RESVERATROL-PHOSPHOLIPID COMPLEXES (PHYTOSOMES)
WITH IMPROVED PHYSICOCHEMICAL PROPERTIES FAVORABLE
FOR DRUG DELIVERY VIA SKIN

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\textbf{ABSTRACT}

Phyto-phospholipid complex (phytosomes) is helpful in enhancing oral bioavailability and transdermal permeation of polyphenols. The present work was aimed to deliver Resveratrol (RSV) via the skin in the form of phytosomes to utilize its anti-inflammatory effect. Resveratrol-phytosomes (RSV-P) were prepared in various ratios of RSV to phosphatidylcholine (PC). These formulations were found more aqueous soluble than pure RSV (0.0770 ± 1.66 mg/ml). RSV-P also showed n-octanol/water partition coefficient in the range 1.16-1.92 favorable for skin penetration. Drug and phospholipid compatibility was established by the DSC and FT-IR study. SEM images and XRD diffractograms revealed the reduction in crystallinity of RSV in the complexes. Also SEM as well as TEM images showed the round and regular vesicular structure of RSV-P with particle sizes in the range of 1\,\mu m. Selected formulation when tested for 24 hours long \textit{ex vivo} skin permeation showed higher skin penetration and retention at dermal sites compared to free RSV. It is hypothesized that the RSVP would give sustained therapeutic effect in acute and chronic inflammation.

\textbf{KEYWORDS:} Resveratrol; phospholipid; phytosome; drug delivery; skin; anti-inflammatory.

\textbf{INTRODUCTION}

In the recent past, considerable scientific works targeted on the development of novel drug delivery system (NDDS) for herbal drugs. NDDS approaches toward phytochemicals to develop polymeric nanoparticles and nanocapsules, liposomes, solid-lipid nanoparticles,
transfersome, pharmacosome, phytosomes and nanoemulsions, etc. have a number of advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improved tissue macrophages distribution, sustained delivery and protection from physical and chemical degradation. Thus novel drug delivery of herbal drugs has significant scope for enhancing the activity and overcoming problems associated with plant medicine of conventional type (Saraf 2010).

Trans-resveratrol (3,4',5-trihydroxy-trans-stilbene) has many beneficial effects for human Health (Fremont 2000). The history of resveratrol can be traced back to the ancient India (more than 2000 years ago). At that time, a well-known “Ayurvedic" medicine, "Darakchasava" was prescribed as a cardiotoxic and for many other diseases. The active ingredient of "Darakchasava" is Vitis vinifera L (Paul et al., 1999). In popular Japanese and Chinese medicine, resveratrol is the active ingredient of the Polygonum cuspidatum powdered root, used in the treatment of dermatitis, atherosclerosis, hyperlipidemia and inflammatory diseases (Arichi et al., 1982). Resveratrol consists of two phenolic rings linked by a styrene double bond to generate 3,4',5-trihydroxystilbene. Presence of the double bond leads to two isomeric forms of resveratrol, trans-and cis-isomeric forms. The trans-isomer is sterically more stable and pharmacologically active (Aggarwal et al., 2004). Prolonged exposure to UV radiation converts the trans-RSV to cis-RSV.

RSV attracted attention for possessing many beneficial effects for human health. It is an antioxidant (Frankel et al., 1993), anti-inflammatory (Khanduja et al., 2004), anticarcinogenic (Qiuney 2004, Narayanan 2002), protects against cerebral ischemic injury (Hung et al., 2004) causing fat reduction in white adipocytes (Horowitz et al., 2004, Picard et al., 2004), effective in multiple sclerosis (Tsunoda et al., 2008). Mechanisms for its various pharmacological activities involve modulating lipid metabolism, platelet aggregation, and inflammatory response (Jarolim et al., 2004; Norata et al., 2007).

