

ENHANCED TRANSDERMAL DRUG PENETRATION OF CURCUMIN VIA ETHOSOMES

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The objective of the present research work is to overcome the barriers of poor oral bioavailability through transdermal drug delivery (TDD). Among the available transdermal systems, ethosomes are the promising ones with increased penetration effect due to the combination of lipid bilayer and ethanol in its structure. Curcumin has poor oral bioavailability and can be given as transdermal ethosomes to enhance bioavailability along with local action of antiinflammation. Curcumin ethosomes were prepared and optimised for lipid and ethanol concentrations based on entrapment efficiency, vesicular size and drug penetration. Drug penetration capability of the ethosomes was compared with aqueous, ethanolic and liposomal solutions. The optimised ethosomes were compared for the cumulative drug penetration (74.2±0.236%) into skin with aqueous (5.61±0.263%), ethanolic (62.31±0.263%), liposomal (59.3±0.44%) and ethosomal curcumin-β-cyclodextrin complex (78.01±0.22). It was found that the penetration was enhanced and maximum in ethosomes incorporated with curcumin-β-cyclodextrin complex because of combined effects of ethanol, lipid bilayer along with increased dissolution of curcumin with β-cyclodextrin complex. This formulation will be suitable to treat local skin inflammations with enhanced penetration via ethosomes incorporated with curcumin-β-cyclodextrin complex.

Keywords: Curcumin, Ethosomes, Transdermal drug delivery, Enhanced penetration

INTRODUCTION

Transdermal drug delivery (TDD) is an effective means of drug delivery for local as well as systemic action. It avoids first pass metabolism and fluctuations in plasma drug concentration (Vaibhav *et al.* 2007). It also has the general advantages of being non-invasive and increasing patient compliance. In spite of several advantages, only few molecules are able to permeate through skin mainly because of stratum corneum (a thick and tough lipid layer) which acts as a barrier. To enhance TDD through skin, various approaches were proposed and they include both physical and chemical methods (Jhong, Zhai and Maibach 2007; Barry 2001). Vesicular systems are one among such approaches. In recent years, research on TDD vesicular systems is focussed on ethosomes, which are modified liposomes. Ethosomes are the vesicular systems which contain lipid bilayer just like conventional vesicles but they contain ethanol in higher concentration in place of cholesterol (Manish, Lifeng and Sui 2011). Ethanol is a well known skin penetration enhancer. It increases the drug penetration into the skin by reduction in the barrier

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property of stratum corneum (Magnusson *et al.* 1997). Ethosomes are soft, malleable vesicles which present an ample chance to deliver the drugs in to the deeper layers of the skin than the conventional vesicles (Vaibhav *et al.* 2007).

Most of common inflammatory skin disorders desire that the drug should be delivered to the deeper skin layers where inflammation occurs (Punith *et al.* 2012). From the herbal hub of India, curcumin extracted from *Curcuma longa* is used for the variety of skin inflammatory conditions including cancer and wound healing effects. It was reported in the literature that curcumin is poorly absorbed from intestine and undergoes metabolism very quickly to different metabolites through reduction and conjugation (Radha *et al.* 2006). Poor absorption, quick metabolism and less solubility in water are the main reasons for the low bioavailability of curcumin (Aggarwal and Sung 2008, Mukherjee *et al.* 2007). To overcome these problems, our investigation focuses on formulation and optimisation of curcumin ethosomes for transdermal delivery.

METHODS

Materials

Curcumin was from S.D. Fine Chemicals (Mumbai). Soya lecithin was a kind gift sample from Dr. Reddy's Laboratories (Bachupally, Hyderabad). Ethanol, Span 20, 60 and 80 were of analytical reagent grade from S.D. Fine Chemicals (Mumbai). All other reagents used were of analytical grade.

Preparation of Ethosomes and Liposomes

Ethosomes were prepared with the concentrations of soya lecithin (from 1% to 4%; w/v) and ethanol (from 25% to 45%; v/v); the formulations (soya lecithin, ethanol) F1 (1, 25), F2 (1, 35), F3 (1, 45), F4 (2, 25), F5 (2, 35), F6 (2, 45), F7 (3, 25), F8 (3, 35), F9 (3, 45), F10 (4, 25), F11 (4, 35) and F12 (4, 45). For the preparation of ethosomes, soya lecithin and curcumin (100 mg) were first dissolved in ethanol. Aqueous phase of water (volume up to 100 mL) was slowly added under continuous stirring at 700 rpm using a mechanical stirrer (Remi Motors, Mumbai) for 5 min at room temperature (Elisabetta, Enea and Rita 2004). For the preparation of liposomes, soya lecithin at 3% (w/v) and curcumin (100 mg) were added to a mixture of ethanol (90.3%; v/v), methanol (5.1%; v/v) and isopropanol (4.6%; v/v). The mixture was injected 5 times the volume of aqueous phase of water at continuous stirring at 700 rpm for 5 min at room temperature.

