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Effect of carvone on the permeation of nimodipine from a membrane-moderated transdermal therapeutic system

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Received October 15, 2002, accepted February 17, 2003

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Pharmazie 58: 559–563 (2003)

The purpose of this investigation was to develop a membrane-moderated transdermal therapeutic system (TTS) of nimodipine using 2% w/w hydroxypropylmethylcellulose (HPMC) gel as a reservoir system containing 10% w/w of carvone (penetration enhancer) in 60% v/v ethanol. The flux of nimodipine through an ethylene vinyl acetate (EVA) copolymer membrane was found to increase with an increase in vinyl acetate content in the copolymer. The effect of a pressure-sensitive adhesive (TACKWHITE A 4MED®) on the permeability of nimodipine through an EVA 2825 membrane (28% w/w vinyl acetate) or an EVA 2825 membrane/skin composite was also studied. An EVA 2825 membrane coated with TACKWHITE 4A MED® was found to provide the required flux of nimodipine (117 ± 5 μg/cm^2/h) across rat abdominal skin. Thus a new transdermal therapeutic system for nimodipine was formulated using EVA 2825 membrane, coated with a pressure-sensitive adhesive TACKWHITE 4A MED®, and 2% w/w HPMC gel as reservoir containing 10% w/w of carvone as a penetration enhancer. Studies in healthy human volunteers indicated that the TTS of nimodipine, designed in the present study, provided steady-state plasma concentration of the drug with minimal fluctuations.

1. Introduction

Nimodipine, a calcium antagonist, is used in the treatment of cerebrovascular disorders, stroke and hypertension [1]. It is subjected to an extensive hepatic first-pass metabolism following oral administration with a systemic bioavailability of about 13% [2, 3]. Because of its short biological half-life (1–2 h), the drug has to be given frequently (30 mg three to four times daily). Thus, the conventional therapy with nimodipine may result in higher fluctuation in the plasma concentration of the drug resulting in unwanted side effects. Hence, the development of a transdermal therapeutic system for nimodipine would circumvent those problems and thus would result in a better therapeutic efficacy in the management of hypertension.

In a previous study, it was reported [4] that an ethanol-water solvent system in the ratio of 60 : 40 v/v was a suitable vehicle for the transdermal delivery of nimodipine. However, it was necessary to improve the permeation rate of nimodipine by suitable penetration enhancers. Recently, it was reported that the 10% w/w of carvone in 2% w/w HPMC gel provides the required permeability of nimodipine across excised rat abdominal skin [4].

Besides the solvent system, gelling agent and penetration enhancer, the skin permeability of nimodipine could be affected by the rate-controlling membrane or the pressure-sensitive adhesives. Thus, the present study was carried out to develop a reservoir-type transdermal therapeutic system of nimodipine, and to investigate the effect of a rate controlling membrane or a pressure-sensitive adhesive on the skin permeation rate of the drug in order to optimize the formulation parameters in fabricating the TTS. Further, a bioavailability study was conducted in human volunteers to find the ability of the developed reservoir type TTS of nimodipine in providing a steady state concentration of the drug.

2. Investigations, results and discussion

HPMC gel formulations containing nimodipine and selected concentrations of carvone (0% w/w to 12% w/w) in 60 : 40% v/v ethanol-water co-solvent system were prepared [4], and evaluated for *in vitro* permeation of nimodipine through excised rat epidermis. As carvone concentration increased from 0% w/w to 10% w/w, the permeability of nimodipine increased. On increasing the carvone concentration further from 10% w/w to 12% w/w, the increase in the permeability was insignificant ($P > 0.05$). The flux of nimodipine was found to be 161 ± 4 μg/cm^2/h with an enhancement ratio of about 4.56 when carvone was incorporated at a concentration of 10% w/w in HPMC gel when compared to HPMC gel without carvone (35 ± 2 μg/cm^2/h). The FT-IR data indicated that carvone increased the drug permeability through the rat skin by disrupting the highly ordered intercellular lipid bilayer structure of the stratum corneum. Based on these studies, HPMC gel (2% w/w) containing 10% w/w of carvone as a penetration enhancer was chosen for further studies to design the TTS of nimodipine.

The HPLC method used for the quantitative determination of nimodipine was found to be precise and accurate as

indicated by less than 2.5% of CV (inter- and intra-day variation) and a high recovery (99.7%). The HPMC gel formulations were found to contain 98.8 to 100.2% of nimodipine showing uniformity of drug content in the gel formulation.

