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RESEARCH PAPER

Enhanced Percutaneous Permeability of Nicardipine Hydrochloride by Carvone Across the Rat Abdominal Skin

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ABSTRACT

The purpose of this study was to investigate the effect of carvone on the permeation of nicardipine hydrochloride across the excised rat abdominal epidermis from 2% w/w hydroxypropyl cellulose (HPC) gel system. The HPC gel formulations containing nicardipine hydrochloride (1% w/w) and selected concentrations of carvone (0 to 12% w/w) were prepared, and evaluated for drug content, stability of the drug, and in vitro permeation of the drug through excised rat abdominal epidermis. The HPC gel was found to contain 99.98 to 101.6% of nicardipine hydrochloride, and the drug was found to be stable in the HPC gels. The permeation flux of nicardipine hydrochloride across rat epidermis was increased markedly by the addition of carvone to the HPC gels. A maximum flux of nicardipine hydrochloride ($243.95.70 \pm 1.90\ \mu g/cm^2/hr$) was observed with an enhancement ratio of 7.9 when carvone was incorporated at a concentration of 12% w/w in the HPC reservoir system. The differential scanning calorimetry and Fourier transform-infrared data indicated that carvone increased the permeability of nicardipine hydrochloride across the rat epidermis by partial extraction of lipids in the stratum corneum. The results suggest that carvone may be useful for enhancing the skin permeability of nicardipine hydrochloride from transdermal therapeutic system containing HPC gel as a reservoir.

Key Words: Nicardipine hydrochloride; HPC gels; Rat abdominal skin; In vitro transdermal permeability; Carvone; FT-IR; DSC.

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INTRODUCTION

Nicardipine hydrochloride, a potent calcium channel antagonist, is used in the treatment of angina pectoris and hypertension.[1] The terminal half-life of nicardipine hydrochloride after single dosage in human subjects is between 2 and 4 hr. After oral administration, nicardipine hydrochloride undergoes extensive first-pass elimination, and inter- and intrasubject variability of plasma concentration is observed. Because of the first-pass elimination, oral bioavailability[2] of nicardipine hydrochloride in human subjects has been reported to be as low as 30–35%. Thus, the conventional therapy may result in higher fluctuations in plasma concentration of the drug resulting in unwanted side effects. Hence, the development of a transdermal therapeutic system for nicardipine hydrochloride that provides a predetermined constant drug delivery would be beneficial for an effective and safe therapy of hypertension. In a recent study, it was reported from our laboratory[3] that ethanol and water solvent system in the ratio of 70:30 v/v was a suitable vehicle for the transdermal delivery of nicardipine hydrochloride. However, it was necessary to improve the permeation rate of nicardipine hydrochloride by using a suitable penetration enhancer.

The greatest obstacle in the transdermal drug delivery is stratum corneum, because it provides a rate-limiting step for drug absorption.[4] It is composed of dead, flattened cells filled with keratin in the form of regular array of protein-rich cells embedded in an intercellular and multicellular lipid domain running parallel to the skin.[5] Many studies showed that lipoid domain, the integral component of the transport barrier, must be breached if the drug is to be delivered transdermally at an appropriate rate.[6,7] Several enhancement techniques have been developed to overcome the impervious nature of the stratum corneum.[8–14] A popular technique is the use of chemical penetration enhancers, which alters reversibly the permeability barrier of the stratum corneum.[15] In this context, azone has been reported to enhance the permeation of nicardipine hydrochloride through the skin.[16] A pronounced promoting effect of cyclic monoterpenes on the percutaneous absorption of nicardipine hydrochloride has been reported.[17–19] These cyclic monoterpenes are of low cutaneous irritancy and therefore considered as good candidates for improving the skin penetration of drug. A variety of terpenes have been shown to increase the percutaneous absorption of hydrophilic[20–27] and lipophilic drugs.[20,28–31]

In the present study, the enhancing effect of a naturally occuring terpene, carvone, on the in vitro percutaneous absorption of nicardipine hydrochloride from the 2% w/w of hydroxypropyl cellulose (HPC) containing 70:30 v/v of ethanol and water across the excised rat abdominal epidermis were investigated. Ethanol, one of the most widely used vehicles, has been found to affect skin permeability.[32] Ethanol, used as a part of cosolvent system with water, has been demonstrated to increase the permeability of variety of drugs through the skin barrier.[33–35] Carvone, a cyclic terpene ($\log P = 2.23 \pm 0.25$), is free from toxic effects and has been used as a penetration enhancer in the transdermal delivery of several drugs.[7,36] A patent exists for the use of carvone as penetration enhancer.[37] Thus, the specific goal of the study is to investigate the usefulness of carvone as a penetration enhancer on the transdermal permeability of nicardipine hydrochloride such that the required flux of the drug could be provided from the HPC reservoir of the transdermal therapeutic system. The amount of carvone needed to provide the required flux of nicardipine hydrochloride through rat skin from HPC gels would be utilized in developing a membrane-moderated transdermal therapeutic system.