Curcumin- β -cyclodextrin inclusion complex was prepared in 1:1 ratio by solvent evaporation method by using ethanol as solvent for curcumin and water as solvent for β -cyclodextrin (Sang *et al.* 2012). The inclusion complex (100 mg curcumin equivalent) was incorporated into optimised ethosomal formulation in place of curcumin.

Vesicular Size Analysis and Entrapment Efficiency

To understand the effect of lipid and ethanol concentrations on vesicular size, the prepared ethosomes and liposomes are studied for vesicular size by using electron microscope attached with digital camera (Lafco, Hyderabad with Sony DSC-W520). For entrapment efficiency, 10 mL of formulation was taken and centrifuged. Sediment and

supernatant was separated. To the sediment, ethanol was added to make up the volume to 5 mL and the amount of curcumin was estimated by UV spectrophotometer (Elico SL 159, Mumbai) at 426 nm.

Drug Penetration Studies Through Skin

Drug penetration studies were conducted using pig ear skin from local slaughter house. Ten mL formulation was placed in donor compartment by using fabricated Franz diffusion cell. Fifty mL phosphate buffer (pH 6.4) was placed in receptor compartment. The experiment was carried out for 24 hours. Five mL sample was withdrawn at predetermined intervals and the same volume was replaced with phosphate buffer. Samples were estimated by UV spectrophotometer for curcumin content at 426 nm.

RESULTS

Drug Entrapment Efficiency and Vesicle Size

With the increased concentration of ethanol there was a gradual increase in the entrapment efficiency from 31.1 ± 0.23 (at 25%; v/v) to 68.1 ± 1.89 (at 45%; v/v). The values of both vesicle size and entrapment efficiency were represented in Figure 1. Higher entrapment efficiency was observed with the increase in the lecithin concentration from 1% to 4% (w/v). At 1%, 2%, 3% and 4% (w/v) of lecithin, the entrapment efficiencies were found to be 34.5 ± 3.2 , 40.14 ± 0.73 , 72.94 ± 3.61 and 63.65 ± 1.89 , respectively. At optimum concentration of lipid (3%), the increase in entrapment efficiency was from 55.49 ± 2.79 (at 25%) to 73.65 ± 1.89 (at 45%). In case of effect of lecithin at optimum concentration of ethanol (at 45%), the entrapment efficiency was increased up to 73.65 ± 1.89 from 34.57 ± 3.2 .

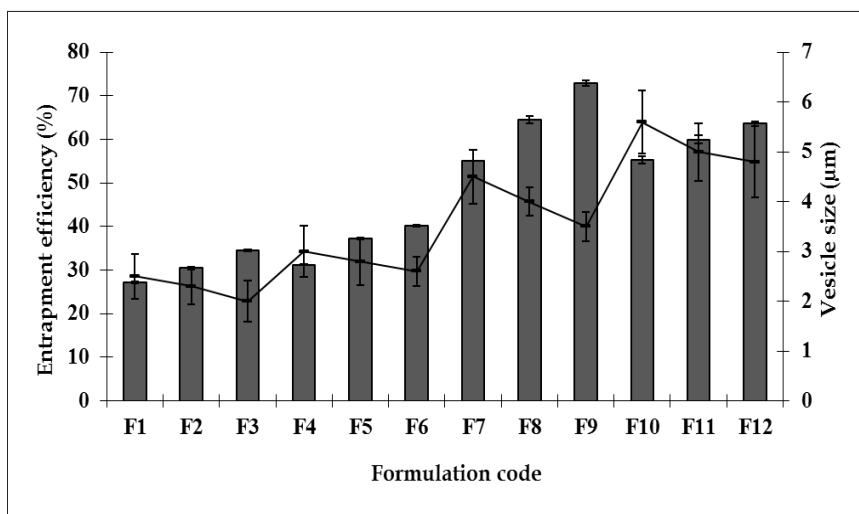


Fig. 1: Entrapment efficiency (bar) and vesicle size (line) changes with different ratios of lecithin and ethanol.

The vesicle size of ethosomes was found to be between 2.0 ± 1.27 to 5.6 ± 1.91 μm . The microscopic and electron photographs of the vesicles were represented in Figures 2 and 3.