2.1. Permeability of nimodipine through EVA copolymer membranes with varying vinyl acetate content

EVA (ethylene vinyl acetate) copolymer membranes were chosen as rate-controlling membranes. The membranes with vinyl acetate contents ranging from 9% w/w to 28% w/w were prepared by the glass substrate technique. The membranes were dried at 30 °C for about 10 h and kept in a desiccator for 72 h over fused calcium chloride. The average thickness of the membrane was 22 ± 2 μm. The diffusion profiles of nimodipine from the HPMC gel (2% w/w) containing 1.5% w/w of drug and 10% w/w of carvone across the EVA membranes with varying vinyl acetate contents were shown in Fig. 1. The membrane permeation rate of nimodipine increased with an increase in the vinyl acetate content of the EVA copolymer membrane. This might be due to the increased water vapor transmission of EVA copolymer with an increase in its vinyl acetate content (9% w/w to 28% w/w). Thus, EVA 2825 membrane with 28% vinyl acetate content has more water vapor transmission than the EVA membrane with 9% (EVA 0906) or 18% (EVA 1802) of vinyl acetate content and might have exhibited more permeability. Also increase in vinyl acetate content (9% w/w to 28% w/w) in the EVA membranes increases the polarity of the EVA copolymers. Thus EVA 2825 containing 28% of vinyl acetate with low lipophilicity might have shown high permeability of nimodipine.

The maximum steady-state permeation rate of nimodipine was 150 ± 3 μg/cm^2/h through the EVA membrane containing 28% vinyl acetate (EVA 2825), and this increase was found to be significant ($P < 0.001$) when compared to 18% w/w vinyl acetate (EVA 1802) or 9% w/w vinyl acetate (EVA 0906). The flux obtained with 10% w/w carvone from HPMC gel across the rat abdominal skin was 161 ± 1 μg/cm^2/h wherein EVA 2825 provided 150 ± 3 μg/cm^2/h, the decrease in flux was significantly different. However, The other membranes EVA 1802 and EVA 0902 provided a flux of 89 ± 4 μg/cm^2/h and 39 ± 1 respectively. Further permeability studies were carried out to determine the influence or possible interaction of carvone with either EVA 2825 membrane or EVA 2825 coated with adhesive. It could be observed that there was no significant ($P > 0.01$) difference in flux of nimodipine obtained from HPMC gel prepared with ethanol-water solvent system without carvone (152 ± 3 μg/cm^2/h) when compared to the flux obtained from HPMC gel with 10% w/w of carvone (150 ± 3) indicating that carvone did not interact with the EVA 2825 membrane.

In the present study, TACKWHITE A 4MED® was chosen as a pressure-sensitive adhesive in the development of membrane-moderated TTS. The water-based acrylic adhesive emulsion (TACKWHITE A 4MED®) was applied uniformly over the EVA 2825 membrane with a glass rod and dried for 2 h. The flux (J), permeability coefficient (k_p) and cumulative amount (Q_{24}) of nimodipine permeated through EVA 2825 membrane or EVA 2825 coated with TACKWHITE A 4MED® and through the EVA 2825 membrane/skin composite are shown in Fig. 2. When the membrane was coated with a TACKWHITE A 4MED®, the permeation of nimodipine from the HPMC drug reservoir decreased when compared to membrane alone. The flux (J) and cumulative amount of drug permeated (Q_{24}) across EVA 2825 membrane were found to be 150 ± 3 μg/cm^2/h and 3520 ± 58 μg respectively, whereas those obtained with the membrane coated with TACKWHITE A 4MED® were found to be 129 ± 2 μg/cm^2/h and 3041 ± 10 μg respectively which were statistically different. The decrease in permeability might be due to the resistance offered by the pressure-sensitive adhesive. The flux across the EVA 2825 membrane coated with