MATERIALS AND METHODS

Materials

Nicardipine hydrochloride and carvone were obtained from ICN Biomedicals (USA) and Merck-Schuchardt (Hohenbrunn, Germany), respectively. Hydroxy propyl cellulose was a gift sample from Dow Chemical Company (Michigan, USA) and was of pharmacopeial quality. Acetonitrile [high-performance liquid chromatography (HPLC) grade] was obtained from Qualigens Fine Chemicals (Mumbai, India). Triple-distilled water was used. Ethanol (analytical grade) and potassium dihydrogen phosphates (analytical grade) were obtained from Qualigens Fine Chemicals.

Solubility Studies

Excess nicardipine hydrochloride was added to 10 mL of 70% v/v of ethanol:water solvent system containing selected concentrations of limonene (1% w/w, 2% w/w, 4%w/w, 8% w/w, or 12% w/w) and vortexed. The test tubes containing the mixture

was immersed in a water bath at 37°C and allowed to equilibrate with intermittent shaking. The samples (0.5 mL) were obtained as function of time (12, 24, and 36 hr) and filtered through a 0.4-μm membrane filter; the filtrate was suitably diluted, and the concentration of nicardipine hydrochloride was estimated by HPLC method.

HPLC Analysis of Nicardipine Hydrochloride

The quantitative determination of nicardipine hydrochloride was performed by HPLC. A gradient HPLC (Shimadzu HPLC Class VP series; Shimadzu, Kyoto, Japan) with two LC-10AT VP pumps, variable wavelength programmable UV/VIS Detector SPD-10A VP, CTO-10AS VP Column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC_{18} (Pelliguard^{TM}, LC_{18}, 2 cm; Supelco, Inc., Bellefonte, PA) and RP C_{18} column (150 mm × 4.6 mm i.d., particle size 5 μm; Flexit, Inc., Pune, India) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)."

The mobile phase used was a mixture of acetonitrile and 0.02 M KH_2PO_4 in the ratio of 60:40. The mobile phase components were filtered (0.45-μm PTFE membrane) and pumped at a flow rate of 1 mL/min. The column temperature was maintained at 40°C. The eluent was detected by UV detector at 239 nm and the data were acquired, stored, and analyzed with the software "Class-VP series version 5.03 (Shimadzu)." A standard curve was constructed for nicardipine hydrochloride in the range of 0.01–2 μg/mL. A good linear relationship was observed between the concentration of nicardipine hydrochloride and the peak area of nicardipine hydrochloride with a high correlation coefficient ($r = 0.9999$). The required studies were carried out to estimate the precision and accuracy of this HPLC method of analysis of nicardipine hydrochloride. The standard curve constructed as described above was used for estimating nicardipine hydrochloride in the skin permeates, HPC gel formulations, and drug content in the rat abdominal skin after 24 hr of study.

Preparation of HPC Gels

The composition of HPC gel formulation is shown in Table 1. To prepare 2% w/w of HPC gel, HPC powder was added to 70% v/v of ethanol while being stirred with a stirrer (Remi Motors, Mumbai, India) at 2,500 rpm, and the resulting mixture was mixed continuously at 37°C for about 1 hr until the formation of gel. Nicardipine hydrochloride (1% w/w) and carvone (2% w/w, 4% w/w, 8% w/w, or 12% w/w) were added to HPC gel and mixed well for complete dissolution. The gel formulations were left overnight at ambient temperature.

Table 1. Composition of HPC gel formulation containing nicardipine hydrochloride and selected concentrations of carvone.

	Quantity present in gel formulation (% w/w)				
Ingredients	I	II	III	IV	V
Nicardipine HCl	1	1	1	1	1
Carvone	—	2	4	8	12
HPC	2	2	2	2	2
Ethanol 70% v/v q.s.[a]	100	100	100	100	100

[a]q.s. = quantity sufficient.

Quantitative Determination of Nicardipine Hydrochloride in HPC Gel Formulation

One gram of the drug reservoir (HPC gel formulation) was accurately weighed, placed in a 100-mL volumetric flask containing 30 mL of mobile phase, stirred for 30 min, and made up to volume. The resultant mixture was filtered through a 0.45-μm membrane filter and injected into the HPLC system. The amount of nicardipine hydrochloride was estimated using the standard curve as described above.

Preparation of Rat Abdominal Skin

Animals used for the preparation of skin were male albino rats (150–200 g) obtained from Ghosh Enterprises (Kolkata, India). They could have free access to food and water until used for the study. Care of the rats was in accordance with institutional guidelines. Rats were euthanized using carbon dioxide asphyxiation before the experiments. Dorsal hair was removed with clippers, and full-thickness skin was surgically removed from each rat. The epidermis was prepared by a heat separation technique,[21] which involved soaking the entire

abdominal skin in water at 60°C for 45 sec, followed by careful removal of the epidermis. The epidermis was washed with water and used for in vitro permeability studies.

