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Transdermal Permeation Enhancement by Drug-Phospholipid Supramolecular Complexation

Surendra Tripathy*[1], Malay K Das[1]
1.Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786004, INDIA

ABSTRACT

Drug delivery of highly lipophilic molecules through the transdermal route is unsuitable when systemic effect is desired. Besides the different physical and chemical approaches for permeation enhancement, a novel method has been drawn for improvement in permeation behaviour. Here, the drug molecule was supramolecularly complexed with a phospholipid molecule to form a novel chemical entity possessing improved permeation behaviour suitable for transdermal application. Curcumin (CMN) was used as model drug for preparation of Curcumin-Phospholipid Supramolecular Complex (CPSC) and was characterized by FT-IR Spectroscopy and X-Ray Diffraction Analysis. Comparative solubility study of CPSC in water and n-octanol was performed. The prepared CPSC was incorporated in polymeric matrix films of Eudragit RL100 and Eudragit RS100 and the physicochemical compatibility was studied. The permeation kinetics and permeation parameters of the films were studied against a control batch formulation loaded with uncomplexed CMN across processed excised pig ear epidermis. Skin irritation test was perfomed on rats for safety assessment of films. Analytical reports suggested supramolecular complex formation. The solubility study showed increased hydrophilicity of the prepared CPSC. The FT-IR Spectroscopy and Differential Scanning Calorimetry (DSC) confirmed no interaction between polymers and CPSC. The formulations loaded with CPSC followed zero order and Higuchi model kinetics and possessed improved permeation parameters and a good enhancement ratio against the control batch. No skin reactions were observed during the skin irritation test. The conducted experiments suggested the supramolecular phospholipid complexation to be an effective permeation enhancement technique for transdermal route.

Keywords: Curcumin, phospholipid, supramolecular complex, skin permeation, enhancement ratio, skin irritation.

*Corresponding Author Email: surendratripathy210@gmail.com
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INTRODUCTION

Transdermal drug delivery is an advantageous system for drug delivery to the tissues underlying the skin and to the systemic circulation, with a good patient compliance for long term therapy. This route is generally preferred for maintaining a constant and prolonged drug level in the plasma by sustaining the drug delivery. The transdermal drug delivery is meant for increasing the flux through intact skin and minimization of retention and metabolism of drug in skin[1,2].

The model drug Curcumin (CMN) is reported to be a hydrophobic phytoconstituent obtained from the dried rhizomes of *Curcuma longa*, belonging to family Zingiberaceae. CMN is reported to have many pharmacological activity including some major ones like anticancer, anti-inflammatory and hypocholesterolemic activity[3,4,5]. As the molecule is having a very high metabolic rate in blood and hydrophobicity, it is hard to find a way for such molecule to deliver it to the systemic circulation. The oral route requires a very large dose for producing the effect and CMN is well tolerated at higher doses up to 2 g/kg via oral route[6]. Some novel approaches for CMN delivery include microspheres[7], solid lipid nanoparticles[8], transferosome[9], transdermal films using penetration enhancers[10]. The phospholipid complexation is a well established technique for delivering phytoconstituents via oral route and topical route for increasing bioavailability[11,12]. By complexing with a phospholipid molecule, the hydrophilicity of the CMN can be increased to some extent, which may help the drug for transdermal delivery. The phospholipid itself has health promoting activity in certain hepatic disorders and native to the biological membrane, hence it is helpful for carrying the complexed drug across the stratum corneum and provides additional stability to the drug by enveloping it[13].

In the present study, an attempt has been made to enhance the transdermal permeability of the model drug CMN by complexing it supramolecularly with phospholipid. The CPSC were prepared and incorporated in the transdermal film fabricated by blending Eudragit RL100 and Eudragit RS100 in different ratio. As the phospholipid is native to the lipid bilayer of the stratum corneum the complex will be easily permeate through it. The skin retaining power of CMN can be reduced as the prepared complex has a balanced lipophilicity and hydrophilicity. The drug interaction with the polymer has been studied to investigate any chemicophysical incompatibility. Comparative skin permeation study was performed to observe the improvement in permeation upon complexation. The skin irritation study was performed on rats to assess the safety profile of the films for long term transdermal use.

MATERIALS AND METHOD

The Phospholipid was procured from ACROS Organics, New York, USA. Curcumin was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Eudragit RL100 and Eudragit RS100 were obtained from Rohm Pharma, Germany. Dichloromethane, 1,4-dioxane, methanol, n-Hexane, Polyvinylalcohol, Di-n-butylphthalate and other chemicals were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India.