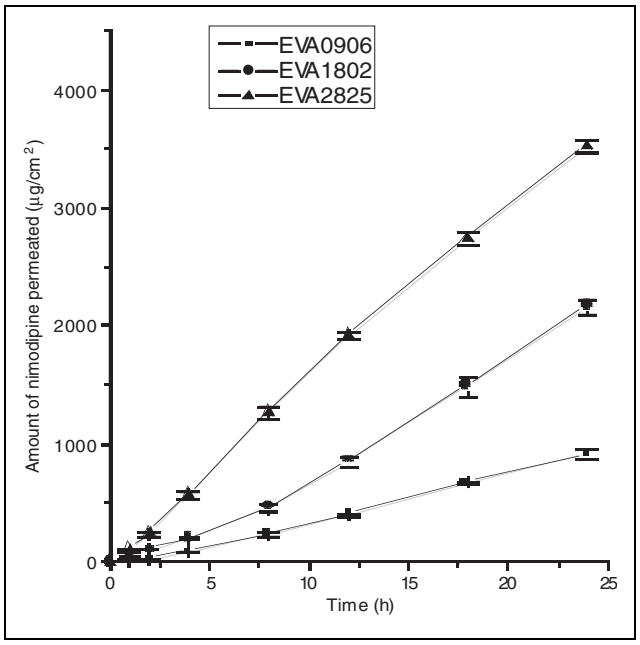

Fig. 1: Mean (± s.d.) amount of nimodipine permeated from 2% HPMC gel containing 10% w/w of carvone across the EVA membrane with 28% w/w (EVA 2825), 18% w/w (EVA 1802) or 9% w/w (EVA 0906) of vinyl acetate (n = 3)

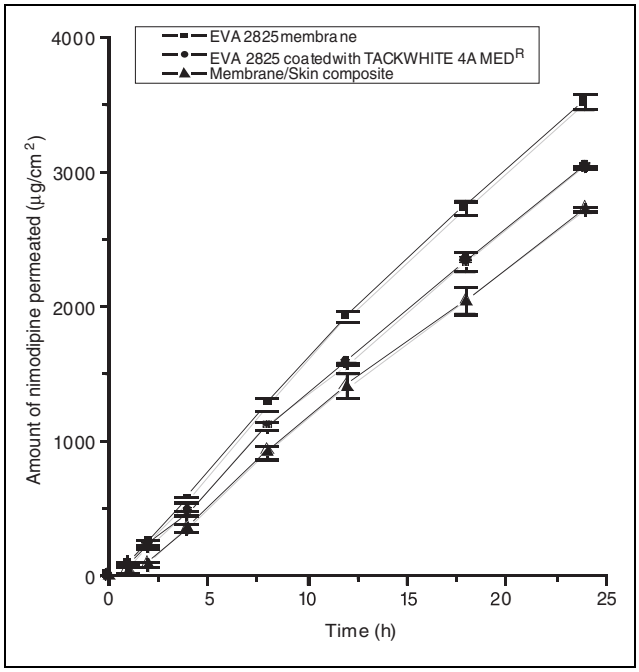

Fig. 2: Mean (± s.d.) amount of nimodipine permeated from 2% HPMC gel containing 10% w/w of carvone across the EVA 2825 membrane, EVA 2825 membrane coated with TACKWHITE A 4MED® and EVA 2825 membrane coated with TACKWHITE A 4MED®/skin composite (n = 3)

TACKWHITE A 4MED® rat abdominal skin composite was found to be 117 ± 5 μg/cm^2/h. The decrease in permeability when compared to the permeability of the drug across adhesive coated EVA 2825 membrane indicates that the skin is offering its own resistance to the permeation of the drug across the adhesive-coated EVA 2825 membrane. Thus it is likely that both the patch and the skin are controlling the transdermal permeation of nimodipine. The permeability studies were carried out to determine the influence or possible interaction of carvone with the adhesive (TACKWHITE A 4MED®). The flux obtained across adhesive-coated EVA 2825 membrane from HPMC gel (without carvone) was 125 ± 1 μg/cm^2/h whereas the flux from HPMC gel (with 10% carvone) was 129 ± 2 indicating that carvone did not interact even with the adhesive coated on EVA 2825 membrane. However, there was 1.4 times more flux than required (83 μg/cm^2/h). Hence, further studies were carried out to develop and evaluate TTS of nimodipine. The TTS patch applied to human volunteers were found intact during the course of study (48 h) indicating a good adhesion of the patch. However, stability studies on the carvone-based TTS patch regarding its drug release, adhesive properties and *in vivo* performance are planned.

