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PREPARATION AND EVALUATION OF DRUG PHOSPHOLIPIDS COMPLEX FOR INCREASING TRANSDERMAL PENETRATION OF PHYTO CONSTITUENTS

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ABSTRACT

Keywords:

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complex, transdermal
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Curcumin is a natural compound found in turmeric and possesses antioxidant, anti-inflammatory and anti-tumor ability. Curcumin is poorly soluble in water and has lower pharmacokinetic properties. In order to improve the bioavailability of curcumin, its complexation with phospholipid (in 1: 2 molar ratios) was carried out. The formation of complex was confirmed by IR spectroscopy and DSC analysis. *In vitro* skin penetration rate of curcumin-phospholipid complex was compared with that of curcumin. Curcumin-phospholipid complex showed almost 60% greater permeation of curcumin through rat skin as compared to that of plain curcumin. It was concluded that the phospholipid complexation of curcumin with phospholipid results in increased transdermal penetration of curcumin.

INTRODUCTION:

Transdermal delivery of drugs through the skin to the systemic circulation provides a convenient route of administration for a variety of clinical indications. The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetration across the skin which is associated primarily with the outermost stratum corneum layer of the epidermis¹. Although many chemicals have been evaluated as penetration enhancers in human or animal skins, to date none has proven to be ideal². The penetration enhancers should work unidirectional, i.e. should allow therapeutic agents into the body whilst preventing the loss of endogenous material from the body. Penetration enhancement occurs through optimization of drug and vehicle properties³. Prodrugs and ion-pairs, chemical potential of drug in vehicle saturated and supersaturated solutions, complexes, liposome and vesicles. Penetration enhancement by stratum corneum modification: Hydration, Lipid Disruption/ Fluidization by Chemical Penetration Enhancers, Interaction with Keratin, Skin Penetration Retarders, Natural drugs are the lead molecule in the pharmaceutical it having potent activity but the main problems with natural drugs is the poor systemic bioavailability and lower pharmacokinetic formation a complex with the phospholipids is the one the technique for penetration enhancement of natural molecule. Formation complexes with the phospholipids these complex markedly increase the bioavailability of natural molecule.

MATERIALS AND METHODS

Chemicals: Phospholipon[®] 90 H (Hydrogenated Phosphatidylcholine from Soybean) obtained as a gift sample from Lipoid, Germany and **Curcumin** procured from Yucca Enterprise, Mumbai, India.

Method for preparation of curcumin- Phospholipids complex

The curcumin-phospholipid complex was prepared at 1:2 molar ratios of curcumin and phospholipid respectively. Fifty mg of curcumin and 100mg Phospholipid were taken in a 100 ml round bottom flask and 40 ml of dichloromethane was added. The mixture was refluxed at a temperature not exceeding 40 °C for 3 hrs, with constant stirring on magnetic stirrer. The resultant clear solution was evaporated in a rotary evaporator to obtain curcumin-phospholipid

complex. The resultant curcumin–phospholipid complex (yield 88%, w/w) was kept in an amber colored glass bottle, and stored in refrigerator⁴.

Evaluation of curcumin-Phospholipid complex

Determination of Curcumin content in Complex:

Drug content of the curcumin in complex was determined by dissolving an accurately weighed quantity of Curcumin-phospholipid complex (about 50 mg) in 25 ml of methanol, transferred to the volumetric flask and the volume was adjusted up to mark. Absorbance of the resulting solutions was measured at 425 nm using double beam spectrophotometer to determine the drug content of the Curcumin-phospholipid complex. Curcumin content was calculated from the linear regression equation obtained from calibration curve of curcumin in methanol.

Differential Scanning Calorimetry (DSC) Analysis:

DSC analysis was done on a Shimadzu DSC 30S. The samples were sealed in the aluminum crimp cell and heated at the speed of 10°C/min from 0 to 350 °C in nitrogen atmosphere (60 ml/min). The peak transition onset temperature of curcumin, phospholipid, curcumin–phospholipid complex and physical mixture of curcumin and phospholipid were determined and compared with each other.

Infrared Spectroscopic Analysis:

FTIR spectra for the samples were obtained on a Shimadzu FTIR spectrometer in the transmission mode with the wave number region 4,000-500 cm⁻¹ using potassium bromide (KBr) pellet technique.