Preparation of Curcumin-Phospholipid Complex (CPSC)

CPSC was prepared by the reflux method used by Maiti *et al*[14] with a little modification. The CPSC was prepared by a trial and error method by refluxing the CMN and PL in a reflux solvent for obtaining maximum yield. The drug phopspholipid ratio, solvent system for reflux, reflux temperature and reflux time were studied individually for obtaining a higher yield. The various reflux parameters and their screening has been given in Table 1. The stoichiometric ratio of 1:1 for CMN and PL was found optimum for maximum yield with a dry end product. The CMN and PL were refluxed in a round bottom flask at 50^{o}C with dichloromethane for 3 hours. The solvent was evaporated slightly and n- hexane (5ml) was added with continuous agitation to enhance the precipitation and for providing hardening effect and remove traces of reflux medium. The complex was then evaporated completely at room temperature and again vacuum dried. The yield of the complex was measured by simple weighing method after maximum drying of the products.

Table 1: Screening of Reflux Parameters for preparation of CPSC.

Reflux Parameters	Optimized Reflux parameters	Product yield and quality
CMN & PL Ratio:		
1:1		
1:2	**1:1**	
1:3		
Reflux medium		
Dehydrated Ethanol		
1,4-Dioxane	**Dichloromethane**	**80%**
Dichloromethane		**Dry, Free flowing**
Reflux Time (hour):		
1		
2	**3**	
3		
Reflux Temperature (°C):		
50		
60	**50**	
70		

Characterization of Curcumin –Phospholipid Complex (CPSC)

Determination of CMN content in the CPSC

5 mg of the dried complex was added to 10 ml methanol and stirred with a magnetic stirrer for 2 hour until the complex was dissolved completely. The sample was then diluted suitably and the drug content was measured spectrophotometrically (SPECTRASCAN UV 2600, Thermo scientific, India) at a λ_{max} of 421nm.

Characterization by instrumental methods

The CPSC was characterized by FT-IR spectroscopy and X-Ray Diffraction Analysis to identify the interaction occurred between the drug and the phospholipid molecule[15,16].

FT-IR Spectroscopy

The FT-IR Spectra of pure CMN, PL, physical mixture of CMN and PL and the CPSC were taken separately by the Attenuated Total Reflectance (ATR) technique with the FT-IR spectrometer (OPUS, Alpha, Bruker, Germany). ATR is a quicker and non-destructive technique for obtaining the IR spectrum of the material surface. This technique required hardly any sample preparation. Intimate optimal contact between the sample and the ATR crystal was necessary. The spectra were compared to show the possible interaction between drug and phospholipid molecule.

X-Ray Diffraction Study

X-Ray diffraction study of curcumin (pure drug), phospholipid, physical mixture of curcumin and phospholipid and CPSC was performed using X-ray Diffractometer (X'Pert pro, PANanalytical, The Netherlands). The voltage and current were 30 kV and 15 mA respectively. The diffractograms were compared to detect any interaction of drug and the phospholipid molecule.

Comparative solubility study of CMN, physical mixture of CMN and PL and CPSC

The phospholipid complexation increases the aqueous as well as oil solubility. To study the comparative solubility profile excess of the drug, physical mixture of drug and phospholipid and the drug-phospholipid complex were added to 5 ml of water (aqueous phase) and 5 ml of n-octanol (organic phase) in separate sample tubes and stirred for 24 hours at a temperature of 37 ± 0.5^{o}C. Then the samples were withdrawn and centrifuged at 5000 rpm for 15 minutes. The supernatant was filtered and 1ml of supernatant was added to 9 ml of methanol and quantified spectrophotometrically at 421 nm[14].

Preparation of matrix type transdermal patch

Matrix type transdermal patch was prepared by solvent evaporation method. Eudragit RL-100

(ERL100) and Eudragit RS-100 (ERS100) were used as the film forming polymer to prepare the patch. Di-n-Butylphthalate was used for providing plasticity. Different polymer ratio selected were Eudragit RL-100 : Eudragit RS-100, 1:1, 1:2, 1:3, 2:1 and 3:1 respectively. The polymeric solution was prepared in dichloromethane. After that plasticizer was added and stirred properly for complete distribution. The CPSC was then incorporated to the polymeric solution and stirred gently for uniform mixing. It was finally poured on aluminium frame of 5cm × 5cm dimension containing a backing membrane for evaporation. The films were carefully removed after complete drying. The control batch formulation was prepared by the same technique and same polymer composition for further study.

Compatibility study between the polymer and CPSC

FT-IR spectra and DSC Thermograms of the physical mixtures, blank formulations and formulation containing the CPSC were studied to investigate any possible drug interactions[17,18,19]. The peaks at different wave numbers were compared for all the cases to derive the results. The DSC Thermogram is an important tool for identifying crystalline property and any kind of interaction between two compounds. The thermograms were obtained at a scanning rate of 20°C/min between 50 and 250°C with a Jade DSC (Perkin Elmer, Switzerland). Temperature calibrations were performed periodically using indium as standard. The different endothermic peaks were matched to observe the Crystallinity and drug-excipient interactions.