Recent researches for anti-inflammatory remedy suggested the usefulness of RSV to treat acute and chronic inflammation. RSV ability to inhibit low-density lipoprotein oxidation, while suppressing the activity of cyclooxygenase 2 and induced nitric oxide synthase contributes to the anti-inflammatory and antioxidant effects (Bourgeois et al., 2008). The cyclooxygenase enzymes are crucial in the production of pro-inflammatory molecules by
both the cyclooxygenase and 5-lipoxygenase pathways and inhibitors are commonly used as anti-inflammatory drugs. Because resveratrol is an effective inhibitor of cyclooxygenase activity in vivo (Subbaramaiah et al., 1998) its anti-inflammatory properties have been investigated and found satisfactory results. Resveratrol significantly reduces both acute and chronic chemically induced edema (Chen et al., 2005), lipopolysaccharide induced airway inflammation (Birrel et al., 2005) and osteoarthritis (Elmali et al., 2005) and helps to prevent allograft rejection (Wu et al., 2005). Additional research links resveratrol with reducing inflammation through NF-kappa B signaling (Rahman 2009).

Poor water solubility of resveratrol is being encountered in many of its improved drug delivery system (Santos et al., 2012). Its hydrosolubility of 0.03 mg/ml (~0.13 mM) makes it “practically insoluble” in water according to the European Pharmacopoeia definition (Vian et al., 2005). Inclusion complexes between trans-resveratrol and β-cyclodextrin or hydroxypropyl-β-cyclodextrin revealed improved solubility and kept the scavenging capacity unaltered (Kumpugdee-Vollrath and Ibold, 2012, Lopez-Nicolas et al., 2006). A casein-RSV complex makes RSV available in stable forms and formulations having long shelf-life and improved solubility, preferably in aqueous media (Chen 2010). A resveratrol nanoemulsion formulation was optimized by drawing the ternary-phase diagrams in which poly(oxyethylene)-hydrogenated castor oil was selected as surfactant, ethanol as co-surfactant and isopropyl myristate as oil phase, and resveratrol loading was 6.18±0.11 mg.1^{-1} (Li et al., 2009). The solubilization of resveratrol in micellar solutions of bile acids was also investigated. Results showed that a micellar solution of 3,7,12-triketocholic acid had the highest affinity for resveratrol solubilization, and its critical micellar concentration was 2.0 mM. (Atanacković et al., 2009). Anzaghi et al. 2005 developed RSV-phospholipid complex with their unique method which they claimed to be a highly bioavailable composition for pharmaceutical and cosmetic use for the treatment of ageing and cellular degeneration. Duan et al., 2010 patented nanoemulsion of resveratrol-phospholipid complex and method for preparing the same by dispersing resveratrol-PC complex in aqueous solution of polyol wherein they achieved particles size less than 200nm applying high pressure homogenizer. The nanoemulsion such prepared was found miscible with water at any ratio, has high stability and high bioavailability, and thus can be widely used for preparation of cosmetics, pharmaceuticals or healthcare products. Agarwal et al. 2014 published an article on process optimization of resveratrol-phospholipid complexes using Box-Behnken statistical design and for the optimized formulation % yield, entrapment efficiency and partition coefficient was
found to be 81.71%, 82.76% and 2.25±0.085, respectively, which they hypothesized to possess good passive absorption and improved oral bioavailability.

Despite the poor water solubility, resveratrol exhibits high membrane permeability and can be considered a class-II compound in the Biopharmaceutical Classification System (Amidon et al., 1995). But RSV after oral administration rapidly absorbed to intestinal lumen and undergo presystemic metabolism by glucuronidation and sulfate conjugation and remaining amount reach to the liver by portal vein where it is again metabolized by glucuronidation and sulfate conjugation. This metabolism has been described as the main cause of the trace amounts of RSV found in the systemic circulation and consequently its low oral bioavailability (Kapetanovic et al., 2010, Walle et al., 2004, Das et al., 2008, Kuhnle et al., 2000). Resveratrol was encapsulated in oil-in-water food-grade nanoemulsions of subcellular size, produced by high-pressure homogenization. These formulations exhibited antioxidant activities, which was comparable to unencapsulated resveratrol dissolved in DMSO, suggesting that nanoencapsulated resveratrol, not being metabolized in the gastrointestinal tract, can be potentially absorbed through the intestinal wall in active form (Sessa et al., 2011).