2.3. In vivo bioavailability studies on TTS patch of nimodipine

The mean plasma concentration of nimodipine at various time intervals following the application of TTS (with 10% w/w of carvone) or oral administration of immediate release tablet dosage form (30 mg) is shown in Fig. 3. The plasma concentration of nimodipine gradually increased and attained an average steady state level of 15 ± 0.2 ng/mL at about 3.67 h (lag period). However, the steady state concentration of the drug declined gradually after 24 h. Thus, the steady state concentration of nimodipine was maintained for 26 h. The pharmacokinetic parameters of nimodipine such as C_{max}, T_{max}, $AUC_{0-\alpha}$ and relative bioavailability following oral administration of immediate release tablet dosage form or application of TTS patch are given in the Table.

The inter-subject variation in plasma concentration of nimodipine observed on oral administration of an immediate release tablet was found high (more than 10% of CV). The low variation (less than 3% of CV) in peak plasma levels following transdermal application of nimodipine could be accounted for uniformity in the skin permeation characteristics of the drug, which are possibly the same for all subjects. The inter-subject variation in plasma levels observed in the volunteers receiving an immediate release tablet could be due to the high variation in gastric emptying and GI absorption etiology of individual subjects [5–7].

The relative bioavailability of nimodipine with the TTS patch was found to be significantly ($P < 0.001$) high indicating the improved bioavailability on transdermal delivery of the drug (Table). The relative bioavailability of nimodipine from TTS patch was calculated by dividing its AUC by that of the immediate release tablet (reference formulation). The reported [2, 3] low bioavailability of nimodipine (13%) might be due to the extensive first pass metabolism. However, the TTS patch (with 10% w/w of carvone), designed in the present study, was found to enhance the bioavailability of nimodipine by 8.5 times (mean relative bioavailability 848 ± 22) with reference to an immediate release tablet dosage form. Considering the reported mean oral bioavailability of nimodipine as 13%, the TTS patch, used in the present study, provided 95.75% of bioavailability. This improved availability may be due to the elimination of hepatic first pass metabolism on transdermal delivery of the drug. Thus, the TTS patch (with 10%w/w of carvone), designed in the present study, was found to provide prolonged steady state concentration of nimodipine with minimal fluctuations and improved bioavailability. The TTS application sites, in volunteers, were examined visually for signs of local irritation after application of the TTS device for two days. No local irritation was observed at the application site indicating that the device was well tolerated on dermal application of the patch application for two days.

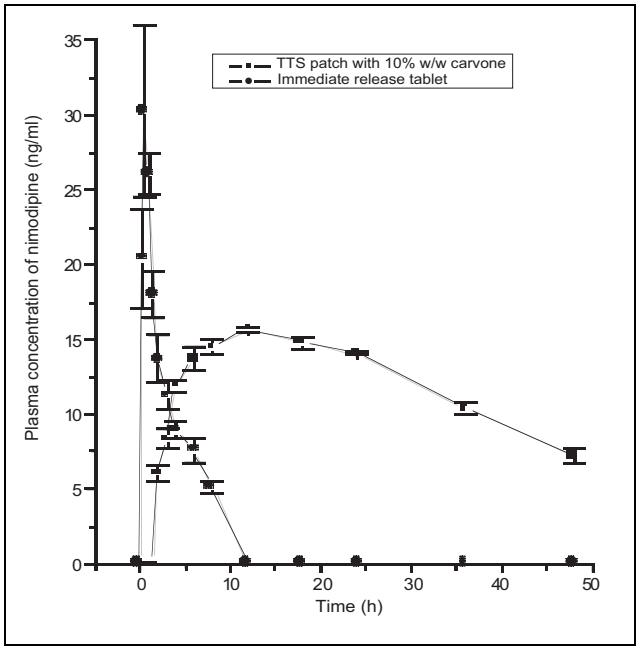

Fig. 3: Mean (± s.d.) plasma concentration of nimodipine following the oral administration of immediate release tablet dosage form or application of TTS patch (with 10% w/w carvone) in human volunteers (n = 6)

3. Experimental

3.1. Materials

Nimodipine and d-carvone were obtained from M/s. Micro Labs, Banglore, India and M/s. Merck-Schuchardt, Germany respectively. Ethylene vinyl acetate copolymer beads of various weight fractions (% w/w) of vinyl acetate were gift samples from M/s. NOCIL India Ltd., India. Nimesulide and

