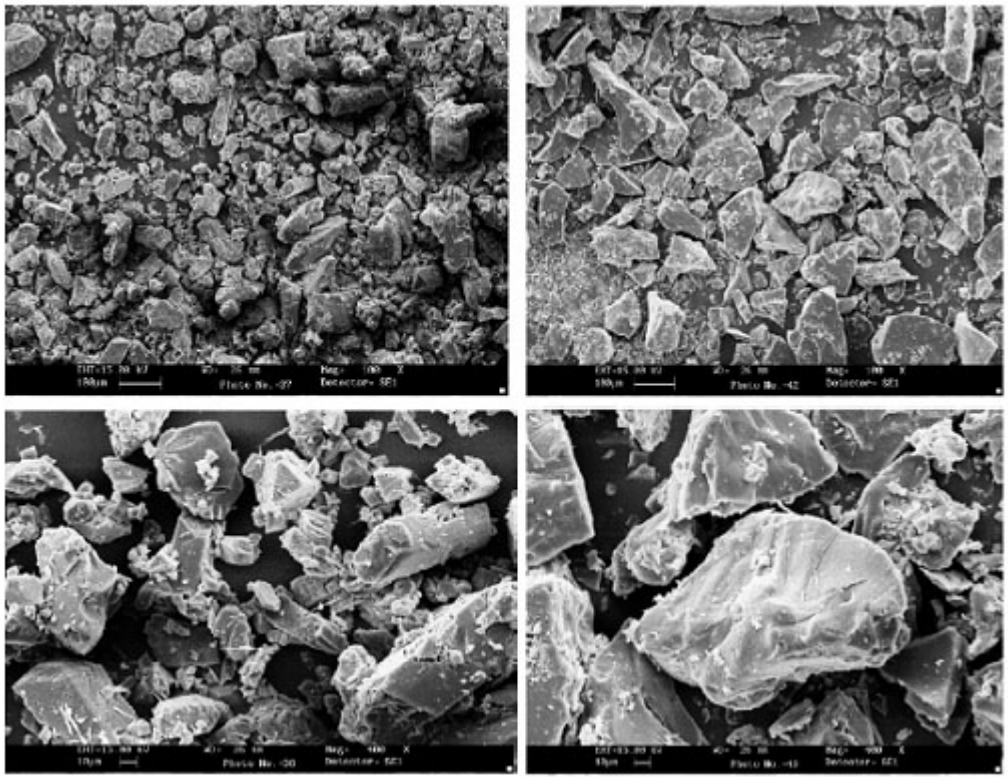

Fig.4 SEM micrographs: Magnification: 100 X and 400 X
(a) Epicatechin (b) Epicatechin-Phospholipid complex

Table 1. Solubility Study (H2O/ n-Octanol) at 25°C

Sample	Aqueous Solubility (µg/ ml)	n-Octanol Solubility (µg/ ml)
Epicatechin	326.32 ± 0.902	378.53 ± 0.76
Epicatechin Complex	427.12 ± 1.025	502.67 ± 0.234

Data expressed as mean values and standard deviations (± SD); n = 3.

characteristics and reduction in molecular crystallinity of the epicatechin [32]. Phospholipids are the amphiphilic surfactant which have been reported to increase the solubility of the drugs [33,34], thus, the complex showed an amphiphilic nature, which in turn may show the improved dissolution and overall absorption of the epicatechin by oral delivery.

Dissolution study (*in-vitro* drug release)

In vitro drug release was developed to predict the *in vivo* performance of the epicatechin in the phospholipid complex. The complex of epicate-chin (Epi-PC) showed a better dissolution profile than its free state (Fig. 5). Unlike free epicatechin or Epicat (which showed only 53.2 % drug release at the end of 24 h) the epicatechin complex showed 87.63 % at the end of 24 h of the disso-lution study. The rate of release of a drug not only depends on the surface area and particle size of the processed powder, but also greatly affected by crystal morphology and wettability. Phospho-lipids being amphi-philic surfactants might have increased the solubility of the drug by the action of wetting and dispersion resulting in improved dissolution. The improved surface morphology and probable amorphization induced by the complexation might have resulted in improved dissolution of epicatechin from its complex [27-31,35].