Ex-Vivo skin permeation study

The permeation behaviour was studied using a modified Franz type diffusion cell containing full thickness excised pig ear epidermis, which is a widely used and most accepted animal model of permeation study across human skin [20,21,22,23]. The pig ear skin after procurement was processed. The epidermis was removed intact and preserved until use. The prepared epidermis was placed by keeping the dorsal surface attached to the drug releasing surface of the matrix. The holder containing the skin and the formulation was then placed on the receiver compartment containing 50% PEG400 maintaining 37°C thermostatically and stirred by a magnetic stirrer. Samples were withdrawn at intermittent period regularly and estimated spectrophotometrically. Cumulative amount of drug permeated per unit area was calculated. Simultaneous permeation study was performed for the control batch containing pure CMN, to compare the cumulative release and skin penetration behaviour.

Permeation Kinetics Study

The data obtained from the *in-vitro* skin permeation studies were fitted to various kinetic equations such as zero-order equation ($Q_t = k_0 t$), First-order equation ($\ln Q_t = \ln Q_0 - k_1 t$) and the

Higuchi equation ($Q_t = k_h t^{1/2}$). In zero-order kinetics, the graph of the cumulative amount of drug permeated versus time (Q_t vs. t) was plotted. The graph of log cumulative amount permeated versus time [($\ln Q - Q_t$) vs. t] was plotted to study the First order kinetics. The Higuchi plot was prepared by taking cumulative drug released against the square root of time (Q_t vs. $t^{1/2}$). The mathematical models are studied to determine the permeation behaviour[24,21]. The permeation parameters like flux, diffusion coefficient, permeability coefficient were determined from the skin permeation data and results of the permeation kinetics study. The Enhancement Ratio of Flux was calculated by dividing the flux of formulation loaded with CPSC with the control batch formulation without complexation with phospholipid[25,26].

Measurement of skin deposition of drug

The skin samples after permeation study was washed with distilled water repeatedly to remove the drug particles attached to the surface. Then the epidermis was cut into small pieces and put into stoppered conical flask containing 10 ml of methanol and stirred for 24 h. The aliquot of 1ml was then taken and diluted with methanol and filtered through membrane filter of pore size 0.22µm. The drug amount was quantified spectrophotometrically.

Skin Irritation study

The guideline for skin irritation test was approved by the Institutional animal ethical committee (Department of Pharmaceutical Sciences, Dibrugarh University, Approval No: IAEC/DU/19). The hair on the dorsal side of Wistar albino rats was removed by marketed depilatory cream preparation 1 day before the experiment[27]. The rats were divided into four groups (n = 6). Group I served as the control, group II received the formulation CPCF5, group III received the control batch formulation PDF5 and group IV received 0.8% (vol/vol) aqueous solution of formalin as a standard irritant[28]. A new film, and new formalin solution, was applied daily for 7 days. Finally, the application sites were graded according to a visual scoring scale, always by the same investigator[29].

Statistical analysis

The data obtained in this study have been expressed as Mean ± S.D (Standard Deviation). The data were subjected to statistical analysis and assessed by one way Analysis of Variance (ANOVA) following Dunnett's t – test. p value of less than 0.05 was considered as evidence of a significant difference.

RESULTS AND DISCUSSION

Preparation of CPSC

The method of preparation of CPSC was developed from the screening of reflux parameters, reflux solvents and drug PL ratio. The product obtained were dry and having good integrity. The maximum yield of CPSC was found to be 80%. Dichloromethane was the solvent of choice for the reflux medium and n-Hexane was the solvent for the precipitation and providing hardening effect to the complex formed. Aprotic solvents are generally chosen for the preparation of the complex, neither protogenic nor protophilic solvents are chosen because if dilute acid is taken as solvent, it may split off the molecule of choline leaving phosphotidic acid where as alkali causes choline to split off, followed by the hydrolysis of phosphotidic acid to give soaps of fatty acids and the salt of glycerol phosphoric acid.

Characterization of prepared CPSC

On spectrophotometric determination the CMN content in the CPSC was found out to be 33.6 %. The FT-IR spectra of CMN, PL, physical mixture of CMN & PL and the CPSC were taken separately and compared to observe the interaction occurred (Figure.1). By comparing the spectrum, in the case of the CPSC the peak of free hydroxyl group (for O – H stretching) of CMN was not there. The stretching vibration has been decreased to 3335.74 cm^{-1} from 3610.21 cm^{-1} and a broad band was observed, which may be due to the intermolecular hydrogen bonding between drug and phospholipid[15,30]. All other peaks were duly interpreted and compared and were not showing any significant changes. The resulting supramolecular complex was formed as the PLs were bifunctional groups having hydrophilic choline part and lipophilic long hydrocarbon chain. In PL molecule one of the two primary methylenes, had a phosphate group, where the hydroxyl group of the polyphenolic compound (CMN) was attached. Chemical analysis indicated that the choline head of the PL molecule binds to these compounds by making some ion-ion, ion-dipole, ion-induced dipole interactions or the most possible hydrogen bond, while the lipid soluble phosphatidyl portion comprising the body and tail which then enveloped the choline bound material. As a result a bond was formed between these two molecules, creating a supra molecularly complexed hybrid molecule which was like a little microsphere or cell, better suited to merge into the lipid phase of the outer cell membrane. Hence, the phytomolecules produce a lipid soluble molecular complex also called phytophospholipid complex with phospholipids which enables absorption of natural products irrespective of their molecular weight[31].