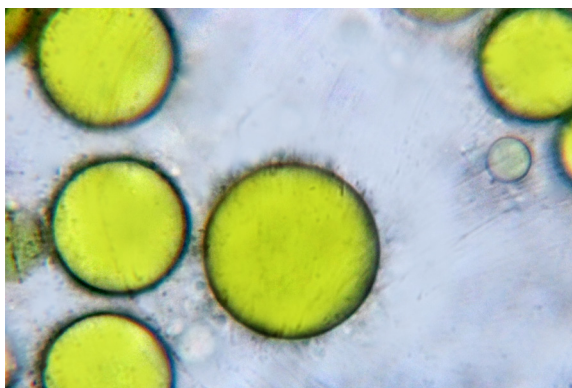


Fig. 2: Microscopic pictures of curcumin ethosomes (45X10 magnification).

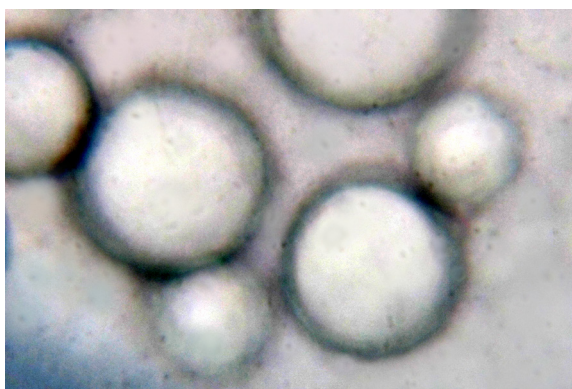


Fig. 3: Electron photograph of the curcumin ethosomes (45X10 magnification).

Drug Penetration Study Through Skin

Drug penetration studies were conducted for ethosomes by varying ethanol and lecithin ratios (Figs. 4 and 5). The maximum amounts of drug permeated at hour 24 were 62.5 ± 2.38 , 68.5 ± 3.12 and 74.2 ± 3.61 at 3% soya lecithin and 25%, 35% and 45% (v/v) ethanol, respectively.

There was good enhancement in drug diffusion into the skin with increased amount of lecithin in ethosomes. It was found that the percentage of drug permeated through skin at 1%, 2%, 3% and 4% of lecithin concentrations were 41.5 ± 0.998 , 53.0 ± 1.080 , 74.2 ± 0.236 and 68.1 ± 0.413 , respectively.

Transdermal flux (given in Table 1) is a crucial parameter for calculating the penetration of drug. By increasing the ethanol volume and the phospholipid concentration up to 3%, flux value was increased. Further increase in the lecithin concentration decreases the flux due to increase in the thickness of phospholipid double layer that retards the release of ethanol from vesicle, i.e. F9 shows highest flux (64.352 ± 0.31).

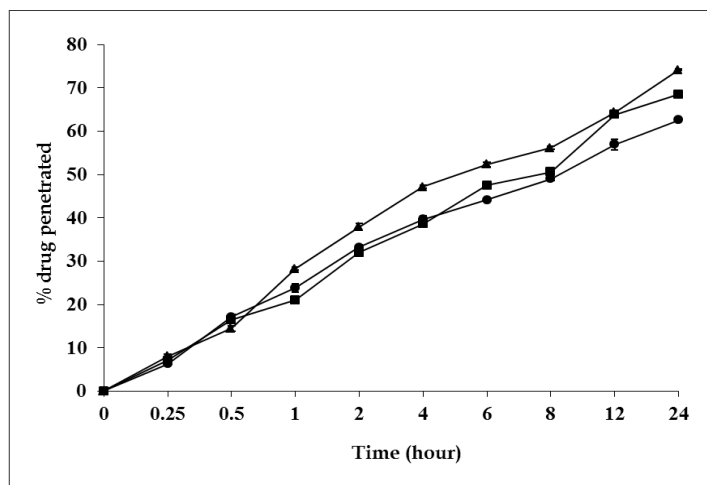


Fig. 4: Percentage of drug penetrated into skin from ethosomes with 3% lecithin and varying concentrations of ethanol at 25% (●), 35% (■) and 45% (▲) [v/v].

