Drug Development and Industrial Pharmacy, 34:1125–1129, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040801974121

informa
healthcare

Effect of Adhesive Matrix Composition and Terpinolene on Indomethacin Bioavailability in Rats from Transdermal Therapeutic System

Krzysztof Cal, Malgorzata Sznitowska, and Stanislaw Janicki
Department of Pharmaceutical Technology, Medical University of Gdansk, Gdansk, Poland

Drug-in-adhesive matrix-type transdermal therapeutic systems for indomethacin (IND) were formulated and evaluated. Silicone and two types of polyacrylates were used as the bases of matrices. Terpinolene was used as a penetration enhancer. The physicochemical properties of matrices were determined. The bioavailability study of IND was performed in rats. The presence of IND in blood was demonstrated for each system. The calculated pharmacokinetics parameters for IND mainly depend on the solubility of IND in the adhesive layer. The positive influence of a penetration enhancer on IND bioavailability was observed only for one type of polyacrylate matrices.

Keywords transdermal therapeutic system; indomethacin; bioavailability; penetration enhancer; terpinolene

INTRODUCTION

Despite some limitations, transdermal therapeutic systems (TTS) are still considered to be a promising drug form. They are intended to be applied onto the intact skin in order to deliver drug substance to the systemic blood circulation after permeation through the skin barrier. Transdermal administration of nonsteroidal anti-inflammatory drugs (NSAIDs) is particularly advantageous and intensively tested because oral therapy, although effective, may cause ulcerations of stoma and intestinal mucosa. Creams, gels, ointments, and solutions containing NSAIDs are commercially available; whereas TTS with NSAIDs are available only on some markets. The drug applied in TTS form is delivered in a controlled amount, the hepatic first-pass metabolism is omitted, and the single application enables to obtain the steady-state, therapeutic level of the drug substance in the blood even for a few days (Krishnaiah, Satyanarayana, & Bhaskar, 2003; Valenta & Auner, 2004).

The flexibility of TTS enables good fit to the skin over the diseased joint or muscle and contributes to the overall adhesion on the skin tissue, but skin adhesion itself is governed by the pressure-sensitive adhesive and other excipients. TTS vary in size and area, and may exist as matrix- or reservoir-type preparations. The drug-in-adhesive matrix-type TTS are most often proposed because the production technology involved is less complex. This type of TTS consists of a backing film, drug-in-adhesive matrix layer, and a liner. The system is characterized by the incorporation of the drug substance directly within the skin-contacting adhesive layer that not only affixes the system onto the skin, but also provides the formulation foundation-adhesive matrix containing the drug and all other excipients.

Stratum corneum (SC) is a rate-limiting barrier in the percutaneous absorption of drug substances. In order to increase SC permeability, an addition of penetration enhancers to the transdermal drug form is sometimes required; however, this depends on physicochemical properties of the drug substance and its pharmacological potency. Among penetration enhancers, terpenes are often used experimentally to increase the skin penetration of NSAID (Chang, Tsai, Wu, & Huang, 2007; Obata, Ozawa, Takayama, & Nagai, 1999; Okabe, Takayama, & Nagai, 1992; Takayama & Nagai, 1994; Williams & Barry, 2004). Terpenes are considered as GRAS (generally regarded as safe) substances. They increase the solubility of the drug substances in the SC, interact with SC lipids disturbing their arrangement in the intercellular spaces, and increase the partition coefficient SC/carrier for penetrants (Gao & Singh, 1997; Obata et al., 1999; Williams & Barry, 1991, 2004). The advantages in using terpenes as penetration enhancers are as follows: they act reversibly on the SC and are quickly eliminated from this layer mainly by evaporation, not by penetration into deeper skin layer (Cal, 2006; Cal, Kupiec, & Sznitowska, 2006; Kang et al., 2007).

The aim of this study was to develop TTS with indomethacin (IND) and to study the influence of adhesive matrix type and the presence of a penetration enhancer on the IND transdermal penetration in rats. As adhesive layers, polyacrylate and silicone components were studied, forming three formulations of TTS. Terpene terpinolene (TERP) was used as a penetration enhancer.