Table: Pharmacokinetic parameters of nimodipine following oral administration of immediate release tablet dosage form (30 mg) or application of TTS patch (10% w/w of carvone) in human volunteers (n = 6)

Formulation	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-\alpha}$ (ng/h/mL)	Relative bioavailability (%)	Lag period (h)
Immediate release tablet	30.34 ± 3.35	0.66 ± 0.23	115.21 ± 9.86	–	0.18 ± 0.01
TTS patch (with 10% w/w of carvone)	15.69 ± 0.31*	11.99 ± 0.15*	848.62 ± 22.4*	736.58 ± 0.86	3.53 ± 0.57*

* Significant at $P < 0.001$ when compared to immediate release tablet

hydroxypropylmethylcellulose (HPMC) were a gift sample from M/s. Dr. Reddy's Labs, Hyderabad. Release liner (3M™ Scotchpak™ 1022) and backing membrane (3M™ Scotchpak™ 9732) were gift samples from 3M drug delivery systems, USA. Pressure-sensitive adhesive, TACKWHITE A 4MED®, was offered by M/s. Ichemco, Italy. Methanol (HPLC grade), acetonitrile (HPLC grade) and water (HPLC grade) were obtained from M/s. Qualigens Fine Chemicals, Mumbai, India. Other materials used in the study such as ethanol were of analytical grade.

3.2. Preparation of HPMC gel

To prepare 2% w/w hydroxypropylmethylcellulose gel, HPMC powder was added to 60 : 40% v/v ethanol-water while being stirred by means of a stirrer (M/s. Remi Motors, India) at 2,500 rpm, and the resulting mixture was mixed continuously at 37 °C for about 1 h until gel formation. Nimodipine (1.5% w/w) and carvone (10% w/w) were added to the HPMC gel, and mixed well for complete dissolution. The gel formulations were left overnight at ambient temperature.

3.3. HPLC

The quantitative determination of nimodipine was performed by HPLC. A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wave length 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™, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA.) and RP C-18 column (150 mm × 4.6 mm I.D., particle size 5 μm; YMC Inc., Wilmington, USA) 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 water (58 : 42 v/v). The mobile phase components were filtered and pumped at a flow rate of 1 mL/min. The column temperature was maintained at 40 °C. The eluent was detected by an UV detector at 237 nm. A standard curve was constructed for nimodipine in the range of 0.1 to 10 μg/mL. A good linear relationship was observed between the concentration of nimodipine and area of nimodipine with a high correlation coefficient ($r = 0.9999$). The required studies were carried out to estimate the precision and accuracy of this HPLC method. The standard curve, constructed as described above, was used for estimating nimodipine either in the skin permeates or in HPMC gel formulations.

3.4. Quantitative determination of nimodipine in HPMC gel formulation

One gram of the drug reservoir (HPMC 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 nimodipine was estimated using the standard curve as described above.

3.5. Preparation of rat abdominal skin

Male albino rats (150–200 g) obtained from M/s Ghosh Enterprises, Kolkota, India, were euthanised using carbon dioxide asphyxiation before the experiments. The dorsal hair was removed with a clipper and full thickness skin was surgically removed from each rat. The epidermis was prepared by a heat separation technique [8, 9]. Whole skin was immersed in water at 60 °C for 45 s, followed by careful removal of the epidermis. The epidermis was washed with water, and used for the *in vitro* permeability studies.

3.6. In vitro skin permeability studies

Modified Keshary-Chien diffusion cells [8–10] were used in the *in vitro* permeation studies. The epidermis prepared as above was mounted between the two compartments of the diffusion cells with stratum corneum facing the donor compartment. The effective diffusional area was 5.6 cm^2. The volume of receiver compartment was 35 mL. Two grams of the HPMC gel containing 30 mg of nimodipine were added to the donor cell. Ethanol and water (60 : 40 v/v) added to the receiver cell in order to maintain sink conditions. The cells were placed on a magnetic stirrer with heater (Remi Equipments, Mumbai, India) and temperature was maintained at 37 ± 0.5 °C. The contents in the receiver compartment were stirred with the help of a magnetic bar at 500 rpm. At predetermined time intervals (1, 2, 4, 8, 12, 18 and 24 h) 0.5 mL a permeate sample was withdrawn from the receiver compartment which was replaced with an equivalent amount of drug-free solvent (60 : 40% v/v ethanol-water) to maintain sink conditions. The samples of the skin permeates were assayed as described above.