In Vitro Transdermal Permeability Studies

Modified Keshary-Chien diffusion cells[19] were used in the in vitro transdermal permeation studies. The rat epidermis, prepared as described above, was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The effective diffusion area was 3.5 cm^2, and the volume of the receiver compartment was 24 mL. Three grams of HPC gel, without or with carvone (2% w/w to 12% w/w) containing 30 mg of nicardipine hydrochloride, were placed in the donor cell. Ethanol (70% v/v) was added to the receiver cell to maintain sink conditions. Cells were maintained at 37 ± 0.5°C by using a magnetic stirrer with heater (Remi Motors). The contents in the receiver compartment were stirred with the help of a magnetic bar rotating at 500 rpm. The permeate samples were withdrawn from the receiver compartments at different time intervals up to 24 hr, and an equivalent volume of drug-free solvent (vehicle) was added to the receiver compartment to maintain a constant volume. Samples were assayed for nicardipine hydrochloride by the HPLC method as described. After 24 hr of the study, the skin sample was removed from the cells and washed briefly in methanol (25 mL) for 15 sec[38,39] to remove the adhering HPC gel drug reservoir. After drying at room temperature for 10 min, the skin was cut into pieces and then homogenized in 4 mL of methanol. Samples were centrifuged, the supernatant layer was filtered through 0.2-μm membrane filter, and analyzed for drug content by the HPLC method.

Stability of nicardipine hydrochloride in the ethanol and water (70:30 v/v) solvent system, or in the HPC gel containing carvone (2% w/w, 5% w/w, 8% w/w, or 12% w/w) was assessed by the HPLC method. The chromatogram was observed for additional peaks, if any.

Preparation of Rat Stratum Corneum

The rat epidermis was incubated for 4 hr with 1% w/v trypsin solution in phosphate-buffered saline (pH 7.4) at 37°C. The tissue was then smoothed out on a flat surface, and the mushy epidermis was removed by rubbing with a moistened cotton-tipped applicator. The transparent stratum corneum so obtained was floated briefly on water, blotted dry, and used in the differential scanning calorimety (DSC)[40] and Fourier transform-infrared (FT-IR) studies.[41]

FT-IR Spectroscopy of Rat Stratum Corneum

A typical FT-IR spectrum of rat stratum corneum shows separate lipid and protein peaks. The study of lipid biophysics by observing the peaks caused by C–H stretching vibrations would be helpful in identifying the influence of the penetration enhancer (carvone) used in the study. The absorbances of stratum corneum lipids occur near 2,851 and 2,920 cm^{-1} for the symmetric and asymmetric C–H stretching vibrations, respectively. Changes in the amount of stratum corneum lipids have been correlated with C–H stretching absorbance intensity.

Stratum corneum lipid extraction leads to a decrease in the C–H stretching absorbance intensity. Evidence for the assignment of the C–H stretching peaks comes from the study by Casal and Mantsch.[42] In the present study, the rat stratum corneum was treated with selected concentrations of carvone (0% w/w, 2% w/w, 4% w/w, 8% w/w, or 12% w/w) in 70% v/v ethanol for 24 hr. The treated stratum corneum samples were vacuum-dried (650 mm of Hg) at 21 ± 1°C for 2 days and stored in a desiccator to remove traces of the solvent.[43] The completely dried samples of the stratum corneum were then subjected to FT-IR (Shimadzu, Japan) study. Attention was focused on characterizing the occurrence of peaks near 2,851 cm^{-1} and 2,920 cm^{-1} for symmetric and asymmetric C–H stretching absorbencies, respectively. FT-IR experiments were performed in triplicate.

Differential Scanning Calorimetric Study of Rat Stratum Corneum

The rat stratum corneum was treated with different concentrations of carvone (0% w/w, 2% w/w, 4% w/w, 8% w/w, or 12% w/w) in 70% v/v ethanol for 24 hr. The treated stratum corneum samples were vacuum-dried (650 mm Hg) at 21 ± 1°C for 2 days and stored in a desiccator to

remove traces of the solvent.[43] Changes in the structure of rat's stratum corneum were assessed by DSC 220C (Seiko Instruments, Inc., Tokyo, Japan) in terms of phase transition temperature of lipid components.[39] Samples were scanned at 1°C/min over the temperature range of 30–110°C.

Permeation Data Analysis and Statistics

Nicardipine hydrochloride concentration in the skin permeate samples was corrected for sampling effects according to the equation described by Hayton and Chen[44]:

$$C_n^1 = C_n(V_T/V_T - V_S)(C_{n-1}^1/C_{n-1})$$

where C_n^1 is the corrected concentration of the nth sample, C_n is the measured concentration of nicardipine hydrochloride in the nth sample, C_{n-1} is the measured concentration of the nicardipine hydrochloride in the $(n-1)$th sample, V_T is the total volume of the receiver fluid, and V_S is the volume of the sample drawn.