Address correspondence to Krzysztof Cal, Department of Pharmaceutical Technology, Medical University of Gdansk, Hallera 107, 80–416 Gdansk, Poland. E-mail: kcal@wp.pl (K. Cal)

MATERIALS AND METHODS

Materials

IND and TERP (purity ≥99.0 and ≥97.0%, respectively) were purchased from Fluka, Buchs, Switzerland. Eudragit E-100 (Röhm Pharma GmbH, Darmstadt, Germany), silicone pressure-sensitive adhesive MD7-4502 solution in ethyl acetate (Dow Corning Co, Midland, MI, USA), water-based acrylic copolymer adhesive emulsion S-56 (I.Ch.P., Warsaw, Poland), aluminum/heat sealable polyester film laminate No 1009, and polyethylene/silicone coated paper liner No 1361 (both 3M Medica Pharma, St. Paul, MN, USA), which were received as gifts from producers or distributors, were used as components of the TTS. All other chemicals were extra pure or of HPLC grade.

Preparation of TTS

IND was sieved through a 0.08-mm sieve. A liquid containing the components of matrix was casted onto the aluminum foil laminated with polyester film (100 cm^2). The composition of the mixtures is presented in Table 1. The matrices were dried at room temperature, and then they were protected with a silicone-coated paper liner. Matrices with TERP or without penetration enhancer (control) were prepared.

Eudragit E-100-based matrices (ET and E0) were obtained according to the procedure received from Röhm Pharma with insignificant modifications. Eudragit E-100, adipic acid, and lauric acid were added to water (85–90°C). The homogenous dispersion was obtained after mixing for 30 min. Glycerol was added to the mixture, and the dispersion was stirred for 15 min at 45°C. IND and TERP were added to the mixture at room temperature.

Polyacrylate-based matrices (PT and P0) were prepared by adding the aqueous solution of macrogol 1000 to the S-56 dispersion. Next, TERP was emulsified in the mixture and IND and triethanolamine were added.

Silicone-based matrices (ST and S0) were obtained by stirring the silicone-adhesive solution with IND and TERP. A small amount of ethyl acetate was added to obtain an appropriate consistency for casting. The absence of ethyl acetate after drying was confirmed by gas chromatography (GC).

TABLE 1
Composition of Dispersions for Adhesive Layers Preparation (g/100 cm^2)

Component: Matrix:	ET	E0	PT	P0	ST	S0
IND	0.60	0.60	0.60	0.60	0.60	0.60
TERP	1.0		1.0		1.0	
Eudragit E-100	0.60	0.60				
S-56 adhesive			6.00	6.00		
Silicone adhesive					2.40	2.40
Adipic acid	0.07	0.07				
Lauric acid	0.37	0.37				
Glycerol	0.08	0.08				
Macrogol 1000			0.30	0.30		
Triethanolamine			0.25	0.25		
Ethyl acetate					2.40	2.40
Water	2.80	2.80	1.00	1.00		

IND, indomethacin.
TERP, terpinolene.

Physicochemical Evaluation of the Matrices

IND in the adhesive layers was assayed using spectrophotometer (Jasco V-530, Jasco Corp., Tokyo, Japan) at 322 nm. The matrices were cut into small pieces and twice extracted with 5.0 mL methanol. The content of TERP in matrices was determined by GC as described earlier (Cal, Janicki, & Sznitowska, 2001). For the determination of TERP, methanolic extracts were analyzed.

In order to determine the solubility of the drug substance in adhesive layers, increasing quantities of IND were added to the dispersions of matrix components and the obtained liquids were casted as thin layers onto microscopic object glasses. After drying, the resulting films were observed under the optical microscope at a magnification of × 600. The highest concentration of IND for which crystals were not present was considered as the solubility of IND in the matrix.

Thickness of the matrices was determined by the optical microscope. Fragments of the matrices were placed crosswise onto the object glass and the thickness was read using the micrometric scale.

The density measurements were performed in a pycnometer using 1 g sample of matrices and ethanol as the immersion fluid.

For water uptake studies, matrices of the area of about 1 cm^2 were weighed and transferred to the conical flasks filled with water. The flasks were tightly closed and placed at temperature of 37°C. After 24 h, matrices were removed from the liquid, blotted with paper-filter, and weighed. Water uptake capacity was expressed in percent.