The X-Ray diffraction study of CMN, phospholipid, physical mixture of CMN and PL and the CPSC were performed separately and compared to observe the interaction occurred (Figure.2). CMN showed sharp crystalline peaks between 60 -80^{o} (2θ), and the PL showed crystalline peaks

between 15 – 30^{o} (2θ). The physical mixture of CMN and PL also nearly possesses the same peaks as in the case of individual components. But in case of CPSC, no crystalline peaks were distinguished and the intensity was reduced significantly indicating some possible interaction between the drug and the PL, resulting in marked decrease in crystallinity of the drug[16].

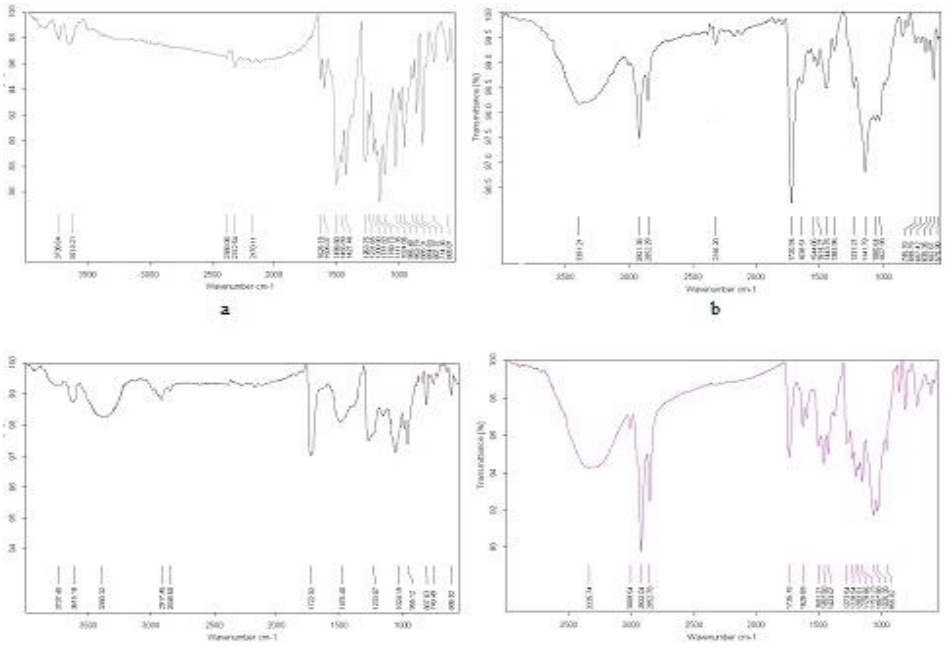


Figure 1 FTIR Spectra of Pure CMN (a), PL (b), physical mixture of CMN &PL(c) and CPSC (d).

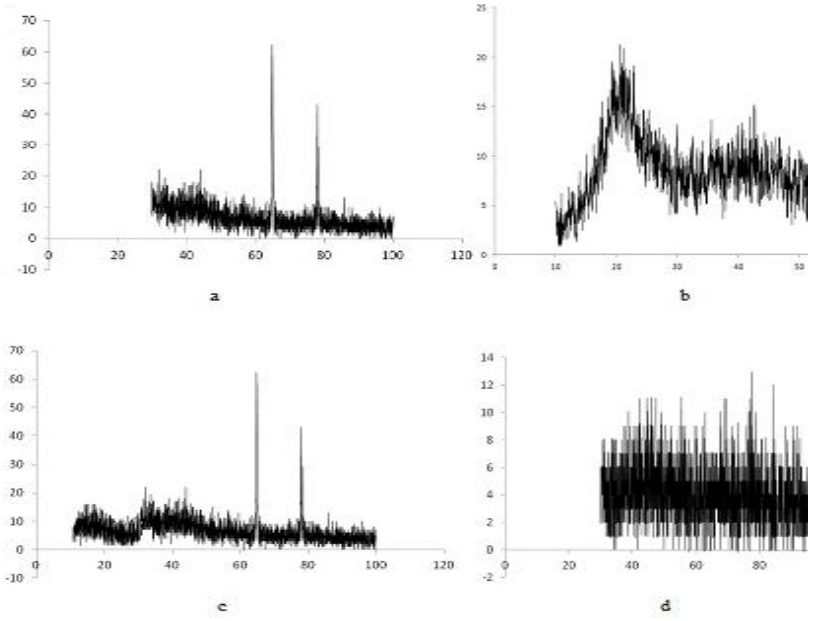


Figure.2 X-Ray Diffraction Study of Pure CMN (a), PL (b), physical mixture of CMN and PL (c) and CPSC (d). The X-Axis represents the Angle (2θ) and Y-Axis represents the Intensity.