The flux (μg/cm^2/hr) of nicardipine hydrochloride was calculated from the slope of the plot of the cumulative amount of nicardipine hydrochloride permeated per cm^2 of skin at steady state against the time using linear regression analysis.[45,46] The steady-state permeability coefficient (k_p) of the drug through rat epidermis was calculated by using the following equation[47]:

$$k_p = \frac{J}{C}$$

where J is the flux and C is the initial concentration of nicardipine hydrochloride in the donor compartment. The penetration-enhancing effect of carvone was calculated in terms of enhancement ratio (ER) and was calculated by using the following equation[48]:

$$ER = \frac{k_p \text{ with penetration enhancer}}{k_p \text{ without penetration enhancer}}$$

Statistical comparisons were made using analysis of variance and Duncan's multiple range test with the help of the STATISTICA program (Release 4.5, StatSoft, Inc., 1993). A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In a recent study, it was reported from our laboratory[3] that ethanol and water solvent system in the ratio of 70:30 v/v was a suitable vehicle for the transdermal delivery of nicardipine hydrochloride. However, it was necessary to improve the permeation rate of nicardipine hydrochloride by using suitable penetration enhancers. Hence, a reservoir gel system with 2% w/w of HPC using 70:30 v/v of ethanol–water as a solvent system containing selected concentrations of menthol, was prepared and evaluated for in vitro skin permeation studies.[19] Results indicated that 8% w/w menthol provided maximum permeability of nicardipine hydrochloride across rat skin. Based on these studies, it was planned to study the effect of carvone, a monoterpene, on the permeability of nicardipine hydrochloride across the rat abdominal skin from the 2% w/w of the HPC gel system containing 70% v/v of ethanol as a solvent system. The HPC gel containing selected concentrations of carvone (2% w/w, 4% w/w, 8% w/w, or 12% w/w) was prepared and evaluated for drug content, stability of the drug, and in vitro skin permeability. The HPLC method used in the quantitative determination of nicardipine hydrochloride was found to be precise and accurate, as indicated by less than 2.5% of CV (inter- and intraday variation) and high recovery (99.98%). The HPC gel formulations were found to contain 99.98–101.6% of nicardipine hydrochloride showing the uniformity of drug content in gel formulation. Stability of nicardipine hydrochloride either in the ethanol–water (70:30 v/v) solvent system or in HPC gel containing carvone (0% w/w, 2% w/w, 4% w/w, 8% w/w, or 12% w/w) was assessed by the HPLC method. The HPLC chromatograms showed no additional peaks without a change in the retention time of nicardipine hydrochloride, indicating stability of the drug both in the solvent system (70% v/v ethanol) and HPC gel containing carvone.

Effect of Carvone on the In Vitro Permeability of Nicardipine Hydrochloride Across Rat Abdominal Skin

In the determination of transdermal permeability of nicardipine hydrochloride, the rat's abdominal skin was used as a skin model. Although human cadaver skin may be the logical choice, as a skin model for the final product to be used in humans, it

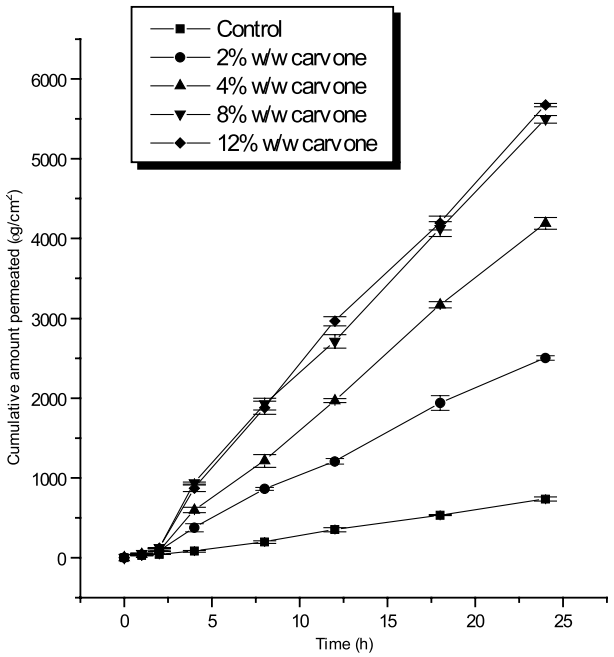

Figure 1. Mean (±SD) amount of nicardipine hydrochloride permeated from HPC gel containing selected concentrations of carvone across the excised rat abdominal epidermis.