To counter low bioavailability due to presystemic metabolism there are reports of RSV delivery via the skin. Gokce et al., (2012) prepared resveratrol loaded solid lipid nanocarrier (SLN) and nanostructured lipid carrier (NLC) and evaluated the antioxidant potential for dermal application. RSV-loaded NLC with smaller particle size and higher drug loading appeared superior to SLN for dermal applications. Anzaghi et al.,(2005) invented a method to provide a bioavailable resveratrol-phospholipids complex, which exhibits increased lipophilicity and consequently, favors gastrointestinal absorption. The composition claimed to be suitable for topical administration in the form of cream, gel, ointment and emulsion and also suitable for oral administration in the form of tablets, capsules, granules. The ex-vivo percutaneous absorption of resveratrol in liposomes (using phosphatidylcholine) and niosomes (using glycerol monooleate/polyglyceryl-3 dioleate, along with two oleins as penetration enhancers) was investigated. Results indicated a high cutaneous accumulation and a low transdermal delivery of resveratrol (Pando et al., 2013). Hung et al., (2008) also advocated the transdermal and topical application of resveratrol after testing its skin permeation profile in in vitro-in vivo models. The objective of the present study was to formulate phyto-phospholipid complexes (phytosomes) of resveratrol and to evaluate the
feasibility for its potential transdermal application in inflammatory conditions for sustained therapeutic benefits.

**MATERIALS AND METHOD**

Resveratrol (RSV) was purchased from Oakwood Products, Inc., West Colombia. Phosphatidylcholine (PC) was purchased from Sigma-Aldrich, Bengaluru.

**Preparation of Resveratrol Phytosomes (RSVP)**

Phytosomes were prepared by refluxing followed by solvent evaporation (Kidd and Head, 2005; Maiti et al., 2007 and Jiang et al., 2001). RSVPs were prepared in different ratios of RSV to PC as shown in Table1. RSV was dissolved in methanol in a 200 ml beaker. In a 500 ml round bottomed flask PC was dissolved in dichloromethane and RSV solution was mixed. The mixture was refluxed for 3 hours at 70°C. After 3 hours the mixture was cooled and then poured to petri dish. The dish was kept open overnight at room temperature for evaporation of solvent. Then the product was kept in hot air oven at 60°C for 2 hour. The dried product was stored in desiccators for further use.

<table>
<thead>
<tr>
<th>Table1: Formulation of RSVPs.</th>
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<td><strong>Ingredients</strong></td>
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<tr>
<td>Resveratrol (gm)</td>
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<tr>
<td>Egg lecithin (gm)</td>
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<tr>
<td>Methanol (ml)</td>
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<td>Dichloromethane (ml)</td>
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**Physicochemical Evaluation of Phytosomes**

**Solubility and partitioning**

Solubility studies were performed by taking an excess of the sample in 5 ml of various solvent viz. water, phosphate buffer (pH 6.8), acetate buffer (pH 4.5) (Chaudhary and Sharma, 2007). Partition coefficient was determined by shake flask method (Berthod and Carda-Broch, 2004) and using the following formula: Partition coefficient = log(amount of drug in n-octanol phase/amount of drug in aqueous phase).

**Drug Entrapment**

A weighed quantity of phytosomes equivalent to 10 mg RSV was added to 50 ml phosphate buffer pH 6.8 in a 100 ml beaker. The contents were stirred on a magnetic stirrer for 4 hours
and then allowed to stand for one hour. Clear liquid was decanted and centrifuged at 5000 RPM for 15 minutes (Model CF10, Daihan Scientific Co. Ltd, Korea). After centrifugation the supernatant was filtered through 0.45μ whatman filter paper and after suitable dilution absorbance was measured in UV-Visible spectrophotometer (Shimadzu 1800, Japan). Calculations were done using the formula: Drug entrapment (%) = (actual amount determined/ theoretical amount present).

Particle size distribution
The prepared phytosomes samples were dispersed in isopropyl alcohol by stirring on a magnetic stirrer for 10 minutes. The dispersion was analyzed in size analyzer (Zeta sizer, Nano series, S90, Malvern).

X-Ray diffraction study
XRD was done on pure RSV and RSVPs in different ratios of drug and PC to see crystallinity in the substance. Sample was scanned in the angular range of 5°– 80° in a PHILIPS X-Pert Pro instrument. Dried powder sample was kept in sample holder (20 mm × 15mm × 2mm) which was fitted into the instrument and X-ray was passed through the sample.