Comparative solubility study of CPSC

CMN itself is poorly soluble in water and has a good solubility in n-Octanol. The objective behind complexing the drug with PL was to increase its bioavailability, for which adequate aqueous solubility is also needed to cross the biological membrane and move into the systemic circulation. The comparative solubility study was predictive parameter to have an idea on the possibility of the complex to proceed towards the systemic circulation after being penetrated into the upper skin layer. The aqueous solubility of curcumin upon complexation increased approximately four folds. The physical mixture was also found to improve the solubility of CMN in both water & n-Octanol which may be due to the surfactant action of the PL and hydrophobic interaction (Table 2).

Table2. Comparative solubility study of CMN, physical mixture of CMN and PL and CPSC.

Drug for solubility study	Solubility in water (µg/ml) (Mean ± S.D.)	Solubility in n-Octanol (mg/ml) (Mean ± S.D.), n = 3
CMN	5.8 ± 0.25	3.8 ± 0.29
Physical mixture of CMN and PL	14.2 ± 0.36	10.2 ± 0.21
Curcumin-Phospholipid Complex (CPSC)	21.7 ± 0.22	29.9 ± 0.33

Preparation of Transdermal films

After the complexation was confirmed by FT-IR and XRD Analysis, the transdermal films were prepared by taking combination of ERL-100 and ERS-100 as film forming polymers and the CPSC was loaded in the films. These polymers were selected because, ERL-100 results in rapid hydration due to higher content of quarternary ammonium groups, where lower proportion of quaternary ammonium group in Eudragit RS-100 was used to control the release of drug from the formulations. Dichloromethane was found to be an excellent solvent because of its non-polar nature for fabrication of the films and no possibility of cleavage of the CPSC was found in the solvent. The films were prepared on previously dried Polyvinylalcohol backing membrane. The patches were completely got dried within 4 hour forming smooth and transparent films. The prepared films were having good chemicophysical properties.

CPSC-polymer compatibility study

The drug-polymer interaction study suggested no interaction between drug and polymers. The FT-IR spectra of physical mixture of polymers and CPSC, blank formulation, CPSC loaded films were observed (Figure.3). The spectrum for blank film was devoid of peaks due to CPSC. The physical mixture showed a peak at 3334.19 cm^{-1} which was very similar with the peak of CPSC confirming no interaction in the physical mixture. On the other hand the spectrum of the

formulation showed a peak at 3394.50cm^{-1}, which was found to be the merged peaks of ERS100 and PL in that particular wave number, but peak due to O-H stretching of CMN was absent, which clearly indicated that the supramolecular interaction was retained in the formulation and the CPSC was molecularly dispersed within the formulation. The DSC thermogram of physical mixture (polymers and CPSC) and drug loaded films were observed (Figure.4). The physical mixture showed an endothermic peak at 146.03^{o}C, which may be due to the CPSC. This peak was absent in the thermogram of formulation suggesting the uniform molecular dispersion of the CPSC in the formulation[32].

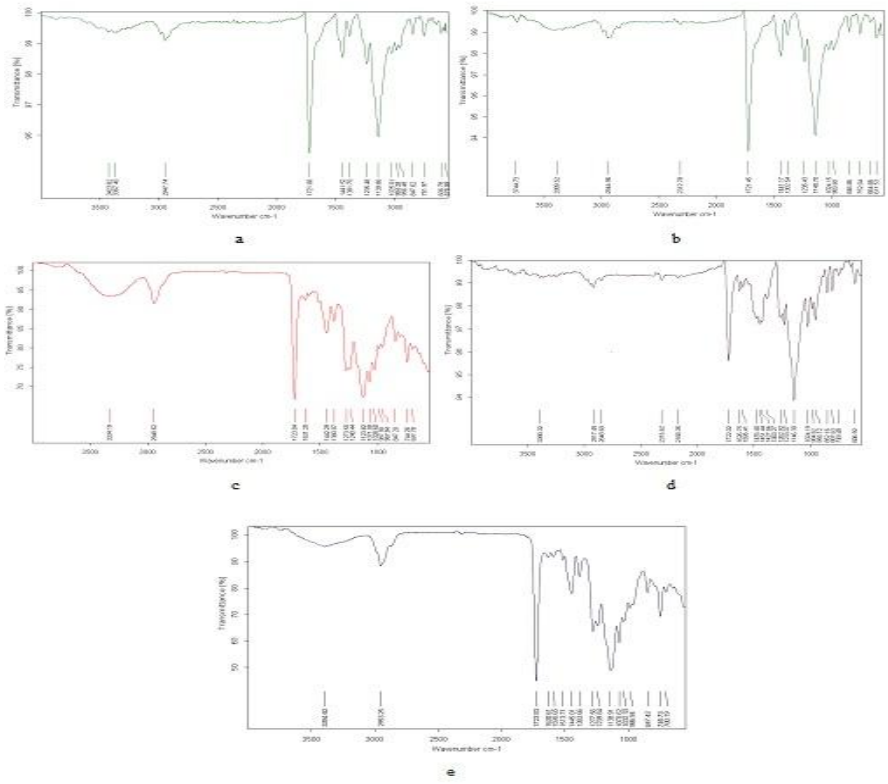


Figure 3 FT-IR Spectra of ERL100 (a), ERS100 (b), Physical mixture of ERL100, ERS100 and CPSC (c), Blank formulation (d) and Formulation loaded with CPSC (e).