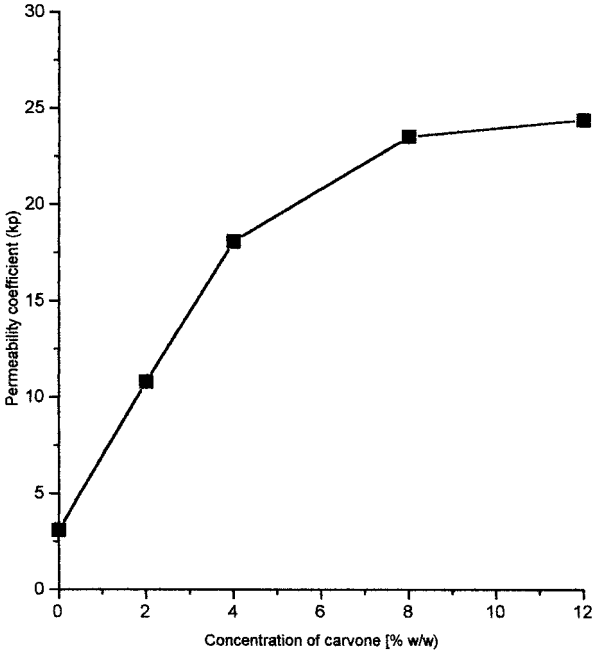

Figure 2. Effect of carvone concentration on the permeability coefficient (k_p) of nicardipine hydrochloride from HPC gel across the excised rat abdominal epidermis.

is not easily available for most of the investigators. Among the animal skins commonly used in the in vitro permeability studies are those obtained from the rat, pig, rabbit, guinea pig, mouse, or snake. Hence, in the present study, the rat's abdominal skin was used after removing the hair. Only the skin of male rats was used, because it was difficult to obtain the required full-length skin from female rats because of the presence of mammary glands.

The cumulative amount of drug permeated across the excised rat abdominal epidermis from the HPC gel containing selected concentrations of carvone is shown in Fig. 1. When data were analyzed, the amount of drug permeated fit for zero-order kinetics right from 3 to 24 hr (with a lag period of about 3 hr). The maximum amount of nicardipine hydrochloride that permeated during the 24 hr of study (Q_{24}) from the HPC gel system (without enhancer) was $734.14 \pm 24.46\,\mu g/cm^2$, and the corresponding flux of nicardipine hydrochloride was $30.91 \pm 0.77\,\mu g/cm^2/hr$. A marked effect of carvone on nicardipine hydrochloride permeation was observed when it was incorporated in HPC gel in varying quantities. The cumulative amount (Q_{24}) of nicardipine hydrochloride permeated over 24 hr was found increased, ranging from $2{,}501.10 \pm 27.37$ to $5{,}672.74 \pm 20.42\,\mu g/cm^2$ from the HPC gels containing 2% w/w to 12% w/w of carvone. The corresponding flux values were ranging from 107.90 ± 1.34 to $243.95 \pm 1.90\,\mu g/cm^2/hr$. However, there was no difference in the lag period when compared with those obtained from various concentrations of carvone. It may be observed from the results (Fig. 2) that there was a constant increase in the permeability coefficient (k_p) of the drug up to 8% w/w of carvone in HPC gel, and such increase in the flux was found to be significant ($p < 0.001$) when compared with control (without carvone). But, with the incorporation of 12% w/w of carvone, there was a slight increase ($p < 0.01$) in the permeability coefficient of nicardipine hydrochloride when compared with that of 8% w/w of carvone (Table 2). As carvone concentration increased from 0% w/w to 12% w/w, the permeability coefficient of nicardipine hydrochloride was found increased (Fig. 2), as indicated by an increase in both the permeability coefficient and enhancement ratio (Table 2). There was a 7.9-fold increase in the permeability of the drug from the HPC gel containing 12% w/w of carvone. There was a slight increase in the enhancement ratio

Table 2. Effect of carvone on the percutaneous parameters of nicardipine hydrochloride in HPC gels.

Carvone (% w/w)	Q_{24} ($\mu g/cm^2$)[a]	% Drug released	J ($\mu g/cm^2/hr$)[a]	k_p ($cm/hr \times 10^3$)[a]	ER[a]	DCS ($\mu g/g$)[a]	Solubility at 37°C (mg/mL)[a]
0	734.14 ± 24.45	8.56 ± 0.29	30.91 ± 0.77	3.09 ± 0.08	1	1,200.10 ± 201.56	224.21 ± 1.00
2	2,501.10 ± 27.37	29.18 ± 0.3	107.90 ± 1.34	10.79 ± 0.14	3.49 ± 0.08*	1,750.05 ± 195.89	225.01 ± 2.12
4	4,190.25 ± 73.40	48.89 ± 0.86	180.59 ± 3.09	18.06 ± 0.31	5.84 ± 0.11*	2,420.20 ± 198.21	224.98 ± 3.09
8	5,494.06 ± 48.15	64.09 ± 0.56	235.09 ± 2.24	23.51 ± 0.23	7.61 ± 0.13*	2,885 ± 128.65	223.10 ± 1.01
12	5,672.75 ± 20.42	66.10 ± 0.24	243.95 ± 1.90	24.40 ± 0.19	7.89 ± 0.17**	2,902.25 ± 201.65	224.10 ± 2.51

