Percutaneous Absorption of Ketoprofen from Acrylic Gel Patches Containing *d*-Limonene and Ethanol as Absorption Enhancers

Hideaki OKABE,*[,a] Kozo TAKAYAMA,[b] and Tsuneji NAGAI[b]

Lintec Corporation, Research Laboratory,[a] Nishiki-cho, Warabi-shi, Saitama 335, Japan and Department of Pharmaceutics, Hoshi University,[b] Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan. *Received December 13, 1991*

The percutaneous absorption of ketoprofen (KPF) from gel patches containing *d*-limonene and ethanol was investigated in rats. Plasma levels of KPF varied with the kind of polymers which constitute the gel patch, and the highest level was observed when the copolymer of ethylacrylate (EA) and diethyleneglycolmethacrylate (DEGMA) was used as a vehicle. The amount of KPF permeating through the rat skin from the gel patch was well correlated with that of ethanol. Permeations were enhanced with increase in the amount of *d*-limonene distributed from the vehicle to the skin tissue. The amount of *d*-limonene accumulated in the skin varied greatly with the kind of polymers; the highest accumulation was observed with the EA–DEGMA copolymer, and decreased with increasing affinity of *d*-limonene to the polymers. The reason EA–DEGMA copolymer showed the highest percutaneous absorption of KPF from gel patches containing *d*-limonene may be the hydrophilic nature of this polymer which showed the lowest affinity to *d*-limonene.

Keywords percutaneous absorption; ketoprofen; *d*-limonene; ethanol; gel patch; acrylic polymer; hydrogel; adhesive tape

Introduction

Transdermal drug delivery is found as a desirable route for drug administration, however, many drugs do not penetrate the skin at a sufficient rate for therapeutic use. The stratum corneum of the skin is generally recognized as the main barrier limiting the entry of foreign substances and water loss.[1,2] One approach to the delivery of an effective dose of drug through the skin is to reduce temporarily the barrier function of the skin with the aid of absorption enhancers. Many reports are now available concerning basic investigations on absorption enhancers such as Azone[1,2] and its analogues,[3] pyrrolidones,[1,2] unsaturated fatty acids,[2] urea analogues and others. We reported the promotional effect of cyclic monoterpenes present in essential oils on the percutaneous absorption of the drugs indomethacin,[4] ketoprofen (KPF)[5] and diclofenac sodium.[6] Percutaneous absorption of these durgs was remarkably enhanced on addition of hydrocarbons such as limonene, menthane, terpinene and terpinolene in the presence of ethanol. In this work, we employed mainly gel ointments containing ethanol as test formulations. Such ointment is acceptable for topical application in practical use of compounds for their enhancing activity, though a solid vehicle is more desirable for systemic application. In this study, we investigated the preparation of acrylic gel patches in which sufficient amounts of *d*-limonene and ethanol can be loaded as absorption enhancers. The role of hydrophobic and hydrophilic components of the acrylic gel patches was determined to find suitable polymers which could enhance drug absorption. KPF was used as a model drug.

Materials and Methods

Materials Reactive monomers used in this study are listed in Table I. Laurylacrylate was supplied by Kyoeisha Chemical Co., Ltd. and diethyleneglycolmethacrylate was from Nippon Oil & Fats Co., Ltd. The other monomers and *d*-limonene were of reagent grade and were purchased from Tokyo Chemical Industries Co., Ltd. Trimethylolpropane triacrylate was purchased from Nippon Kayaku Co., Ltd. Benzyldimethylketal was purchased from Ciba-Geigy Corp and KPF from Wako Pure Chemical Industries Co., Ltd. Other chemicals used were of reagent grade.

Preparation of Adhesive Tapes Adhesives consisting of a copolymer of 95 parts 2EHA and 5 parts AA were prepared.[7] A glass reactor was charged with 2EHA, AA, ethylacetate and 0.05 mol% of 2,2′-azobisisobutyronitryl free radical initiator from Wako Pure Chemical Industries Co., Ltd. (concentration of monomer: 40% (w/w)). The reactor was purged with nitrogen and placed in a water bath at 60 °C for 12 h to prepare the polymer. KPF and *d*-limonene were dissolved in the polymer solution, and the mixture was coated on an aluminum-vapor-deposited polyester film (38 μm) to provide a dry coating thickness of 30 μm. After drying in an oven at 90 °C for 2 min, the coated film was laminated with a silicone coated polyester film (38 μm).

Prepration of Gel Patches The polymer sheet for hydrogel as drug reservoir was synthesized by photoradical polymerization of the comonomers. Trimethylolpropanetriacrylate as a crosslinking agent (0.1 mol%) and benzyldimethylketal (0.5 mol%) were dissolved in a mixture of a hydrophobic monomer and hydrophilic monomer (60 : 40 weight ratio). After the air in the solution was replaced with nitrogen gas, the mixture was photopolymerized for 10 min by an ultraviolet lamp (Eye Graphics

TABLE I. Acrylic Monomers Used in Preparation of Hydrogel

Hydrophobic monomer		Hydrophilic monomer	
Ethyl acrylate	(EA)	Acrylic acid	(AA)
Buthyl acrylate	(BA)	Hydroxyethyl acrylate	(HEA)
2-Ethylhexyl acrylate	(2EHA)	Diethyleneglycol methacrylate	(DEGMA)
Lauryl acrylate	(LA)	*N*,*N*-Dimethyl acryl amide	(DMAA)
		N-Vinly pyrrolidone	(VP)