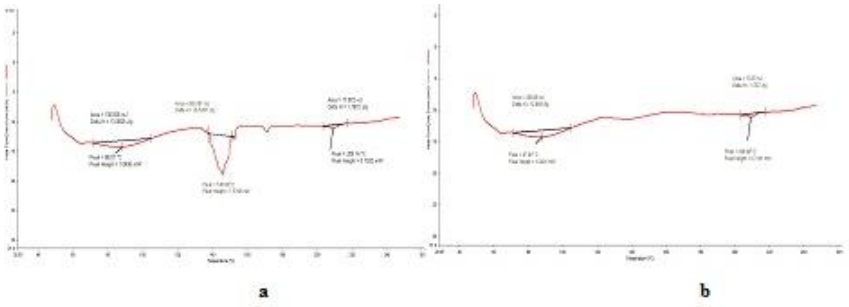
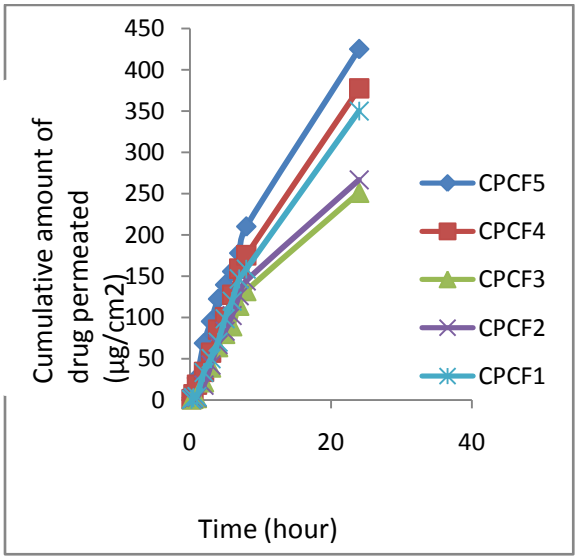
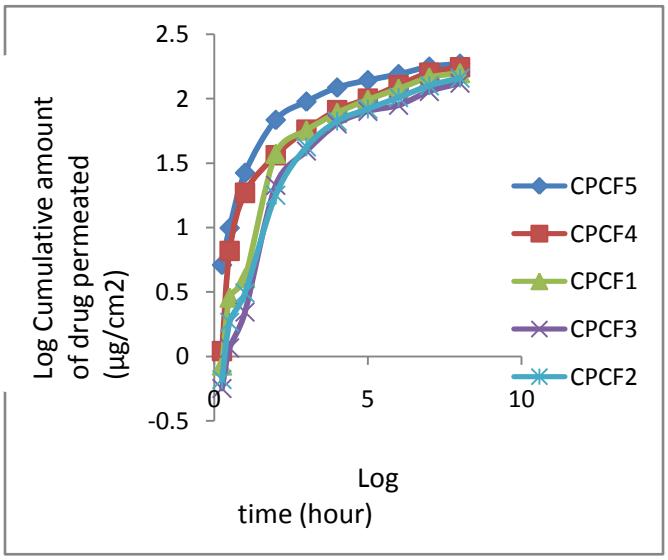


Figure 4 DSC Thermograms of physical mixture of ERL100, ERS100 and CPSC (a) and Formulation loaded with CPSC (b). Ex-vivo Skin permeation and kinetic study