Q_{24}, cumulative amount of nicardipine hydrochloride after 24 hr; DCS, drug content in skin after 24 hr; ER, enhancement ratio of nicardipine hydrochloride.
[a]Mean ± SD ($n = 3$).
*Significant at $p < 0.001$ when compared with control.
**Significant at $p < 0.01$ when compared with 8% w/w carvone.

of the drug from the gel containing 12% w/w of carvone when compared with a gel containing 8% w/w of carvone. However, such an increase was statistically significant ($p < 0.01$). The results of the study indicate that, carvone at a concentration of more than 8% w/w in HPC gel, has shown a plateau effect (although such an increase in flux was statistically significant, $p < 0.01$) in the permeability of nicardipine hydrochloride across the rat abdominal skin.

The total drug used in the study was accounted for when the drug content in the skin, donor compartment, and receptor compartment was summed. The mean total recovery of nicardipine hydrochloride in various studies was 99.13 ± 2.09%, indicating a mass balance of the drug used in the study. It is interesting to note that carvone increased the skin content of nicardipine hydrochloride significantly ($p < 0.001$) in proportion to the concentration of carvone in the reservoir at the end of 24 hr of the in vitro permeability study when compared with that obtained from HPC gel without carvone (Table 2). With increased concentration of carvone in the HPC gel drug reservoir, there had been an increase in the skin content of the drug that might have resulted in the increased permeability of the drug. This might be because of the occupation of nicardipine hydrochloride in the partially delipidized stratum corneum of the rat's abdominal skin. The solubility studies (Table 2) showed that carvone did not increase the solubility of nicardipine hydrochloride, thus indicating that the penetration enhancer (carvone) did not increase the permeability by affecting the solubility of the drug.

It was reported that terpenes increase the drug percutaneous permeation mainly by disrupting the intercellular packing of the stratum corneum lipids.[25,30,48] Hence, DSC and FT-IR studies were carried out to confirm such a hypothesis on the observed penetration-enhancing effect of carvone, a cyclic terpene, on the permeability of nicardipine hydrochloride through rat epidermis from the HPC gel.

FT-IR STUDIES

FT-IR studies provide an insight into the effect of carvone on the biophysical properties of the rat stratum corneum.[43,49–51] The extraction of stratum corneum lipids by chloroform–methanol solvent system led to dramatic decrease in the intensity (>95%) for the C–H stretching peaks, and increased the stratum corneum permeability by several orders of magnitude compared with that of untreated tissue. There are several other reports on the use of FT-IR study to measure the changes in stratum corneum lipids with different solvent systems and penetration enhancers.[21,30,52] The treated stratum corneum of the rat was vacuum-dried, and stored in a desiccator for FT-IR study.[43,49–51] This resulted in the evaporation of carvone and ethanol, and allowed studying the changes in the C–H stretching absorbance caused by carvone. Figure 3 depicts IR spectra from 3,000–2,700 cm^{-1} of the stratum corneum pretreated with different concentrations of carvone (0% w/w to 12% w/w). Table 3 shows the peak heights under the asymmetric and symmetric C–H stretching. The lipid extraction resulting from