Permeability test of the matrices for water vapor was performed as follows: the open neck (1.9 cm i.d.) of the conical flask containing 20 mL of water was tightly closed with a matrix. The system was weighed and the flask was placed in an air-conditioned chamber at a temperature of 37°C and relative humidity 50%. After 24 h, the flask was weighed again. The permeability was expressed as mg of water permeating for 1 h through 1 cm^2 matrix area.

In Vivo Skin Penetration Study

Each investigated formulation was studied using four male Wistar rats (200–220 g) under thiopental anesthesia (75 mg/kg). The hair of the dorsal surface was carefully removed with scissors; the IND containing matrix (2 × 5 cm^2) was applied and immediately secured with an adhesive tape (Leukotape, BDF, Hamburg, Germany). At times 0 (before application), 4, 6, 8,

and 24 h, blood samples (0.5 mL) were withdrawn from the tail veins into heparinized glass vessels with teflon caps (Chromacol, Trumbull, USA). The blood was stored frozen (–20°C) until analysis.

Determination of IND in Blood

IND was determined by HPLC (Merck-Hitachi, Tokyo, Japan) equipped with LiChrospher 100 RP-18 (5 μm) column (Merck, Darmstadt, Germany). Blood samples were mixed with 1.5 mL of methanol and 50 μL of methanolic solution of flufenamic acid (20 μg/mL) as an internal standard (IS). The samples were mechanically shaken for 15 min and centrifuged at 2,000 × g for 15 min (Heraeus, Osterode, Germany). The supernatant was filtered through 0.22-μm membrane filter (Sartorius, Goettingen, Germany) and evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 200 μL of the mobile phase. The mobile phase consisted of methanol and acetate buffer pH 3.6 (65:35 vol/vol) and the flow rate was 1 mL/min. About 50 μL sample volume was injected into the column; detection was performed at a wavelength of 320 nm. The retention times were 5.2 and 8.3 min for IND and IS, respectively. The specificity, accuracy, and linearity of the method were confirmed. The lower limits of detection and quantitation were 50 and 100 ng/mL, respectively.

Data Analysis

On the basis of the obtained experimental data, the following pharmacokinetics parameters for IND were calculated according to the well-known equations with the use of MS Excel spreadsheet: maximum drug concentration in the blood (c_{max}); time of reaching the maximum concentration (t_{max}); and area under concentration/time profile curve ($AUC_{0\rightarrow 24h}$), calculated by the trapezoid method. Results are expressed as the mean ± *SD* of four experiments. Statistical analysis for calculated pharmacokinetics parameters was performed using the Wilcoxon test and differences were considered significant at $p < .05$.

RESULTS AND DISCUSSION

Drug-in-adhesive matrix-type TTS composed of three different adhesive materials and loaded with 6 mg/cm^2 IND, and TERPs were prepared by casting of mixtures of dispersed or dissolved components onto the backing foil. The drug substance/terpene ratio (1:1) is often used for the percutaneous penetration of NSAID from semi-solid or liquid drug forms (Takayama et al., 1991). TERP was chosen as a penetration enhancer considering its small in vitro permeation through the skin, compared with other terpenes cumulation in the skin layers and the large enhancing effect on the IND permeation (Cal et al., 2001; Takayama et al., 1991). TTS with or without penetration enhancers were prepared.

The properties of the obtained adhesive layers such as content confirmation, solubility of drug substance in the adhesive layer, thickness, density, water uptake capacity, and permeability for the water vapor were examined (Table 2). The final concentrations of IND and TERP in matrices were about 6 mg/cm^2 (Table 2). The thickness of the matrices was within the range 140–290 μm for ET and PT, respectively, and the differences result in different diffusion pathway for drug substance and penetration enhancer. The highest solubility of IND in the adhesive layers (120 μg/cm^2) was determined for PT and P0 matrices, which were prepared on the basis of aqueous polyacrylate dispersion. For other adhesives, the solubility of IND was 1.5–4 times smaller and always lesser in the presence of TERP. The greatest water uptake capacity was found for matrices PT. This parameter is always higher when the matrix contains terpene. The permeability for water vapor is higher for PT and P0 matrices compared with ST and S0. In contrast to the water uptake capacity, the water vapor permeation did not depend on TERP content. The densities of ET, E0, PT, and P0 adhesives are similar (about 1 g/cm^3), but for ST and S0 matrices, they are 15 and 35% smaller, respectively. The water uptake and water vapor permeability tests were not performed for ET and E0 adhesive because they dissolved during the tests. The physicochemical data obtained show more clearly the hydrophilic properties of polyacrylate matrices (ET, E0, PT, and P0) compared with the silicone matrices (ST and S0).