Preparation of Gel formulation for Curcumin and Curcumin-Phospholipid Complex:

Gels were prepared by dispersing 2.5% w/w Carbopol 934 in warm distilled water, being kept under mechanical stirring at high speed. The dispersion was then neutralized (pH 7.4) by addition of 0.2M NaOH. Then add curcumin DMSO solution in gel. Any entrapped air in the gel was allowed to escape by allowing the gels to stand overnight.

Determination of Curcumin content in Gel:

Drug content of the gel were determined by dissolving an accurately weighed quantity of curcumin and Curcumin-phospholipid complex gel (about 50 mg) in 25 ml of methanol, the resulting solutions were filtered through whatman filter paper and quantitatively transferred to the volumetric flasks and the volume was adjusted as required. Absorbance of the resulting

solutions was measured at 425 nm using double beam spectrophotometer to determine the drug content of the Curcumin-phospholipid complex. Drug content was calculated from the linear regression equation obtained from calibration curve of curcumin in methanol.

In-vitro skin permeation studies through Rat skin

Preparation of curcumin standard solution for calibration curve:

2.5mg curcumin was dissolved in 25 ml methanol. From this stock solution, solutions of curcumin were at different concentrations 1 µg/ml, 2 µg/ml, 3 µg/ml, 4 µg/ml, 5 µg/ml, 6 µg/ml were absorbance of std. solutions was measured at 425 nm using Shimadzu UV-1800 UV/VISIBLE double beam spectrophotometer. Graph of absorbance vs concentration was plotted and from the equation of linear regression curcumin content in the test sample of curcumin was calculated.

Skin membrane preparation:

Abdominal hair of Wister rats (male/female, weighing 160 ± 25 gm) was shaved using electric and hand razors (CPCSEA KB/10/208). Abdominal skin of rat was surgically removed after anesthetizing the rat with solvent ether^{5,6}. Adhering subcutaneous fat was carefully removed. Before starting the diffusion experiment the skin was kept in saline solution for 1 hour to remove extraneous debris and leachable enzymes. Gel (500 mg gel) was applied to the surface of the preweight glass rod and the rod was re-weighed. The rod was used to spread the gel evenly on the skin and it was again weighted to determine the amount of the formulation applied to the skin.

Assembling of the diffusion cell:

The prepared skin was mounted between two halves of the simple glass type Franz diffusion cell with the stratum corneum facing the donor compartment. Diameter of the Franz diffusion cell was measured using vernier caliper; area of diffusion cell was calculated. The receptor compartment was filled with 25 ml of phosphate buffer pH 7.4. The receptor chamber contents were continuously agitated using magnetic bead driven by magnetic stirrer.

Method of determination of curcumin permeated through the skin sample:

Following application of the curcumin gel to the skin, 1 ml of the aliquots were withdrawn up to 8hrs at an interval of 1 hr. each using an injection syringe and recipient compartment was replenished each time with 1 ml of phosphate buffer at 7.4 pH. The absorbance of the withdrawn

samples was measured at 425nm and the content of curcumin in the withdrawn aliquots was determined by linear regression equation of std. curcumin. The cumulative % drug release at each collection time of point was calculated.

Statistical Analysis

The cumulative drug release data generated in the study were analyzed by one-way ANOVA followed by Tukey's multiple comparison test to determine significant difference between groups at $p < 0.05$.

RESULTS:

Evaluation of drug-phospholipid complex

Content of curcumin in the complex, as estimated by UV spectrophotometry, was 30.04% (w/w). Differential scanning calorimetry (DSC) is a fast and reliable method to ascertain possible interactions between 2 or more compounds in the mixture. In DSC, an interaction is concluded by elimination of endothermic peak(s), appearance of new peak(s), change in peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy. The DSC thermograms of pure curcumin (a), phospholipid (b), physical mixture of curcumin and phospholipid (c) curcumin-phospholipid complex (d) as shown in Figure 1. The thermogram of curcumin showed a single endothermic peak at 178°C. Thermogram of phospholipids exhibit two different peaks; the first one at 220°C is sharp, which appears because of the hot movement of phospholipids polar head group. Physical mixture of curcumin and phospholipids showed one peak at 205°C due to depression in melting point owing to presence of curcumin. The thermogram of the complex exhibits a single peak at the 245°C which differs from the peak of curcumin, phospholipids and their physical mixture. It is evident that the original peaks of curcumin and phospholipids disappear from the thermogram of complex and the phase transition temperature was higher than that of phospholipids. Curcumin can interact with phospholipids and the interaction was hydrophobic in nature. There was also some contribution of hydrogen bonding apart from hydrophobic interaction in the curcumin-phospholipid complexation interaction. The -OH groups of the phenol rings of curcumin were involved in hydrogen bonding where as the aromatic rings could be involved in hydrophobic interaction. The interaction of curcumin with the polar part of phospholipids molecules make the long hydrocarbon tail of phospholipids to turn freely and envelop the polar head of phospholipids containing the curcumin molecule. As a