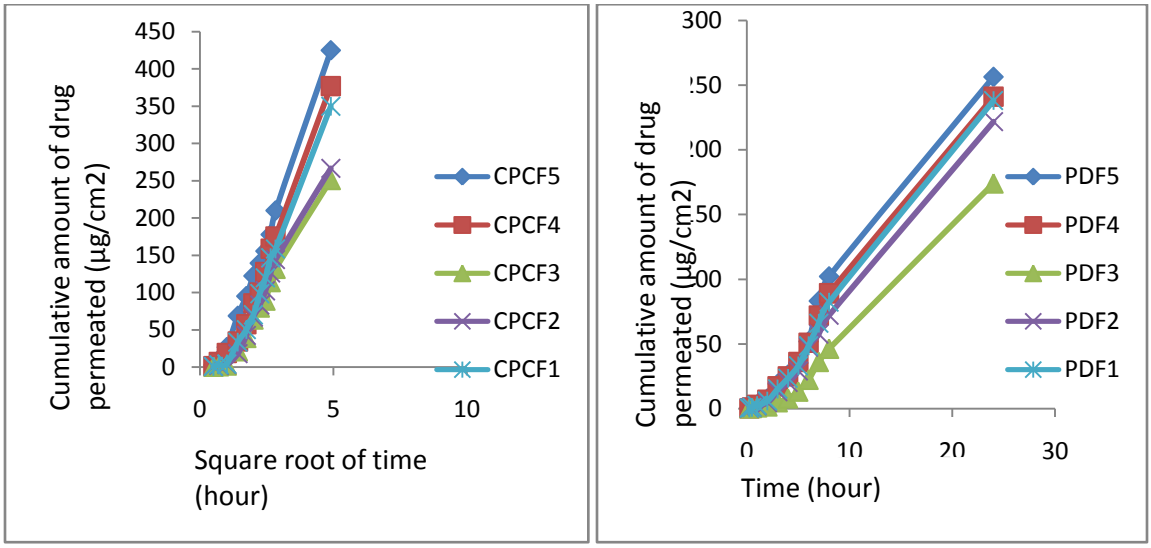
The pig ear epidermis was removed and processed. The thickness of pig ear epidermis was determined to be 112.25 µm from the microscopic study. The permeation kinetics of formulations loaded with CPSC were studied and were compared with a formulation containing only pure CMN for cumulative release and permeation profile study. Results of the permeation study showed that the formulation loaded with CPSC had a marked increase in the maximum cumulative release after 24 hours. The formulation CPCF5 had a maximum Flux of 25.90µg/cm^2/h, which was more than the formulation PDF5, having a Flux of 11.84 µg/cm^2/h. The Enhancement Ratio (ER) was calculated for each formulation and were found between the value 2.14 – 3.34, which indicates that the formulations were superior to the control batch formulations containing uncomplexed or pure CMN. The formulation CPCF5 had a greater Diffusion Coefficient (D) of 1.05 × 10^{-4} cm^2/h and Permeability Coefficient of 2.43 × 10^{-2}µg/ cm^2/h than that of the control formulations (Table 3). The result suggested that the dissolution of the polymer matrix didn't play a major role, rather the complexed drug was more soluble in the eluting medium and more permeable through the porcine skin. The Permeability Coefficient and Diffusion Coefficients were studied for all the formulations, which indicated that the the formulation loaded with CPSC were superior. The permeation kinetics data showed that the formulation loaded with the CPSC followed the zero order kinetics (r^2= 0.986 – 0.995) and the Higuchi model kinetics (r^2= 0.971 – 0.990) (Figure.5). The formulations didn't follow first order kinetics (Table 4)



(a)



(b)



(c) (d)

Figure. 5 Graphical representation showing zero order permeation kinetics of films loaded with CPSC (a), first order kinetics of films loaded with CPSC (c), Higuchi model kinetics of films loaded with CPSC (c), zero order permeation kinetics of control batch formulation loaded with pure CMN (d).

Table 3. Permeation parameters of Transdermal films.

Formulation code	Lag time (hour) (Mean ± S.D.)	Diffusion Coefficient X 10^{-4} (cm^2/h) (Mean ± S.D.)	Permeability Coefficient X 10^{-2} (cm/h) (Mean ± S.D.)	Flux (µg/ cm^2/h) (Mean ± S.D.)	Cumulative % release after 24 h.
CPCF1	0.45 ±0.06	0.46 ±0.005	1.08 ± 0.09	21.55±1.02	38.18
CPCF2	0.50±0.08	0.42 ± 0.004	0.972 ±0.15	19.33±2.14	32.51
CPCF3	0.82 ±0.03	0.26 ±0.007	0.608 ±0.05	17.48±3.58	29.92
CPCF4	0.30 ±0.01	0.71 ±0.029	1.62 ±0.12	22.70±2.98	40.69
CPCF5	0.20 ±0.05	1.05 ±0.041	2.43 ±0.12	25.90±3.66	47.67
Control batch Formulations containg pure CMN					
PDF1	1.50 ±0.09	0.14±0.08	0.51 ±0.12	9.83 ±2.26	21.11
PDF2	1.30 ±0.1	0.16 ± 0.08	0.58 ±0.18	8.76 ±1.20	19.02
PDF3	1.70±0.12	0.12 ±0.07	0.45 ±0.05	5.22 ±1.35	13.67
PDF4	1.20±0.08	0.17 ±0.02	0.63 ± 0.09	10.60±1.11	23.46
PDF5	1.4 ±0.08	0.15 ±0.03	0.54 ±0.19	11.84±1.98	25.77

S.D.= Standard Deviation, n=3. (CPCF represents formulation loaded with CPSC and PDF represents formulations loaded with Pure drug.)

Table 4. Permeation Kinetics modelling of the Transdermal films.