TABLE 2
Physicochemical Properties of Adhesive Matrices

Parameter: Adhesive:	ET	E0	PT	P0	ST	S0
Indomethacin (IND) content (mg/cm^2)	5.9	5.8	5.8	5.8	5.8	5.9
TERP content (mg/cm^2)	6.4	–	6.2	–	6.0	–
IND solubility (μg/cm^2)	30	60	120	120	60	85
Thickness (μm)	140	180	290	280	180	240
Density (g/cm^3)	1.081	1.001	1.049	1.045	0.859	0.674
Water uptake (%)	(*)	(*)	41	30	31	12
Permeability for water vapor (mg/cm^2 h)	(*)	(*)	1.433	1.467	1.021	0.996

(*) – matrices dissolved during the test.

For all prepared TTS, in vivo skin penetration experiments were conducted. The systems (10 cm^2) were adhered onto the dorsal rats' skin, and the blood samples were collected at the specified time points up to 24 h. The presence of IND in the rats' blood was demonstrated for each TTS (Table 3, Figure 1). For most of the hydrophilic adhesives ET and E0, no significant effect of the penetration enhancer on IND absorption was observed. The c_{max} was reached after 6 h for ET and E0 and was about 2 μg/mL for both matrices. The constant blood concentration of IND during 24 h of application was demonstrated. The calculated AUC values are statistically similar and were 22 and 24 μg/mL/h for ET and E0, respectively. These unexpected parameters can be a result of the composition of adhesives. The ET and E0 matrices contain a few modifying excipients (adipic and lauric acids, glycerol) that can also act as potential penetration enhancers. The data obtained show a high partition coefficient ratio SC/matrix for IND, independent of the presence of TERP.

Among all the investigated TTS, the highest concentration of IND in blood was determined for PT adhesives, but the t_{max} was significantly longer than that of other matrices (Table 3, Figure 1). The AUC value for the PT system (43 μg/mL h) is about eight times greater than that of the control P0 (without penetration enhancer) and about two to seven times greater than that of other matrices. All above-mentioned differences are statistically significant. The PT and P0 matrices contained macrogol 1000 that has a melting point of 36°C. It probably caused the formation of the liquid phase during the contact of adhesive with the rats' skin surface. This process can result in increased solubility of the penetration enhancer and drug substance in SC and, in consequence, increased SC/matrices partition coefficient, for both IND and TERP.

The silicone adhesives (ST and S0) contained no additional compounds that can increase IND skin permeation. The solubility of IND in these matrices was in between the IND solubilities determined for both polyacrylate adhesives. The results of the tests of water uptake and permeability for water vapor show that they have less hydrophilic properties compared with the polyacrylate matrices. The IND c_{max} (1.1 μg/mL) after application of ST (with penetration enhancer) is about four times higher as compared with control S0, but it is unfortunately about 2–2.5 times lesser than that of polyacrylate systems containing a penetration enhancer. Excepting c_{max}, the influence of TERP on other pharmacokinetics parameters was not demonstrated. It seems that poor bioavailability of IND from the silicone matrices is a result of low partition coefficient SC/matrices for the lipophilic IND.