result there was a decrease in the sequence of the phospholipids hydrocarbon chains and the second sharp peak of phospholipids disappears and lowers the phase transition temperature.

Infrared Spectroscopy

The formation of the complex can be confirmed by the IR spectroscopy comparing the spectrum of the complex with the spectra of the individual components and their mechanical mixtures. FT-IR spectra were recorded on a Shimadzu FTIR spectrometer in the transmission mode with the wave number region $4,000-500\text{ cm}^{-1}$. FTIR spectra showed the changes in peaks in complexes and positions from that of Curcumin and Phospholipid. FT-IR spectra of complex was significantly different from that of physical mixture as shown in figure 2. The peak at 3512 cm^{-1} in the physical mixture indicates the presence of free $-\text{OH}$ group. As seen in the IR spectrum of curcumin-phospholipid complex, this peak was transformed into a broad shallow peak indicating the involvement of $-\text{OH}$ group in the complex formation.

Determination of Curcumin content in Gel

Plot graph of absorbance vs concentration from obtained standard solution reading. From the figure 3 linear regression derived was $Y = 0.1873x$ with $r^2 = 0.9941$. The curcumin content was calculated from linear regression equation and was found to be 1.69 % w/w in curcumin gel and 1.31 % w/w in curcumin-phospholipid complex gel.

In-vitro skin permeation study

Calibration curve of curcumin in phosphate buffer was prepared by plotting absorbance vs concentration of standard solutions of curcumin and the linear regression equation was derived as $Y = 0.1382x$ with a correlation coefficient of 0.9937 as shown in figure 4..

Skin Permeation Study

Permeation of curcumin and curcumin-phospholipid complex gel through rat skin was studied using franz diffusion cell. The results were shown in table 1 and figure 5. As evident from the results of skin permeation study, the curcumin-phospholipid complex gel showed significantly greater permeation ($p < 0.01$) of curcumin as compared to curcumin gel. At the end of 8 hrs, cumulative release of curcumin from curcumin gel was 18.73% as compared to 29.94 % from curcumin-phospholipid complex gel, which was 60% higher than that from the plain curcumin gel.

Table:1 In-vitro skin permeation study

Time (hr)	Curcumin Gel % Cumulative release*	Curcumin-phospholipid Complex gel
1	8.85±0.28	14.28±0.68
2	9.65±0.53	16.56±0.64
3	10.95± 0.69	19.47±0.87
4	12.26±0.78	21.59±0.87
5	13.55±0.93	24.26±0.93
6	15.66±0.93	25.59±0.84
7	17.31±0.46	27.68±2.00
8	18.73±0.40	29.94±2.10

* Mean ± S.E.M (n=2).