Differential scanning calorimetry (DSC)
DSC studies for pure RSV, phosphatidylcholine (PC), physical mixture of RSV and PC and phytosomes were performed on a Perkin Elmer (USA) (Model JADE DSC) differential scanning calorimeter by heating samples over a temperature range of 50-300°C in closed metal pans at the rate of 10°C per minute under the environment of nitrogen gas.

Fourier Transform Infrared spectroscopy (FT-IR) Study
FT-IR studies were performed taking pure RSV, PC and RSVP in an Alpha FT-IR spectrophotometer (Bruker, Germany). A small quantity of sample was placed just below the probe on to which the probe was tightly fixed and scanned in the wave number region 4000-500 cm⁻¹. The obtained IR spectra were interpreted for functional groups at their respective wave number (cm⁻¹).

Scanning electron microscopy (SEM)
Drug and phytosomes samples were coated with gold in a Fine Coat Ion Sputter JFC-1100. Analysis was done on the coated sample by placing a pinch of sample in the JEOL (JSM 6360) scanning electron microscope and surface morphology was viewed and photographed.
Transmission electron microscopy (TEM)
Sample was dispersed in water and a drop was placed on a carbon coated copper grid to form a thin film. The film was stained with 2% uranic acid and allowed to dry by air drying. The stained film was viewed and photographed in a JEOL (JEM 2100) transmission electron microscope.

Ex vivo skin permeation study
Skin permeation of selected phytosomes formulation (F4) was compared with skin permeation of free RSV, as F4 was found superior in terms of aqueous solubility, partition coefficient, crystallinity and drug entrapment than other RSVPs (F1, F2, F3 and F5). For the study, a modified Franz diffusion cell with a diffusional area of 1.766 cm² was used. Rat abdominal skin excised after sacrificing the animal was used in the study. Skin hairs were shaved and subcutaneous fats were removed carefully. Skin was mounted in the diffusion cell with the stratum corneum facing the donor compartment and the dermis facing the receptor compartment. To the donor compartment 10 mg RSV or RSVP equivalent to 10 mg RSV was applied on the skin after dispersing in 0.5 ml water. The receptor compartment was filled with 32 ml phosphate buffer (pH 6.8) and maintained at 37 ± 0.5°C under continuous stirring with a magnetic bar. From the receptor compartment 2 ml samples were withdrawn at predetermined time intervals up to 24 hours. Equal volume of receptor compartment fluid was replaced after each sampling. Experiment was carried out in triplicate following the same procedure. Samples were analyzed in a UV-Visible Spectrophotometer at the λmax of the drug using phosphate buffer (pH 6.8) as blank. The cumulative amount permeated at each time interval was calculated and a plot of cumulative amount permeated (%) versus time (h) was constructed. The skin after 24 hours study was taken out of the diffusion assembly and was cut into small pieces and extracted with methanol by homogenizing in a tissue homogenizer. Aliquots of the extract were analyzed in the UV-Visible Spectrophotometer after suitable dilution with phosphate buffer (pH 6.8).

RESULTS AND DISCUSSIONS
Preparation of RSVP: We prepared RSVPs in five different ratios of drug to phosphatidylcholine. All formulations appeared off white in color and were in lumps, i.e. not free flowing. Formulations with higher phospholipid content (0.5:1, 0.75:1 and 1.0:1.0) found to be more viscous and sticky lumps. It was assumed that in preparation of phytophospholipid complex obtaining a clear solution of drug and PC is a prerequisite because
insoluble drug or PC will not interact intimately. However no single solvent for dissolving the both was not found. Dichloromethane was chosen for dissolving PC but RSV is insoluble in this mixture. RSV is soluble in methanol. Dichloromethane and methanol are miscible with each other at any volume. RSV and PC were dissolved separately in methanol and dichloromethane respectively and the two solutions were mixed and then refluxed (Table 1). During laboratory procedures proper care was taken to protect the drug and complexes from direct exposure to light knowing the UV-light sensitivity of RSV.