Conclusion

The physicochemical properties of epicatechin changed significantly after it was complexed with the phospholipid and these characteristics especially, the improved solubility, permeability and dissolution might contribute to improve oral absorption of the drug. The water and n-octanol solubility of epicatechin was improved from 326.32 to 427.12 µg/ml and 378.53 to 502.67 µg/ml, respectively in the complex. The dissolution was also improved significantly in the

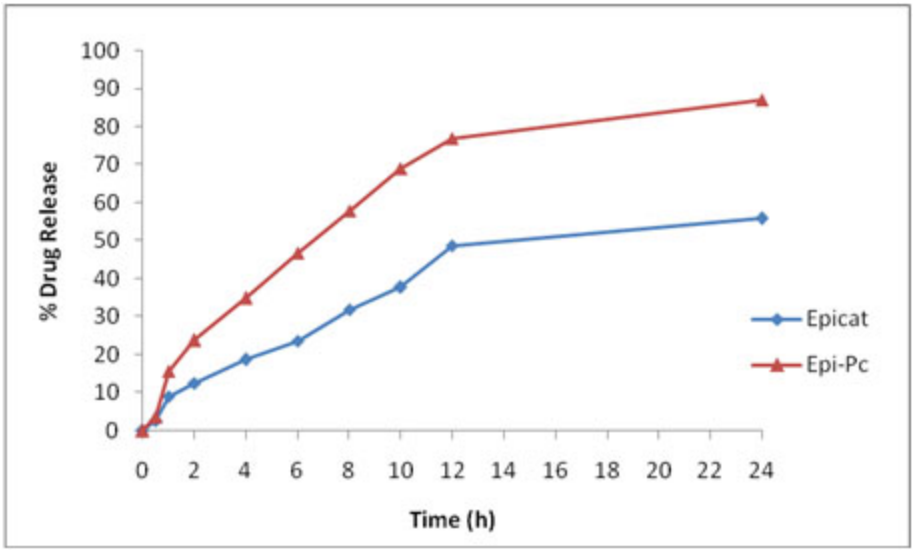

Fig.5 Dissolution pattern of epicatechin and its complex

phospholipid complex.

The developed system or the complex can be used as an effective drug delivery for their diffusion across lipid membranes or into the target cells. As the bioavailability of most of the phytoconstituents are limited by their poor biopharmaceutical properties, the phospholipid complex can lead to simple, safe, stable and efficient drug delivery for better therapeutic performance.