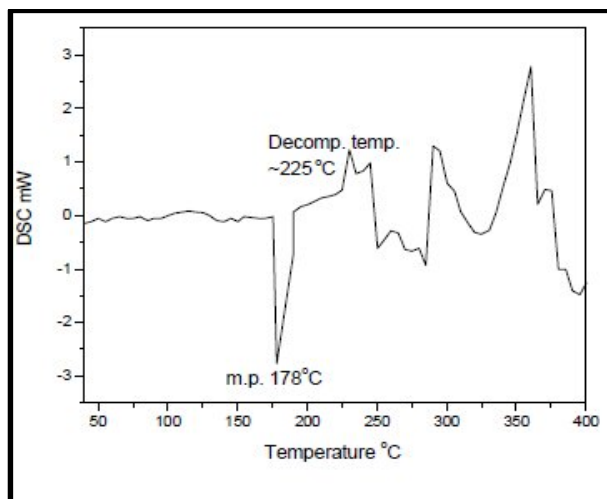


Fig.1 (a) DSC thermogram of Curcumin

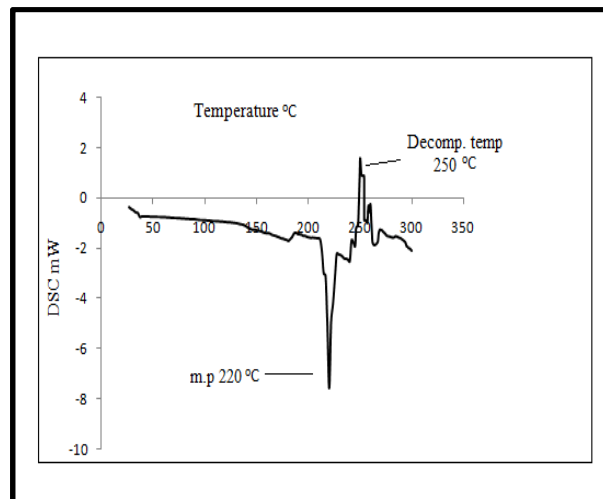


Fig. 1(b) DSC thermogram of Phospholipid

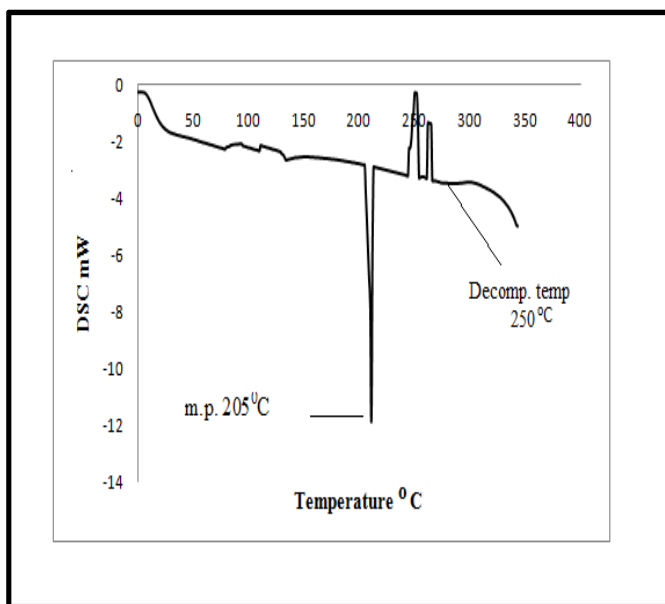


Fig.1 (c) DSC thermogram of Physical Curcumin-Phospholipid