**Solubility and Partitioning:** RSV as reported in many literatures (Polonini *et al*., 2014; Amri *et al*., 2012) found to be highly hydrophobic with water solubility $0.0770 \pm 1.66 \text{ mg/ml}$. While its aqueous solubility in basic pH is higher ($0.102 \pm 0.11 \text{ mg/ml}$) than in acidic pH ($0.0527 \pm 1.5 \text{ mg/ml}$). Solubility further enhanced by the addition of cosolvent (PEG 400) ($0.4228 \pm 0.24 \text{ mg/ml}$). RSV phytosomes were found to be better soluble than pure RSV with the order of solubility in phosphate buffer pH 6.8 > water > acetate buffer pH 4.5 (Table 2). The amount soluble in mg/ml significantly varied as the ratio of RSV to PC varied. Highest solubility was observed for F4 where the molar ratio of RSV to PC is 1:0.75. Results show that F4 solubility in phosphate buffer (pH 6.8) is much higher ($0.7852 \pm 0.05 \text{ mg/ml}$) than in water ($0.6820 \pm 0.44 \text{ mg/ml}$) and acetate buffer pH 4.5 ($0.26 \pm 0.62 \text{ mg/ml}$). Compared to F4, in case of F1, F2 and F3 aqueous solubility in basic, neutral and acidic medium were found very less (Table 2). The reason may be the higher quantity of PC that remained unbound where RSV: PC ratios were 0.5:1(F1), 0.75:1(F2) and 1:1 (F3). The unbound PC might form extra layers surrounding the RSVP vesicles. On the other hand less solubility also observed in case of F5 in comparison to F4 (Table 2).The reason may be attributed to higher quantity of RSV that remained unbound where RSV: PC ratio was 1:0.5 (F5).

Partition coefficient can be considered as an important factor for predicting skin permeability from an aqueous environment to the lipophilic stratum corneum (Cal, 2006). For transdermal absorption the permeant should possess octanol-water partition coefficient in the range -1.0 to 4.0 (Chandrasekhar and Shobharani, 2008; Panchagnula, 1997). The results of the partition coefficient study are presented in Table 2. Partition coefficient values of pure RSV were $2.68 \pm 0.11$ (octanol/water), $2.62 \pm 0.80$ (octanol/phosphate buffer pH 6.8) and $2.73 \pm 0.32$ (octanol/acetate buffer pH 4.5). The results indicate lipophilic nature of RSV. Higher value of partition coefficient may retard drug absorption via skin due to difficulty in permeating the hydrophilic epidermis/dermis beneath the SC (Schneider *et al*., 2009; Hung *et al*., 2010).
Keeping this fact in mind the most satisfactory partition coefficient value for RSVP was exhibited by F4 (1.16 ± 0.48) taking phosphate buffer pH 6.8 as the aqueous medium. Aqueous solubility of drug as well as n-octanol/water partition coefficient are important factors in designing formulations for transdermal application and deciding the fate of permeant for transdermal absorption. However, skin permeation of drug not solely dependent on lipophilicity, but there may be multiple factors and the way the viable dermis respond to a particular permeant.

**Particle size analysis:** Phyto-phospholipid complexes showed average particle size in the range of 1022 – 1285 nm for different combinations of RSV and PC (Table 3). Particle size of RSVP was found to increase as the RSV fraction was increased in the complexes. However, RSVP results from molecular complexation with high cohesiveness and therefore the chance of existence of single particles is very less. The particle size observed in the present study most probably belongs to particle aggregates or floccules.

**Drug entrapment:** All phytosomes formulations contain near to 100 % of drug (Table 3). The results indicate uniform binding of drug and phosphatidylcholine. In formulation F1 and F2 the relatively lower drug content (96.5% and 97.32%) may be due to the presence of unbound phosphatidylcholine where RSV was in 0.5 and 0.75 fraction. In formulation F3, F4, and F5 molar content of RSV was higher than PC and that is why drug and PC obtained sufficient opportunity to interact with each other as PC provide more than one site for drug binding resulting in higher drug content of 99.75%, 99.35 % and 101.32, respectively.