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References

1. **Wells, J. (2006).** Pharmaceutical preformulation: the physicochemical properties of drug substances. In: Pharmaceutics: The Science and Dosage Form Design, 2nd ed, Aulton ME, Ed; Churchil Livingstone: Edinburg; pp. 113-116.
2. **Semalty, A., Semalty, M., Rawat M.S.M. (2011).** Essentials of Pharmaceutical Technology, Pharma Med Press, Hyderabad, India, pp. 4-5.
3. **Ozkan, Y., Atay, T., Dikmen, N., Isimer A., Hassan Y., Enein A., (2000).** Improvement of water solubility and in vitro dissolution rate of gliclazide by complexation with β-cyclodextrins. Pharm. Acta Hel., 74:365-370.
4. **Perrut, M., Jung, J., Leboeuf, F., (2005).** Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes. part I. micronization of neat particles. Int. J. Pharm., 288: 3-10.
5. **Nijlen, T.V., Brennan, K., Mooter, V., Blaton, N., Kinget, R., Augustijns, P., (2003).** Improvement of the dissolution rate of artemisinin by means of supercritical fluid technology and solid dispersions. Int. J. Pharm., 254: 173-181.
6. **Khazaeinia, T., Jamali, F., (2003).** Comparision of gasterointestinal permeability induced by diclofenac phospholipid complex with diclofenac acid and its sodium salt. J. Pharm. Pharm. Sci., 6: 352-9.
7. **Goddard, P.J., Kao, Y.C., Lichtenbereger, L.M., (1990).** Luminal surface hydrophobicity of canine gastric mucosa is dependent on a surface mucous jel. Gastrointerology, 98: 361-70.
8. **Lichtenbereger, L. M., Graziani, L.A., Dial, E.J., Butler, B.D., Hills, B.A., (1983).** Role of surface-active phospholipids in gastric cytoprotection. Science, 219: 1327-9.
9. **Semalty, A., Semalty, M., Rawat, B.S., Singh, D., Rawat, M.S.M., (2009).** Pharmacosomes: the lipid based novel drug delivery system. Expert Opin. Drug Deliv., 6(6): 599-612.
10. **Semalty, A., Semalty, M., Rawat, M.S.M., Federico, F., (2010).** Supramolecular phospholipids-polyphenolic interactions: The PHYTOSOME strategy to improve the bioavailability of phytochemicals. Fitoterapia, 81: 306-14.
11. **Lee, U.L., Choi, S.W. (2011).** The chemopreventive properties and therapeutic modulation of green tea polyphenols in oral squamous cell carcinoma. ISRN Oncol. http://www.ncbi. nlm.nih.gov/pubmed/22084729 (May 23, 2011).
12. **Natsume, M., Osakabe, N., Yasuda, A., Baba, S., Tokunaga, T., Kondo, K., Osawa, T., Terao, J. (2004).** *In vitro* antioxidative activity of (-)-epicatechin glucuronide metabolites present in human and rat plasma. Free Rad. Res., 38(12): 1341-1348.
13. R**ein, D., Lotito, S., Holt, R.R., Keen, C.L., Schmitz, H.H., Fraga, C.G. (2000).** Epicatechin in human plasma: *in vivo* determination and effect of chocolate consumption on plasma oxidation status. J. Nutr., 130(8S):2109S-14S.
14. **Roy, B., Chakraborty, A., Ghosh, S.K., Basak, A. (2009).** Design, synthesis and bioactivity of

catechin/epicatechin and 2-azetidinone derived chimeric molecules. Bioorg. Med. Chem. Lett., 19(24): 7007-10.