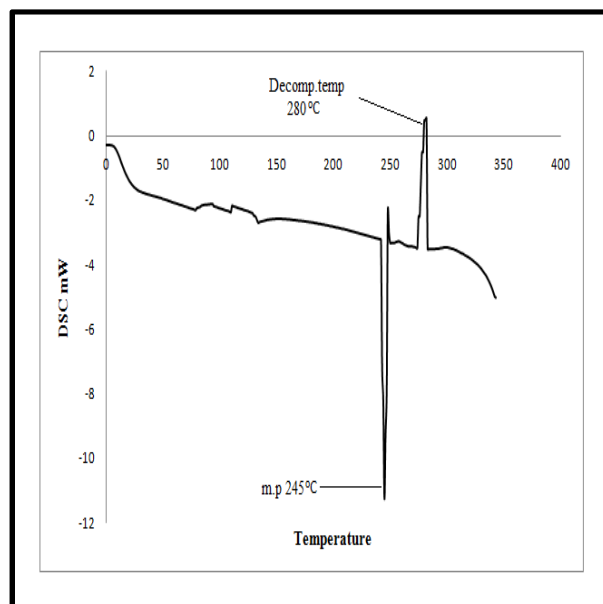


Fig.1 (d) DSC thermogram of mixture of Curcumin-Phospholipid complex

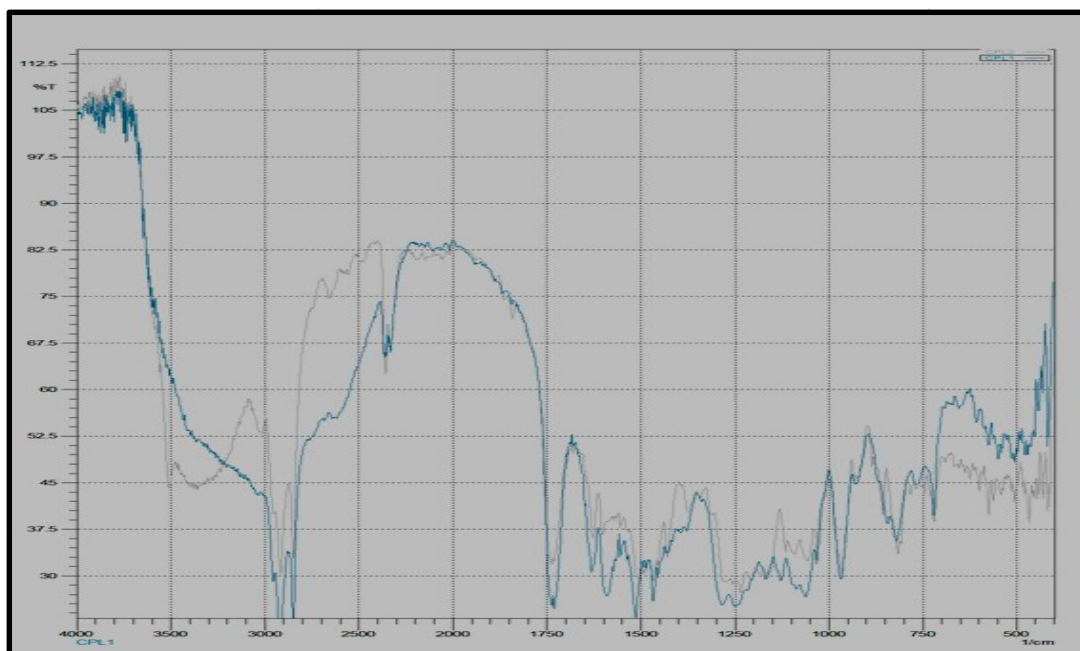


Fig.2. Overlay spectra of curcumin-phospholipid Physical Mixture(Gray) and curcumin-phospholipid complex(Blue)