**Table 2: Data obtained in solubility and partition coefficient determination (Mean±SD (n=3)).**

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Solubility (mg/ml)*</th>
<th>Partition coefficient (logP)*</th>
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<tr>
<td></td>
<td>Water</td>
<td>Phosphate buffer (pH 6.8)</td>
</tr>
<tr>
<td>RSV</td>
<td>0.0770 ± 1.66</td>
<td>0.102±0.11</td>
</tr>
<tr>
<td>F1</td>
<td>0.1674 ± 0.32</td>
<td>0.3225±1.04</td>
</tr>
<tr>
<td>F2</td>
<td>0.1875 ± 1.12</td>
<td>0.3562±1.43</td>
</tr>
<tr>
<td>F3</td>
<td>0.3311 ± 0.07</td>
<td>0.5844±1.06</td>
</tr>
<tr>
<td>F4</td>
<td>0.6820 ± 0.44</td>
<td>0.7852±0.05</td>
</tr>
<tr>
<td>F5</td>
<td>0.6166 ± 0.48</td>
<td>0.7133±0.07</td>
</tr>
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</table>
Table 3: Average particle size and drug entrapment of different formulations of RSVP

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Average particle size (nm) ± SD (n=3)</th>
<th>Drug entrapment (%) ± SD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytosome (0.5:1) (F1)</td>
<td>1022 ± 157</td>
<td>96.56 ± 1.08</td>
</tr>
<tr>
<td>Phytosome (0.75:1) (F2)</td>
<td>1084 ± 137</td>
<td>97.32 ± 0.83</td>
</tr>
<tr>
<td>Phytosome (1:1) (F3)</td>
<td>1107 ± 43</td>
<td>99.75 ± 0.82</td>
</tr>
<tr>
<td>Phytosome (1:0.75) (F4)</td>
<td>1160 ± 48</td>
<td>99.35 ± 1.22</td>
</tr>
<tr>
<td>Phytosome (1:0.5) (F5)</td>
<td>1285 ± 58</td>
<td>101.32 ± 1.05</td>
</tr>
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</table>

X-Ray diffraction study: XRD of RSV shows intense crystalline peaks (Figure 1), indicating higher crystallinity of the drug. Such peaks are relatively less intense in phytosomes formulations. Among the complexes, F4 exhibited least number of crystalline peaks, and it was interpreted that F4 (RSVP 1:0.75) would be more amorphous than phytosomes prepared in other ratios of RSV:PC, i.e. 0.5:1, 0.75:1, 1:1 and 1:0.5. Drug amorphization increases aqueous miscibility of the compound which was seen in the solubility study. At the same time because of its amphoteric nature PC also maintains lipophilicity of RSV as was seen in the partition coefficient results.

![Fig. 1: X-Ray diffraction pattern of drug and complexes](image)

FT-IR spectroscopy: RSV showed IR absorption (Figure 2) at wave number (cm⁻¹) 3189 (-OH Stretching), 1610 (C-C aromatic double bond stretching), 1589 (C-C olefinic stretching), 1385 (C-O stretching), 1499 (Aromatic ring) and 964 (trans-olefinic). These spectral informations have good similarity with the reports of Bertacche et al., (2006). For PC, IR
absorption bands were observed at wave number 2922, 1733, 1210 and 1043 cm\(^{-1}\). The RSV-PC complexes showed the characteristic peaks of both RSV and PC; however intensity of peaks for RSV decreased. This eliminates the possibility of any incompatibility of these combinations. Further, it can also be read that RSV-PC complexes (phytosomes) involve only weak physical bonding. These results are in good agreement with those of Freag et al., (2013) who reported that some weak physical interactions took place between diosmin (-OH) and the phospholipid phosphate group during complex formation.

![FT-IR spectra of RSV, PC and RSVP.](image)

Fig. 2: FT-IR spectra of RSV, PC and RSVP.
**Fig. 3: DSC thermograms of RSV, PC and RSVP**

**DSC study**

Resveratrol showed a sharp endothermic event at 271°C. DSC thermogram of PC shows endothermic peaks at 150.4°C, 172.7°C and 180.8°C. The peaks for PC at 172.7°C and 180.8°C were not detectable in the complex. The DSC thermogram of phytosomes gave two endothermic peaks at 124.93 and 139.84. The peaks for the single compounds PC and RSV disappeared or depressed. For the complexes the phase transition temperature was lower than the phase transition temperature of PC. Such interaction results from combination of hydrogen bonds or van der Waals forces (Xu et al., 2009), but the interaction does not lead to the formation of new compound. After the combination of RSV and the PC through weak bonding, the carbon–hydrogen chain in phosphatidylcholine could turn freely and enwrap the phospholipids molecule polarity parts to which drug is bonded, which made the sequence decrease between phospholipids aliphatic hydrocarbon chains, made the endothermic peak of
phospholipids (at 180.8°C) disappear and depressed the phase transition temperature (Figure 3).