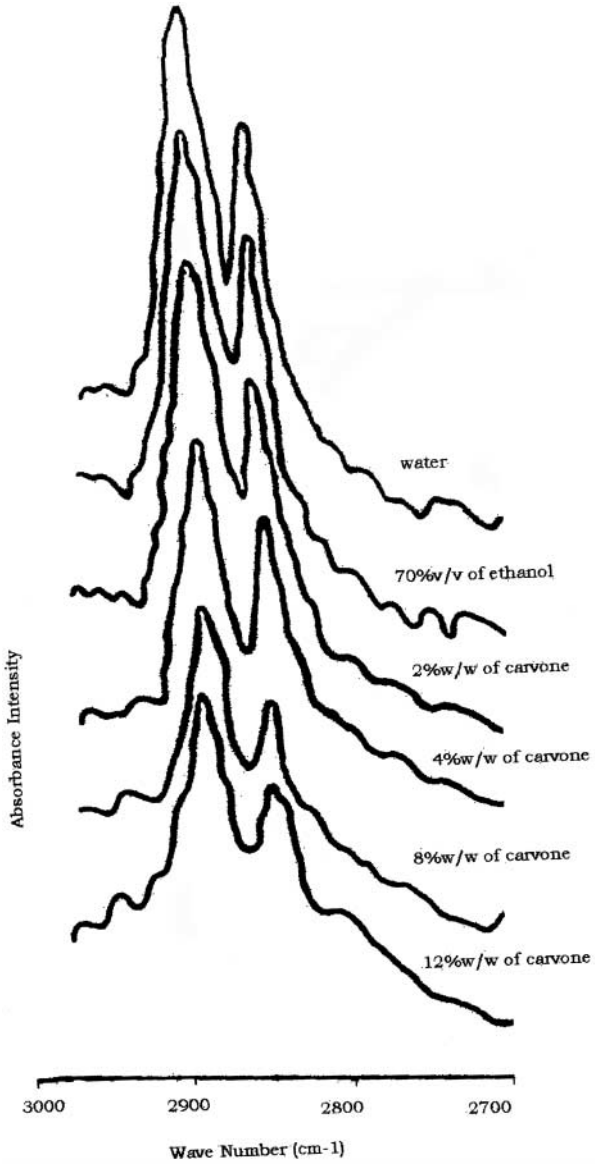

Figure 3. FT-IR spectra of the stratum corneum of rat abdominal skin showing asymmetric and symmetric C–H stretching absorbances treated with various concentrations of carvone in 70% v/v of ethanol and 70% v/v of ethanol and water alone.

the terpene treatment (2% w/w to 12% w/w carvone) was evaluated by comparing the intensities of the asymmetric and symmetric C–H stretching absorbances after treatment with carvone to those corresponding peaks with ethanol–water (70:30 v/v) treatment. Also, the influence of solvent system (70:30 v/v) on the stratum corneum lipid extraction was assessed by comparing the percentage decrease in the peak intensities with that of stratum corneum alone (water treated).

The results of the FT-IR showed that the treatment of stratum corneum with selected concentrations of carvone in ethanol–water (70:30 v/v) solvent system did not produce blue shift in the asymmetric and symmetric C–H stretching peak positions. However, they all showed a decrease in absorbance intensities for both asymmetric and symmetric C–H stretching absorbances in comparison with the stratum corneum treated with the ethanol–water (70:30 v/v) solvent system. The ethanol–water solvent system (70:30 v/v) decreased peak heights for asymmetric and symmetric C–H stretching absorbances by 22.3% and 23.9% (same values), respectively, in comparison with water-treated stratum corneum (Table 3). Carvone, at a concentration of 8% w/w, produced a greater decrease in peak heights for C–H stretching absorbances in comparison with the stratum corneum treated with 2% w/w or 4% w/w. The decrease in peak height may be because of the extraction of the stratum corneum lipids.[51] There was no significant effect on the stratum corneum with a higher concentration of carvone (12% w/w), when compared with 8% w/w carvone. Extraction of the lipids of stratum corneum leads to the enhanced percutaneous absorption of drugs.[53] Our findings suggest that extraction of the stratum corneum lipids by carvone/ethanol led to an increase in the permeability of nicardipine hydrochloride. The increase in permeability may be predominantly because of increased solute diffusivity through the partially delipidized stratum corneum.[54] As expected, the partially delipidized stratum corneum was highly permeable to the nonpolar drug used in this study.

DSC Studies

To obtain more supporting information of lipid components of the stratum corneum treated with different concentrations of carvone (0% w/w–12% w/w), a DSC study was carried out. The DSC study is useful for characterizing the phase transition of the lipid bilayer.[55] Endothermic peaks obtained in the DSC study on carvone-treated stratum corneum are shown in Fig. 4. Endothermic peaks corresponding to the phase transition of constituent lipids of stratum corneum was observed at 58.6°C, 70.6°C, and 86.2°C in both 70% v/v ethanol-treated (vehicle) and water-treated stratum corneum. However,

Table 3. Mean (±SD) peak height of asymmetric and symmetric C–H stretching absorbances of rat stratum corneum lipids ($n = 3$).

	Asymmetric C–H stretching		Symmetric C–H stretching	
Stratum corneum treated with	Peak height	% Decrease in peak height*	Peak height	% Decrease in peak height[a]
Water treated	0.32 ± 0.01	—	0.25 ± 0.01	—
70% v/v ethanol*	0.25 ± 0.01	21.80 ± 0.008	0.19 ± 0.01	24.00 ± 0.008
2% carvone/70% v/v ethanol**	0.19 ± 0.01	24.00 ± 0.006	0.13 ± 0.01	31.57 ± 0.005
4% carvone/70% v/v ethanol**	0.14 ± 0.01	44.00 ± 0.007	0.10 ± 0.01	47.37 ± 0.009
8% carvone/70% v/v ethanol**	0.10 ± 0.01	64.00 ± 0.009	0.07 ± 0.01	63.16 ± 0.009
12% carvone/70% v/v ethanol**	0.09 ± 0.01	64.00 ± 0.008	0.07 ± 0.01	63.16 ± 0.007

[a]% Decrease in peak height = (peak height from ethanol–water-treated stratum corneum − Peak height from enhancer-treated stratum corneum)/Peak height from ethanol–water-treated stratum corneum × 100.
*Significant ($p < 0.001$) when compared with water-treated stratum corneum.
**Significant ($p < 0.001$) when compared with water-treated stratum corneum.