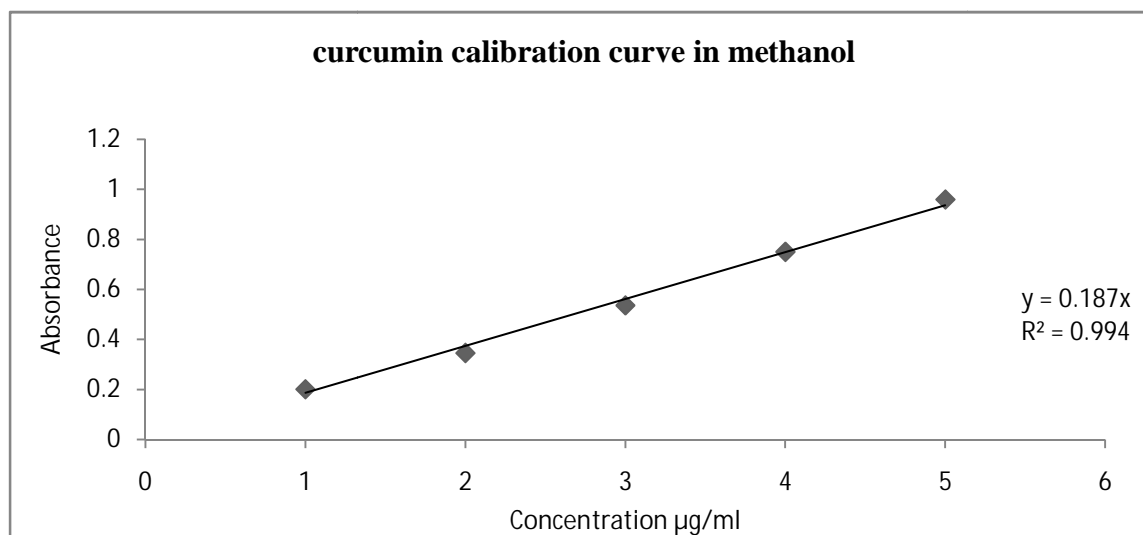


Fig.3. calibration curve of curcumin in methanol

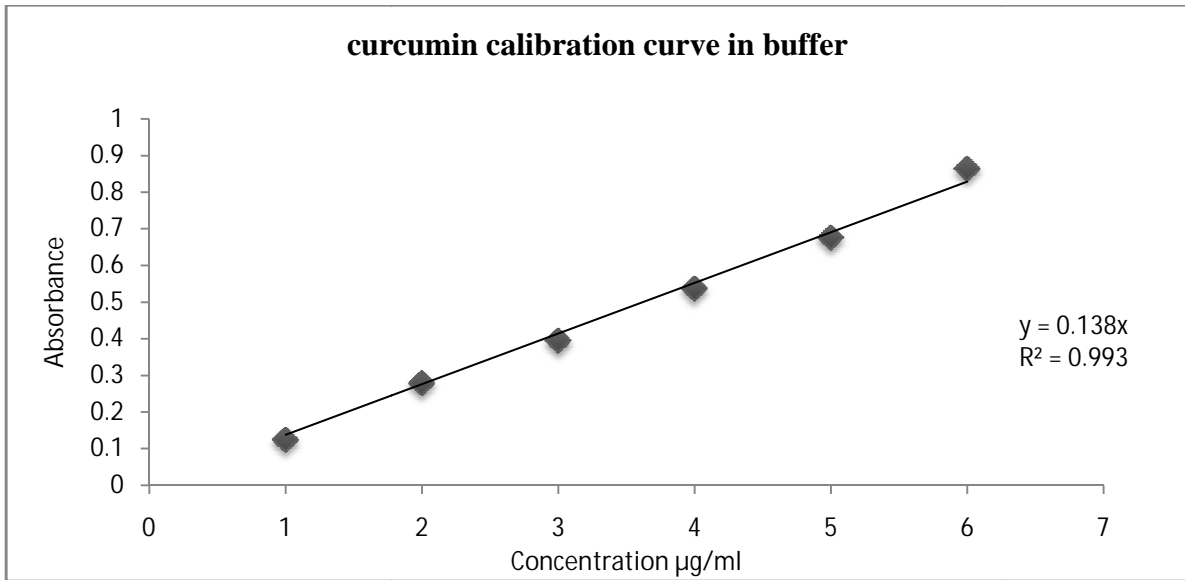


Fig. 4. Calibration curve of curcumin in phosphate buffer pH 7.4

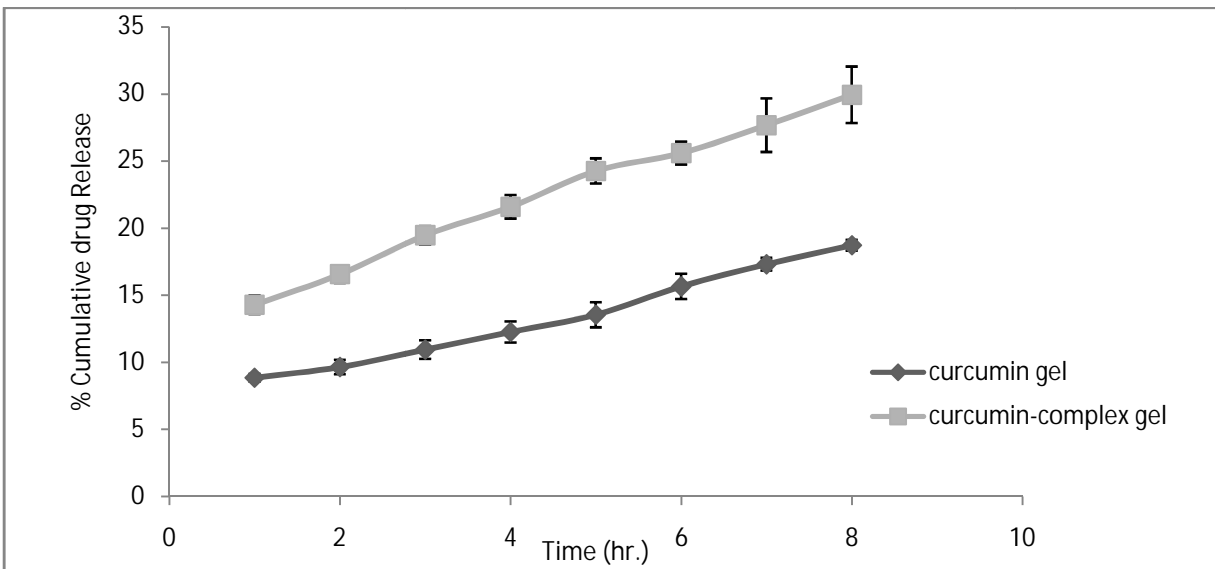


Fig.5. Curcumin and curcumin-phospholipid complex gel drug release profile

DISCUSSION

There is considerable interest in the skin as a site of drug application both for local and systemic effect. However, the skin, in particular the stratum corneum, possesses a formidable barrier to drug penetration thereby limiting topical and transdermal bioavailability. Skin penetration enhancement techniques have been developed to improve bioavailability and increase the range of drugs for which topical and transdermal delivery is a viable option².