**Scanning electron microscopy (SEM)**

Fig. 4: SEM photomicrographs of RSV (A and B), RSVP (C and D)

SEM photographs give important insight into the solid state properties and surface morphology of drug and drug complexes. The highly crystalline state of RSV was visualized in the SEM photograph as numerous flakes (Fig. 4A and 4B). Such crystalline structures were totally converted to amorphous regular spherical structures in formulation F4 (Fig. 4C and 4D). For the remaining formulations a mix mass of amorphous and crystalline structures were observed. These electron microscopic images suggest that formulation F1, F2, F3, F5 may be not the correct stoichiometric combinations for the formation of good quality RSVP.
Transmission electron microscopy

TEM images depict the formation of vesicular structure of RSVPs. In the vesicles there is strong physical entanglement of polyphenolic resveratrol and phosphatidylcholine (Kidd 2009). RSVPs appeared as ring and in many of these rings there was a break with the two ends close to each other.

*Ex vivo skin permeation study*

Skin uptake of RSVP was found to be greater than RSV. At the end of 24 hours, 16.47 ± 0.42 % of RSV was released whereas a higher amount of RSV (54.66 ± 2.04 %) was found in case of RSVP in the receptor fluid (Figure 6). The results show higher permeability of RSVPs, where phosphatidylcholine played a major role in bringing the RSV via the lipophilic stratum corneum to epidermal dermal site and passing the viable dermis which is hydrophilic in nature. RSV as well as RSVP were lipophilic as discussed in partition coefficient results; do not find easy passage through the viable dermis, which may be the reason of lower cumulative (%) permeation after 24 hours. It is to mention here that the total amount of drug that permeates through the skin is the summation of drug quantified in the receptor medium and the quantity of drugs retained in the dermis (superficial dermis: 300–500 µm, profound dermis: 500–1000 µm; and pro-found dermis + beginning of the subcutaneous tissue: >1100 µm), and also that the skin in *in vivo* case is vascularized, and thus the drug within it is able to reach the bloodstream (Reichling *et al.*, 2006). But in *ex vivo* situation skin has its microcirculation obliterated; the dermis can retain compounds that would penetrate *in vivo* otherwise (Sallovitz *et al.*, 2012). This increases the possibility of accumulation of RSV either in free form or as complex at the epidermal dermal site beneath the stratum corneum, as it was reported in earlier works that phyto-phospholipid complexes enhance the passage through the outer lipophilic horny layers (Semalty *et al.*, 2008; Chen *et al.*, 2012). Also
Hung et al., (2008) reported that the viable epidermis/dermis but not the SC layer acted as a diffusion barrier for resveratrol permeation. The skin extract obtained from the skin used for permeation study of free RSV revealed that it contain 21 ±1.06 % drugs. Whereas the skin extract obtained from permeation study using F4 showed 37 ± 1.52 % RSV content. These findings are in good agreement to the report of Polonini et al., (2014). In their work transdermal emulsion of resveratrol when applied in an initial quantity 49.30 µg to the skin, 9.50 µg were quantified in the receptor medium, 20.48 µg was retained at the SC (do not account as permeated) and 21.41 µg was retained at the viable epidermis + dermis (account as permeated), totalizing 30.9 µg of resveratrol permeated after 24 h of application (62.6%).

Fig. 6: Comparison of skin permeation profile of RSV and RSVP. All data points represents average of three observations (n=3).

CONCLUSION
Poor aqueous solubility and presystemic metabolism are the hindrances in oral administration of RSV. Physicochemical evaluation carried out in the present work supports the scope of skin targeted delivery of RSVP. Since the effective treatment by transdermal and dermal route demands once-a-day drug application, this parameter is of major concern in product development. The results suggest that the resveratrol phytosomes are better able to penetrate the highly impermeable stratum corneum than free RSV. Retention of this higher quantity of RSV will be available for slow passage through the viable dermis and prolonged anti-inflammatory effect at superficial as well as deep skin and adjacent muscular tissues and bone interlocks for getting relief in arthritis, rheumatism, athletic aches and other chronic inflammation.
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