15. **Babich, H., Krupka, M.E., Nissim, H.A., Zuckerbraun HL. (2005).** Differential *in vitro* cytotoxicity of (-)-epicatechin gallate (ECG) to cancer and normal cells from the human oral cavity. Toxicol. *In Vitro*., 19(2): 231-42.
16. **Granado-Serrano, A.B., Martín, M.A., Haegeman, G., Goya, L., Bravo, L., Ramos, S., (2010).** Epicatechin induces NF-kappaB, activator protein-1 (AP-1) and nuclear transcription factor erythroid 2p45-related factor-2 (Nrf2) via phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) and extracellular regulated kinase (ERK) signalling in HepG2 cells. Br. J. Nutr., 103(2):168-79.
17. **Yokozawa, T.; Rhyu, D.Y.; Cho, E.J.; Aoyagi, K. (2003).** Protective activity of (-)-epicatechin 3-O-gallate against peroxynitrite-mediated renal damage. Free Radic. Res., 37(5): 561-71.
18. **Matsubara, K., Saito, A., Tanaka, A., Nakajima, N., Akagi, R., Mori, M., Mizushina, Y. (2007).** Epicatechin conjugated with fatty acid is a potent inhibitor of DNA polymerase and angiogenesis. Life Sci., 80(17): 1578-85.
19. **Tommasi, N.D., Piacente, Sonia., Rastrelli, L., Mahmood, N., Pizza, C., (1998).** Anti-HIV activity directed fractionation of the extracts of Margyricarpus setosus. Pharm. Biol., 36(1): 29-32.
20. **Baba, S., Osakabe, N., Yasuda, A., Natsume, M., Takizawa, T., Nakamura, T., Terao, J. (2000).** Bioavailability of (-)-epicatechin upon intake of chocolate and cocoa in human volunteers. Free Rad. Res., 33(5): 635-641.
21. **Xu, J.Z., Yeung, S.Y., Chang, Q., Huang, Y., Chen, Z.Y., (2004).** Comparison of antioxidant activity and bioavailability of tea epicatechins with their epimers. Br. J. Nutr., 91(6): 873-81.
22. **Baba, S., Osakabe, N., Natsume, M., Muto, Y., Takizawa, T., Terao, J., (2001).** *In vivo* comparison of the bioavailability of (+)-catechin, (-)-epicatechin and their mixture in orally administered rats. J. Nutr., 131(11): 2885-2891.
23. **Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M., (2010).** Preparation and characterization of phospholipid complexes of naringenin for effective drug delivery. J. Incl. Phenom. Mac. Chem. 67: 253-60.
24. **Semalty, A., Semalty, M., Rawat, B.S., Singh, D., Rawat, M.S.M., (2010).** Development and evaluation of pharmacosomes of aceclofenac, Indian J. Pharm. Sci., 72: 571-575.
25. **Singh, D., Rawat. M.S.M., Semalty, A., Semalty, M., (2011).** Gallic acid-phospholipid complex: drug incorporation and physicochemical characterization. Lett. Drug Des. Discov., 8: 284-91.
26. **Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M., (2012).** Phyto-phospholipid complex of catechin in value added herbal drug delivery. J. Incl. Phenom. Macrocycl. Chem., 69: 253-60.
27. **Li, Y., Yang, D.J., Chen, S.L., Chen, S.B., Chan, A.S.C., (2007).** Comparative physicochemical characterization of phospholipids complex of puerarin formulated by conventional and supercritical methods. Pharm. Res., 25: 563-77.
28. **Singh, D., Rawat, M.S.M., Semalty, A., Semalty, M., (2012).** Quercetin-phospholipid complex: An amorphous pharmaceutical system in herbal drug delivery, Curr. Drug Disc. Technol. 9(1): 17-24.
29. **Singh, D., Rawat, M.S.M., Semalty, A., Semalty, M., (2013).** Chrysophanol-phospholipid complex, J. Therm. Anal. Calorim., 111: 2069-2077.
30. **Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M. (2009).** Development and physicochemical evaluation of pharmacosomes of diclofenac, Acta Pharma. 59: 335-344.
31. **Yanyu, X., Yunmei, S., Zhipeng, C., Qineng, P. (2006).** The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. Int. J. Pharm., 307: 77-82.
32. **Singh, D., Rawat, M.S.M., Semalty, A., Semalty, M. (2012).** Emodin-phospholipid complex,

J. Therm. Anal. Calorim., 108: 289-298.

33. **Lichtenberger, L.M., Wang, Z.M., Romero, J.J., Ulloa, C., Perez, J.C., Giraud, M.N., Barreto, J.C. (1995).** Non steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. Nature Med., 11: 154-158.
34. **Lichtenberger, L.M., Ulloa, C., Romero, J.J., Vanous, A.L., Romero, J.J., Dial, E.J., Illich, P.A., Walters, E.T., (1996).** Zwitterionic phospholipids enhance aspirin's therapeutic activity, as demonstrated in rodent model systems. J. Pharmacol. Exp. Ther., 277: 1221-1227.
35. **Singh, D., Rawat, M.S.M., Semalty, A., Semalty, M., (2012).** Rutin-Phospholipid Complex: An Innovative Technique in Novel Drug Delivery System- NDDS, Curr. Drug Deliv., 9(3): 305-14.