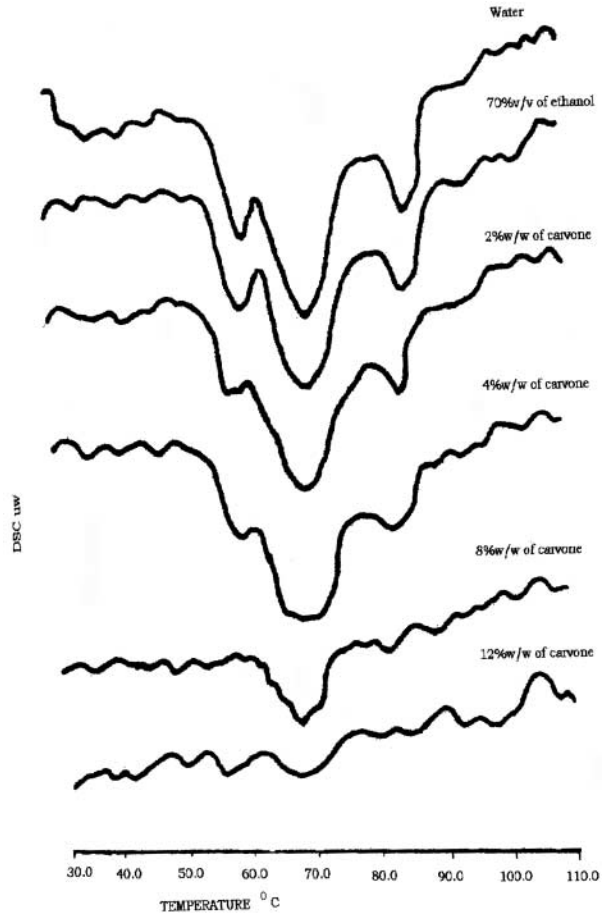

Figure 4. DSC thermograms of the stratum corneum of rat abdominal skin treated with various concentrations of carvone in 70% v/v of ethanol and 70% v/v of ethanol and water alone.

there was broadening of the peaks of the stratum corneum treated with 4% w/w, 8% w/w, or 12% w/w carvone. The results of the study indicated that treatment with carvone (4% w/w–12% w/w) showed pronounced effect on the extraction of lipids in the stratum corneum. Ethanol (70% v/v), the vehicle, also resulted in the extraction of lipids in the stratum corneum in comparison with the water-treated stratum corneum, indicating an additional effect of carvone beyond ethanol (70% v/v) alone. The extraction of the lipids of stratum corneum might have enhanced percutaneous absorption of nicardipine hydrochloride, as was evidenced by increased flux (Table 2). The observed lag period in the permeation may be because of the delay in the action of solvent and carvone on the stratum corneum lipids resulting in the delay of drug saturation in the rat skin.

It was reported[19] earlier that the permeability flux of nicardipine hydrochloride was obtained with the incorporation of 8% w/w of menthol (227.70 ± 1.30 μg/cm^2/hr) in HPC gel as a penetration enhancer when compared with control (30.91 ± 0.77 μg/cm^2/hr). But in the present study, the permeability flux of nicardipine hydrochloride (235.1 ± 2.2 μg/cm^2/hr) was obtained with incorporation of 8% w/w of carvone as a penetration enhancer. In the case of menthol, beyond 8% w/w of menthol, a fine layer of precipitate was observed on the HPC gel, whereas in the present study such an effect was not observed (carvone is miscible with the HPC gel even at a concentration at 12% w/w). Both carvone and menthol have shown plateau effect beyond 8% w/w. This indicates that carvone and menthol show more or less the same permeability enhancement of

nicardipine hydrochloride across the rat abdominal skin. This is in accordance with the report[56] that lipophilic index values of carvone (lipophilicity as denoted by log P is 2.23 ± 0.25) and menthol (lipophilicity as denoted by log P is 2.02 ± 0.15) are almost same. Moreover, the effect of enhancers on the permeation of a drug usually depends on the physicochemical characteristics of both the dug (permeant) and the enhancer.[36]

The enhanced permeability flux of nicardipine hydrochloride by carvone at 8% w/w or 12% w/w level through rat abdominal epidermis, observed in this study, may be useful in the selection of a relatively safe penetration enhancer to aid transdermal drug delivery. However, permeability of nicardipine hydrochloride from HPC gel containing 8% w/w or 12% w/w of carvone as penetration enhancer through the skin/membrane composite needs to be studied in the development of a transdermal therapeutic system. Further studies are also required to find out the influence of carvone on the permeability of nicardipine hydrochloride in human volunteers, and such studies are in progress.

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