3.7. Preparation of EVA membranes

The membranes were prepared by solvent extrusion using a glass substrate technique. A "membrane casting apparatus" fabricated in our laboratory was used for the preparation of the membranes. Briefly, the procedure involves pouring of 5% w/v polymer (EVA) solution on to a glass frame (100 cm^2) and allowing the solvent to evaporate slowly. After complete evaporation of the solvent the membrane was removed carefully and dried.

3.8. Permeability studies across the EVA membranes

The experimental conditions to study the permeation of nimodipine across the EVA copolymer membranes were the same as those outlined above with respect to the skin permeation studies, except that the EVA copolymer membrane with various weight factions of vinyl acetate (VA) was used in the place of rat abdominal skin.
The EVA 2825 membrane coated with a pressure-sensitive adhesive such as TACKWHITE A 4MED® was mounted on the skin and the permeation of nimodipine across the membrane/skin composite was also determined. The experimental conditions were the same as those outlined above, except that the membrane/skin composite was used instead the EVA membrane.

3.9. Fabrication of membrane-moderated transdermal therapeutic system of nimodipine

The nimodipine TTS was fabricated by sandwiching the HPMC reservoir gel system between drug-impermeable backing laminate (3M™ Scotchpak™ 9732, a polyster film laminate with ethylene vinyl acetate heat-sealable layer) and a rate-controlling EVA membrane (20 cm^2) coated with water-based pressure-sensitive acrylic adhesive emulsion (TACKWHITE A 4MED®). The reservoir system consisted of 1.5% w/w of nimodipine and the penetration enhancer (10% w/w of carvone) in 2% w/w of HPMC gel prepared with an ethanol-water (60 : 40% v/v) solvent system. The rate-controlling membrane was EVA 2825 copolymer (containing 28% w/w vinyl acetate). To ensure an intimate contact of the transdermal patch to the skin, a pressure-sensitive adhesive polymer was coated on to the EVA 2825 membrane.
The EVA 2825 membrane was coated with acrylic adhesive emulsion (TACKWHITE A 4MED®), allowed to dry completely and a release liner (3M™ Scotchpak™ 1022, a polyester film coated with fluoropolymer) was pressed over the EVA 2825 membrane. Two g of the HPMC gel containing 30 mg of nimodipine and 10% w/w of carvone in 60 : 40 ethanol-water were placed over the EVA 2825 membrane/adhesive/release liner composite placed on a slightly grooved surface, and then the backing laminate (3M™ Scotchpak™ 9732, a polyester film laminate with ethylene vinyl acetate heat-sealable layer) was placed on it. The composite was heat-sealed and cut to the appropriate sizes (20 cm^2). Then, each patch was kept in a sealed aluminum pouch to minimize the loss of solvent (ethanol).

3.10. In vivo evaluation of the TTS patch

After approval of the ethics committee, the study was conducted at M/s. Sipra Labs Pvt. Ltd., Hyderabad, India. Six healthy male volunteers (60–65 kg, age between 25–30 years) participated in the study, and all were nonsmokers and non-alcoholics. The biochemical examination of the volunteers revealed normal function of the kidney and liver. The nature and purpose of the study were fully explained to them. An informed written consent was obtained from every volunteer. None of the volunteers were on drug treatment one week prior to the participation of the study. The volunteers were divided into two groups (Group-I and Group-II), and a cross over study was carried out. An immediate release tablet dosage form containing 30 mg of nimodipine was chosen as a reference formulation, and was administered to 3 volunteers (group I). Group II ($n = 3$) volunteers applied TTS patch (with 10%w/w of carvone) of 20 cm^2 to the anterior surface of the forearm near the elbow. After a washout period of 10 days, to group I volunteers a TTS patch was applied (with 10% w/w of carvone) and group II received the reference formulation (immediate tablet capsule dosage form). The volunteers were allowed to remove the patch in case of any sign of irritation at the application site. Blood samples were collected from the volunteer's cubical vein of the forearm via a hypodermic syringe (rinsed with dilute heparin solution) over a period of 48 h (0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 36 and 48 h). The blood samples were mixed well, immediately centrifuged at 5000 rpm, plasma was separated and stored at −40 °C until analysis by HPLC.