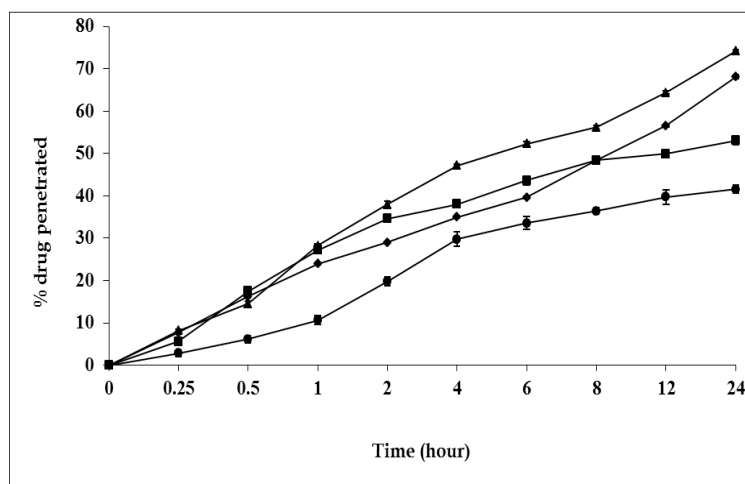
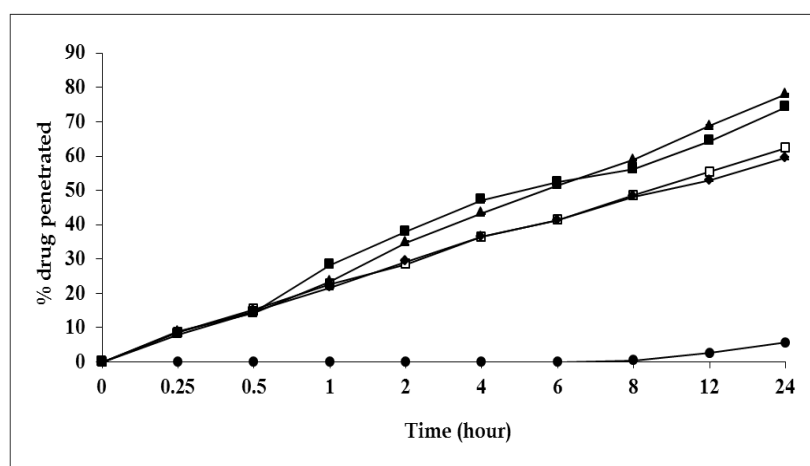


Fig. 5: Percentage of drug penetration of ethosomes through skin with varying lecithin concentrations at 1% (●), 2% (■), 3% (◆) and 4% (▲) [w/v] at 45% [v/v] of ethanol.

The percentage of drug penetrated from various systems was represented in Figure 6. After 24 hours, aqueous solutions of curcumin achieved a maximum penetration of drug of $5.61 \pm 0.263\%$. The penetration of curcumin, when given as ethanolic solution was $62.31 \pm 0.263\%$. Drug penetration with liposomes of curcumin was $59.3 \pm 0.44\%$. There was a slight enhancement in drug penetration with vesicles. As ethosomes, the penetration of curcumin in skin was $74.2 \pm 0.236\%$. The ethosomes prepared by enclosing cyclodextrin inclusion complex was found to have a drug diffusion of $78.01 \pm 0.22\%$ indicating the role of both dissolution and penetration in drug diffusion through skin.

Table 1: Transdermal flux values for the prepared ethosomes.

Formulations (soya lecithin %, ethanol %)	Transdermal flux ($\mu\text{g/hr/cm}^2$)
F1 (1, 25)	10.952 \pm 2.05
F2 (1, 35)	15.408 \pm 0.26
F3 (1, 45)	18.764 \pm 0.34
F4 (2, 25)	17.805 \pm 0.65
F5 (2, 35)	21.626 \pm 0.17
F6 (2, 45)	23.762 \pm 0.60
F7 (3, 25)	40.742 \pm 0.77
F8 (3, 35)	54.743 \pm 0.07
F9 (3, 45) [selected ethosomes]	64.352 \pm 0.31
F10 (4, 25)	41.286 \pm 0.27
F11 (4, 35)	49.877 \pm 0.17
F12 (4, 45)	51.725 \pm 0.23

**Fig. 6:** Comparison of transdermal penetration of curcumin from various systems: aqueous (●), ethanolic (□), ethosomal (■), liposomal (◆) and ethosomal cyclodextrin complex (▲).

DISCUSSION

Entrapment is expected to increase with the increase in the amount of lecithin. Generally hydrophilic drugs are entrapped in the aqueous core inside lipid layer while the lipophilic drugs are retained in lipid lamella. Encapsulation of a hydrophilic drug depends on the captured aqueous volume and that of lipophilic drug depends on the number of bilayers of lipid (Manish *et al.* 2011). Curcumin is a lipophilic moiety (Mukherjee *et al.* 2007). Being

lipophilic in nature curcumin's entrapment efficiency was more dependent on the lipid concentration than that of the ethanol. At optimum concentration of lipid (3%), the increase in entrapment efficiency with increased ethanol concentration was less. In case of effect of lecithin at optimum concentration of ethanol (at 45%) the entrapment efficiency was increased significantly. This indicates the higher influence of lipid concentration on entrapment efficiency of lipophilic curcumin than ethanol. At 4% of lipid the increase in entrapment efficiency was decreased. This may be due to saturation effect on increase in solubility of curcumin in lipid.