Kinetic model ➡	Zero Order (Q vs t)		First Order (logQ vs t)		Higuchian (Q vs $t^{1/2}$)	
Formulations	**Best fit equations**	**R^2**	**Best fit equations**	**R^2**	**Best fit equations**	**R^2**
CPCF1	Q=21.55t – 10.68	0.994	Q=0.263t +0.48	0.841	Q=82.03t – 72.98	0.971
CPCF2	Q=19.33t – 12.17	0.992	Q=0.28t +0.317	0.830	Q=64.94t – 54.52	0.973
CPCF3	Q=17.48t – 9.86	0.992	Q=0.290t+ 0.22	0.806	Q=60.50t – 50.99	0.977
CPCF4	Q=22.70t - 7.00	0.995	Q=0.221t +0.77	0.755	Q=87.22t – 73.90	0.971
CPCF5	Q=25.90t +5.63	0.986	Q=0.192t +1.06	0.721	Q=96.83t – 66.98	0.990
PDF1	Q=9.837t -7.62	0.942	Q=0.277t -0.01	0.896	Q=27.31t – 18.24	0.789
PDF2	Q=8.762t - 6.79	0.943	Q=0.273t -0.03	0.905	Q=24.34t – 16.26	0.791
PDF3	Q=5.221t - 5.32	0.863	Q=0.298t- 0.47	0.941	Q=14.11t – 10.34	0.686
PDF4	Q=10.60t -7.99	0.942	Q=0.278t +0.02	0.868	Q=29.49t – 19.50	0.791
PDF5	Q=11.84 - 9.47	0.914	Q=0.268 +0.11	0.896	Q=32.69t – 21.96	0.757

The increase in permeation of the complex was due to the major contribution of its outer lipophilic part which is native to the biological membrane, besides that the improved solubility of the complex further helped it to pass into the aqueous media.

Drug deposition in the excised pig ear epidermis after permeation study

The drug content in the epidermis were measured after the permeation study by extracting the skin sample with methanol. The formulation loaded with CPSC showed a higher concentration of drug in the skin as the complex had become more lipophilic as well as hydrophilic upon complexation, and probably due to this activity, the retained drug in the skin slowly transferred to the systemic circulation and hence the formulations were having maximum flux rate and cumulative release after the permeation study. The pure drug CMN had lipophilic property but due to lack of hydrophilicity it was retained in the skin for longer period instead of being eluted into the aqueous medium of the receptor compartment.

Skin irritation study

The increased skin penetration was confirmed from the permeation behaviour study on excised pig ear epidermis but the safty aspect of the complex via transdermal route was studied duly on

the rats. The formulation CPCF5 and PDF5 were tested for skin irritation study on wistar rats to get a reproducible result. Both the formulation were scored for formation of erythema and edema. The scores obtained for both was less than 2, which indicated that the formulations were free from any kind of skin irritation. The value less than or equal to 2 are considered to be negative[33,34] (Table 5). The statistical data showed the values were significantly less from that of the formalin ($p < 0.05$).

Table 5. Skin Irritation study of the prepared formulations.

Rat Num.	Control		CPCF5		PDF5		Formalin	
	Erythema*	Edema#	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	1	0	0	1	2	3
2	0	0	0	2	1	1	3	2
3	0	0	1	1	1	0	3	3
4	0	0	0	2	1	1	3	3
5	0	0	0	1	1	2	3	1
6	0	0	2	0	0	1	3	2
Average			0.66± 0.23[a]	1.00± 0.40[a]	0.66± 0.12[a]	1.00± 0.33[a]	2.83± 0.36[a]	2.33± 0.58[a]

*Erythema Scale: 0= none, 1= slight, 2= well defined, 3= moderate, 4= scar formation.

Edema Scale: 0= none, 1= slight, 2= well defined, 3=moderate, 4= severe.

[a] Significant difference found when compared with formalin ($p < 0.05$).

The conventional techniques for penetration enhancement like use of penetration enhancers and physical means causes local cellular damage for the entry of drug molecule. Apart from that the stability of drug in the dosage form and in systemic circulation are important issues for drug delevery. The supramolecular phospholipid complexation provides good drug stability in formulation as well as systemic circulation as the drug is enveloped within the lipophilic sheath of phospholipid and anchored to the choline part by a chemical interaction which is generally not found in any other carrier systems including liposomes, transferosomes, ethosomes meant for transdermal application. The phospholipid is a nutritional aid in certain hepatic disorders and having its own health promoting effect[35]. The transdermal therapeutic system of CPSC was having good permeation profile and showed a sustained release behaviour and is possibly fit for prolonged therapeutic use.

CONCLUSION

From the different experiments conducted, this can be concluded that the supramolecular phopspholipid complexation may be a potential method to deliver polyphenolic phytoconstituent via transdermal route. As the supramolecular complex is solely changing the physical and

chemical properties of a drug molecule but retaining its therapeutic effect, the water insoluble drug molecules can be administered by transdermal route by this technique. The supramolecular complex preparation is a relatively easier and reproducible method. In the present study the CPSC was found compatible with the polymers used and the physicochemical parameters of the films were sufficiently good. The permeation study confirmed the increased penetrating power of CPSC than the pure curcumin through the porcine epidermis. Formulations were not found to cause any kind of skin irritation in rats. Hence, inference can be drawn from this piece work that the supramolecular complexation of polyphenolic drug molecules can be a successful and novel field for delivering them transdermally without the use of any chemical penetration enhancer or any physical mean.

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