Transdermal delivery of drugs through the skin to the systemic circulation provides a convenient route of administration for a variety of clinical indications. The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetration across the skin which is associated primarily with the outermost stratum corneum layer of the epidermis³.

Development of valuable drug delivery system from natural resources is very much necessary because of the beneficial role of herbal drug in the management of varied diseases^{7,8}. Continuing research is very much essential to explore the therapeutic efficacy of the natural molecules as well as to develop proper delivery system for enhancing the therapeutic potential of those molecules⁹.

In recent years, it has been reported that some poorly soluble drugs combined with phospholipids could result in an increase of oral bioavailability and/or improvement of the biological effects, such as silybin, curcumin and puerarin^{4,10,11}. However, detailed information about the preparation of these complexes is limited. Peng et al used a central composite design approach for the optimization of ursodeoxycholic acid-phospholipid complex (UDCA). The yield (%) of UDCA present as a complex was the unique evaluation index for the preparation¹².

In the literature, researchers have demonstrated that phospholipid complexes are not new chemical compounds or simple physical mixtures. Drugs and phospholipids should have some interactions, such as hydrogen bonding or Vander Waals interactions, when they form complexes^{10,13,14}. It was performed some experiments to prove this point. In the structure of curcumin and the phospholipids, there were no chemical groups that could react with each other under our preparation conditions.

In the present experiment curcumin–phospholipid complex was prepared by a simple and reproducible method. Molar ratio of curcumin and phospholipid in the complex was 1:2 respectively. Prepared curcumin-phospholipid complex was evaluated using DSC and IR spectroscopy. It was confirmed by DSC thermograms that there was formation of curcumin-phospholipid complex. IR spectra also revealed that there was involvement of –OH group in formation of the curcumin-phospholipid complex. Gel formulations were prepared for curcumin and curcumin-phospholipid complex using Carbopol 934 as gelling agent. *In-vitro* skin penetration study of prepared gel formulations were performed using rat skin on a Franz diffusion cell. The released curcumin from prepared formulations were measured using UV-Visible spectrophotometry method. Released pattern of curcumin from the curcumin-phospholipid complex gel had shown more than 60% than that from curcumin gel, after 8 hrs. So, it was concluded that complexation of curcumin with phospholipid enhances transdermal penetration of curcumin. From the present study concluded that complexation of curcumin with phospholipid resulted in increased transdermal penetration of curcumin.

Preparation of complexes with phospholipid is one of the techniques of enhancement of transdermal penetration. Curcumin is an important lead molecule with good anti-inflammatory activity but main problem is its poor bioavailability. Drug-phospholipid complex increases bioavailability of drug. So, it was decided to prepare curcumin-phospholipid complex. Curcumin-phospholipid complex was prepared as the reported method and evaluated using of DSC (Differential Scanning Calorimetry) and IR (Infrared spectroscopy). DSC thermograms of physical mixture of curcumin-phospholipid and curcumin-phospholipid complex confirmed formation of curcumin-phospholipid complex. IR spectra also revealed the formation of curcumin-phospholipid complex and involvement of the –OH group in formation of complex. Gel formulations were prepared for curcumin and curcumin-phospholipid complex using Carbopol 934 as gelling agent. *In-vitro* skin penetration study of prepared gel formulations were performed using rat skin on a Franz diffusion cell. The curcumin released from both the gels was measured using UV-Visible spectrophotometric method and % cumulative curcumin release was calculated. Pattern of curcumin released in plain curcumin gel and curcumin-phospholipid complex gel had shown 60% more release of curcumin in curcumin-phospholipid complex gel than that from curcumin gel, after 8 hrs.

CONCLUSION

It is concluded that complexation of curcumin with phospholipid enhances transdermal penetration of curcumin. Therefore, complexation of curcumin with phospholipid resulted in increased transdermal penetration of curcumin.

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