Ethosomes are spherical vesicles with lamella extending throughout the entire volume of vesicles to the core (Godin and Touitou 2004). This multi lamellarity of ethosomal vesicles along with the ethanol allows the better solubility of both lipophilic and hydrophilic molecules (Dayan and Touitou 2000). This might be the reason for the increased entrapment efficiency of ethosomes when ethanol concentration was increased. According to literature, ethosomal systems are generally composed at 20%–45% of ethanol concentration. If ethanol concentration above 50% (v/v) was used, that may lead to the excess increase in the fluidity so that the entrapment efficiency decreases due to leakage (Zhang *et al.* 2012).

Both lecithin and ethanol have significant effect on vesicle size. Increased lecithin concentration was found to increase the vesicle size. At 1% the vesicle size was around 2 μm and at 4% the size was 5 μm . This may be because of the lipid leading the formation of vesicle wall (Vaibhav *et al.* 2007; Barry 2001). The increased concentration of the lipid in solution might have caused the deposition of multimolecular layer of the surfactant at the vesicle wall causing increased thickness of wall and ultimately the vesicle size.

Ethanol at increased concentration was found to have decreasing effect on size. This may be due to solubilisation of lipids in ethanol or by the formation of hydrocarbon phase with interpenetrating properties (Rahul *et al.* 2012). Because of interpenetration, the size decreases. Another fact behind the decreased size is due to modification of the surface charge of ethanol that confers some degree of stabilisation and thus, it may finally decrease the mean vesicle size (Elsayed *et al.* 2007).

There is a clear evidence of increased drug diffusion into the skin with increased concentration of ethanol. There is increased diffusion with decreased vesicle size (Zhang *et al.* 2012). This is because of the increased surface area available for diffusion. In this study, the ethosomes size decreased with increased concentrations of ethanol (25% to 45%; v/v). It is assumed that when the size is around 100 nm they can penetrate into the skin by nanoporus pathway present in stratum corneum (Cevc and Richardson 2002). As the size of ethosomes becomes larger, this may not be possible. Ethanol was found to increase the fluidity of the lipids in skin and hence can be easily absorbed into the skin (Cevc 2004). Ethanol also increases the fluidity of lipids of the vesicle, making them soft and less rigid than liposomes. Being soft and flexible, the ethosomes can penetrate easily in to the deeper layers of skin. Ethanol being an aliphatic chain, disrupts the stratum corneum of skin by extracting the lipid (Bommaman, Potts and Guy 1991).

There is enhanced transdermal drug diffusion due to the presence of lipid bilayer in the vesicle structure, which is similar to the structure of skin. Lipids can also penetrate into the skin by follicular transport pathway (Dayan and Touitou 2000) and hence there is an enhanced diffusion with increased concentration of the lecithin in ethosomes.

Aqueous solutions of curcumin were found to achieve a negligible penetration of drug after 24 hours. This may be because of poor water solubility (Aggarwal and Sung 2008). When given as ethanolic solution, the penetration of curcumin was increased

indicating the ability of the ethanol to increase drug penetration into the skin by various mechanisms (Cevc 2004). Liposomes of curcumin were found to have a drug penetration near to that of ethanolic solution. This may be because of the phospholipid bilayer of the vesicle which increases the penetration ability of curcumin. As ethosomes the penetration of curcumin in skin was almost doubled and this indicates the synergistic effects of ethanol and vesicular system for the enhanced delivery (Cevc 2004).

Topical skin diseases like psoriasis, inflammations and deep fungal infections require the delivery of the drug into the deeper layers of the skin (Bhalaria, Sachin and Misra 2009; Yi *et al.* 2009). Ethosomes loaded with curcumin- β -cyclodextrin inclusion complex results in increased dissolution of the curcumin complex and can be used to enhance drug penetration.

CONCLUSION

In our present investigation, the prepared ethosomes with smaller vesicle size and maximum drug penetration which makes it suitable delivery system for curcumin to treat inflammatory diseases of skin. In inflammatory conditions there is a need to deliver the drug to deeper layers. This is possible with ethosomes which release drug at various points along the penetration pathway.

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