3.11. HPLC analysis of nimodipine in human plasma

A standard graph was constructed by the addition of 0.1 mL of internal standard (nimesulide, 5 μg/mL) spiking solution and an appropriate volume of nimodipine spiking solution to 0.5 ml of human plasma to cover the concentration range from 10 to 200 ng/mL. The mixture was vortexed for 5 min to ensure thorough mixing. After mixing, 0.2 mL of acetonitrile was added, the resulting mixture was vortexed for 30 s, centrifuged at 3000 rpm for 10 min, the supernant layer was separated out into a clean amber-colored vial and then injected into the HPLC column (RP C-18, 250 × 4.6 mm I.D, particle size 5 μm; Merck Lichrospher, USA) with a 20 μL loop. The mobile phase (filtered through a 0.2 μm P.T.F.E membrane filter) comprised of 72 : 28v/v of methanol and water was pumped at a flow rate 1 mL/min which yielded a column back-pressure of 155–161 kg/cm^2. The

detection was by UV absorption at 237 nm. The sensivity of the detector was set at 0.0002 AUFS. A good linear relationship ($r = 0.9994$) was observed between the concentration of nimodipine and the peak area ratio of nimodipine to the internal standard. This method was found to be precise (CV less than 3%) and accurate (99.8% to 99.95% of recovery). The standard curve constructed as described above was used for estimating nimodipine in the samples of human plasma.

3.12. Data analysis

The nimodipine concentration in the skin permeate samples was corrected for sampling effects according to eq. (1) [11]:

$$C^1_n = C_n\,(V_T/V_T - V_S)\,(C^1_{n-1}/\,C_{n-1}) \quad (1)$$

where 'C^1_n' is the corrected concentration of the n^{th} sample, 'C_n' is the measured concentration of nimodipine in the n^{th} sample, C_{n-1} is the measured concentration of the nimodipine 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 ($\mu g/cm^2/h$) of nimodipine (J) was calculated from the slope of the plot of the cumulative amount of nimodipine permeated per cm^2 of skin at steady state against the time using linear regression analysis [12]. The steady state permeability coefficient (k_p) of the drug through rat epidermis was calculated with eq. (2) [13]:

$$k_p = J/C \quad (2)$$

where 'J' is the flux and 'C' is the initial concentration of nimodipine in the donor compartment.

The plasma concentration of nimodipine at different time intervals was subjected to pharmacokinetic analysis to calculate maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}) and area under the curve ($AUC_{0-\infty}$). The values of C_{max} and T_{max} were directly read from the arithmetic plot of time versus plasma concentration of nimodipine. The area under the curve of time versus plasma concentration of nimodipine ($AUC_{0-\infty}$) was calculated using the trapezoidal rule. The relative bioavailability of nimodipine from TTS patch (with 10% w/w of carvone) when compared to reference formulation (immediate release dosage form) was calculated by dividing its $AUC_{0-\infty}$ with that of immediate release tablet dosage form (reference formulation).

The *in vitro* permeation data were subjected to student's t-test to find the statistical significance of the observed differences. The statistical significance of the observed difference in the permeability of the drug through EVA 2825 membrane coated with pressure-sensitive adhesive and membrane/skin composite was tested using analysis of variance (ANOVA) and Duncan's multiple range test with the help of STATISTICA computer program (Release 4.5, StatSoft Inc., 1993). The observed difference in mean pharmacokinetic parameters of nimodipine after application of TTS patch and immediate release capsule dosage form was subjected to the paired t-test to find the statistical significance. In all the cases, a value of $P < 0.05$ was considered statistically significant.

Acknowledgements: The authors highly acknowledge the financial support received from Government of India, Department of Science and Technology (DST) for granting a research project under SERC scheme (Grant No: SP/SO/B70/97, dt. 28. 1. 1999 and 7. 3. 2002). The financial support received from AICTE (MODROBS and TAPTEC) and UGC, New Delhi, India is greatly acknowledged in establishing the basic infrastructure needed for this study. The authors acknowledge M/s. NOCIL, India, M/s. 3M drug delivery systems, USA, M/s Adhesive Inc., UK and M/s. Ichemco, Italy for the gift samples of EVA copolymer, Release liner (3M™ Scotchpak™ 1022), backing membrane (3M™ Scotchpak™ 9732), and TACKWHITE A 4MED® respectively. The authors are thankful to M/s. Sipra Labs Pvt. Ltd., Hyderabad, India in conducting bioavailability studies.

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