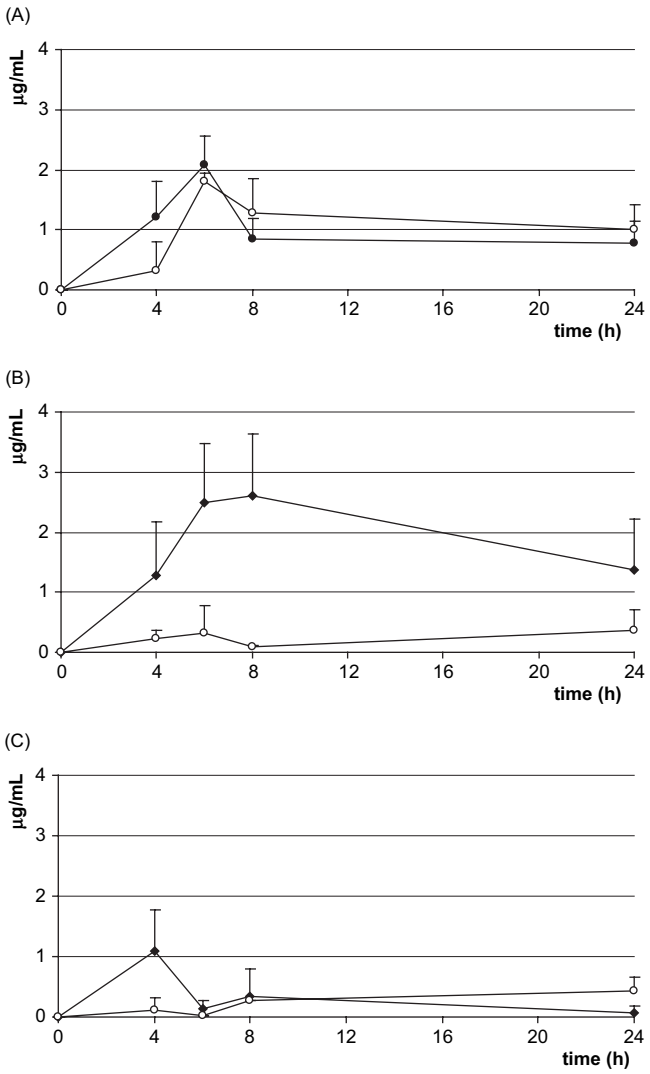

FIGURE 1. Concentration of indomethacin (IND) in blood during 24 h application of transdermal therapeutic systems (TTS) containing TERP (♦) or without (o) (mean ± *SD*, *n* = 4): (A) ET and E0; (B) PT and P0; (C) ST and S0.

At the end of each experiment, the macroscopic observation of skin being in the occlusion condition and subcutis was performed. Observations of rat skin did not show maceration of

TABLE 3
Calculated Indomethacin Pharmacokinetics Parameters

Parameter: System:	ET	E0	PT	P0	ST	S0
c_{max} (μg/mL)	2.1 ± 0.5	1.8 ± 0.1	2.6 ± 1.0	0.3 ± 0.4	1.1 ± 0.7	0.2 ± 0.2
t_{max} (h)	6.0	6.0	7.5 ± 1.0	6.0	4.0	4.0
$AUC_{0\rightarrow24h}$ (μg/mL h)	21.6 ± 4.2	24.0 ± 2.8	43.3 ± 11.6	5.1 ± 3.5	7.3 ± 4.1	6.2 ± 5.1

the skin; however, some changes in the fasciae tissue (noticeable as discoloration) under the application site, especially for the matrices containing TERP, were found.

Surprisingly, the significant positively effect of penetration enhancer on IND bioavailability from TTS was demonstrated only for PT-P0 matrices. It is well known that substances can permeate through the SC barrier when they are in a dissolved form (Smith & Surber, 1999). Among the investigated matrices, the drug substance was dissolved in the highest degree in the PT matrix; thus the dissolution of the drug substance in the carrier seems to be, from a technological point of view, one of the major factors influencing the skin permeation of substances.

In this study, the concentrations of IND in rats' blood are similar or lower than those reported in other studies on liquid or semisolid preparations containing this drug substance (Takayama et al., 1991). It is noteworthy that the proposed liquid or semisolid formulations with NSAID often contain ethanol (or other solvents) that acts not only as a solvent but also as a strong penetration enhancer or a co-enhancer. Terpenes–ethanol synergism in increasing skin permeation for drug substances has been discussed widely for years and has often been applied and practically used in commercial drug products (Okabe et al., 1992; Sugibayashi et al., 1995).

Summarizing presented results, it is possible to obtain TTS that deliver IND through the skin and that can ensure constant concentration of IND in the blood during at least 24 h. This effect may be used in the treatment of arthritis and myositis. The calculated pharmacokinetics parameters clearly show that among two different types of matrices, polyacrylate adhesives are more propitiative for IND delivering through the skin than silicone adhesive.

ACKNOWLEDGMENTS

Before the submission of this article, Professor Stanislaw Janicki passed away. The other authors wish to dedicate this article to his memory and lament the loss of such a highly regarded scientist and brilliant man.

REFERENCES

Cal, K., Janicki, S., & Sznitowska, M. (2001). In vitro studies on penetration of terpenes from matrix-type transdermal systems through human skin. *Int. J. Pharm., 224*, 81–88.

Cal, K. (2006). How does the type of vehicle influence the *in vitro* skin absorption and elimination kinetics of terpenes? *Arch. Dermatol. Res., 297*, 311–315.

Cal, K., Kupiec, K., & Sznitowska, M. (2006). Effect of physicochemical properties of cyclic terpenes on their ex vivo skin absorption and elimination kinetics. *J. Dermatol. Sci., 41*, 137–142.

Chang, J.-S., Tsai, Y.-H., Wu, P.-C., & Huang, Y.-B. (2007). The effect of mixed-solvent and terpenes on percutaneous absorption of meloxicam gel. *Drug Dev. Ind. Pharm., 33*, 984–989.

Gao, S., & Singh, J. (1997). Mechanism of transdermal transport of 5-fluo-rouracil by terpenes: carvone, 1,8-cineole and thymol. *Int. J. Pharm., 154*, 67–77.

Kang, L., Poh, A. L., Fan, S. K., Ho, P. C., Chan, Y. W., & Chan, S. Y. (2007). Reversible effects of permeation enhancers on human skin. *Eur. J. Pharm. Biopharm., 67*, 149–155.

Krishnaiah, Y. S. R., Satyanarayana, V., & Bhaskar, P. (2003). Influence of menthol and pressure-sensitive adhesives on the in vivo performance of membrane-moderated transdermal therapeutic system of nicardipine hydrochloride in human volunteers. *Eur. J. Pharm. Biopharm., 55*, 329–337.

Obata, Y., Ozawa, Y., Takayama, K., & Nagai, T. (1999). Effect of terpenes on skin permeation of non-ionized and ionized ketotifen. *STP Pharma Sci., 5*, 497–503.

Okabe, H., Takayama, K., & Nagai, T. (1992). Percutaneous absorption of ketoprofen from acrylic gel patches containing d-limonene and ethanol as absorption enhancers. *Chem. Pharm. Bull., 40*, 1906–1910.

Smith, E., & Surber, C. (1999). The absolute fundamentals of transdermal permeation (Drug delivery for dummies). In B. Gabard, P. Elsner, C. Surber, & P. Treffel (Eds.), *Dermatopharmacology of topical preparations* (pp. 23–35). Berlin: Springer.

Sugibayashi, K., Kobayashi, D., Nakagaki, E., Hatanaka, T., Inoue, N., Kusumi, S., Kobayashi, M., Kimura, M., & Morimoto, Y. (1995). Differences in enhancing effect of l-menthol, ethanol and their combination between hairless rat and human skin. *Int. J. Pharm., 113*, 189–197.

Takayama, K., Kikuchi, K., Obata, Y., Okabe, H., Machida, Y., & Nagai, T. (1991). Terpenes as percutaneous absorption promoters. *STP Pharma Sci., 1*, 83–88.

Takayama, K., & Nagai, T. (1994). Limonene and related compounds as potential skin penetration promoters. *Drug Dev. Ind. Pharm., 20*, 677–684.

Valenta, C., & Auner, B.G. (2004). The use of polymers for dermal and transdermal delivery. *Eur. J. Pharm. Biopharm., 58*, 279–289.

Williams, A. C., & Barry, B. W. (1991). Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. *Pharm. Res., 8*, 17–24.

Williams, A.C., & Barry, B.W. (2004). Penetration enhancers. *Adv. Drug Deliv. Rev., 56*, 603–618.