TABLE II. Formulation of Impregnated Solution

	Control solution (without *d*-limonene)	Sample solution
Ketoprofen	10	10
d-Limonene	—	10
Ethanol	60	60
Water	30	20
	100 g	100 g

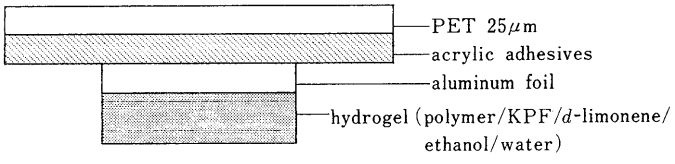

Fig. 1. Schematic Representation of Ketoprofen (KPF) Gel Patch Investigated in Percutaneous Absorption Experiment

Co., Ltd.; 6600 mJ/cm^2 irradiation). The polymer sheet prepared was cut into a disk (20 mm diameter and 2 mm height), and the remaining monomer was washed with a large amount of methanol/chloroform solution for 24 h. After drying in a vacuum, the hydrogel was prepared by immersing in an impregnating solution (Table II). The polymer disk was immersed for an appropriate time and weighed carefully after removing the residual solution on the surface with gauze. The total amount of impregnating solution loaded in each polymer disk was adjusted to $40 \pm 1\%$ (w/w) of the final weight of gel. A cross section of the gel patch is shown in Fig. 1.

Percutaneous Absorption through Rat Abdominal Skin After anesthetization with ethyl carbamate saline solution (25%; 3 ml/kg; i.p.), male Wistar rats (160—190 g) were secured on their backs and the hair on the abdominal skin was removed with an electric animal clipper. Adhesive tape or a gel patch was placed on the shaved skin (effective surface area was 3.14 cm^2 in both systems). Blood samples (0.5 ml) were taken *via* the jugular vein at 2, 4, 6 and 8 h after the administration.

Determination of KPF in Blood Samples After centrifugation of 0.5 ml of blood sample, plasma (200 μl) was removed and thoroughly mixed with methanol (500 μl) containing an appropriate amount of *p*-hydroxybenzoic acid *n*-butyl ester as an internal standard. This mixture was again centrifuged for 5 min. The supernatant solution was filtered using a desposable filter unit (Gelman Science Japan, Ltd., Ekikuro-Disk 3CR), and the concentration of KPF in the filtrate was determined by high performance liquid chromatography (HPLC) (Waters HPLC model 510—490). Ultraviolet detection at 254 nm was employed; the column (4.6 × 150 mm) was packed with Nucleosil 10-C18 (M. Nagel, Germany), and elution was at room temperature with a mobile phase consisting of a mixture of 0.1% phosphoric acid and methanol (35 : 65); the flow rate was 1 ml/min.

Determination of Swelling Ratio of Copolymers in *d*-Limonene and Ethanol The polymer disk prepared as above was immersed in a sufficient amount of *d*-limonene or ethanol, respectively. Following 1 h immersion, the disk was carefully weighed after removing *d*-limonene or ethanol from the surface. Swelling ratio was calculated as follows:

$$\text{swelling ratio} = \frac{\text{weight after immersion} - \text{weight before immersion}}{\text{weight before immersion}} \times 100\ (\%)$$

Determination of KPF, *d*-Limonene, Ethanol and Water in Hydrogel Weighed hydrogel was immersed in a mixture of methanol and acetone (4 : 1 weight ratio; approximately 100 times the weight of hydrogel) for 24 h. The amount of KPF extracted was determined by HPLC (Hitachi HPLC, model L6000). *d*-Limonene and ethanol were extracted by immersing the hydrogel in toluene (approximately 100 times the weight of hydrogel) for 24 h. Extracted amounts were determined by gas chromatography (GC) (Hitachi Gas Chromatogram model 163) using ethylcelosolve as an internal standard. The column (3 mm × 4 m) was packed with LAC-2R 446 (GL Science Co., Ltd.), using a nitrogen carrier gas (30 ml/min) with an injection temperature of 200 °C and column temperature of 120 °C. Water content was determined by subtracting the extracted amounts of KPF, *d*-limonene and ethanol from the total amount initially loaded in the hydrogel.

***In Vitro* Experiment on Permeation through Rat Skin** The abdominal skin of rats (male Wistar, weighing 160—190 g) was shaved with animal clippers. After sacrificing the animals with ether inhalation, this skin was excised and mounted in Franz type cells[8] as the permeation membrane (permeation area was 1.76 cm^2). Gel patches containing KPF, *d*-limonene and ethanol were applied to the skin as the donor side, and the receiver side was filled with aqueous phosphate buffer (pH = 7.2, 16 ml). The receiver solution was stirred with a magnetic stirrer and permeation was studied at 37 °C in a water bath. Receiver solutions were withdrawn 8 h after the experiment began. The amounts of permeated KPF and ethanol were determined by HPLC and GC, respectively.

Determination of Ethanol and *d*-Limonene in the Skin Rat skin used as the permeation membrane was taken immediately after the experiment, cut into an appropriate size and weighed after patting with gauze. The skin was dipped in toluene, and the amounts of ethanol and *d*-limonene extracted by the toluene solution were determined by GC as described previously.

Results and Discussion

Percutaneous Absorption of KPF from Adhesive Tapes and Gel Patches

Figure 2 shows plasma concentration of KPF after the application of adhesive tapes containing this drug. Percutaneous absorption of KPF was not enhanced by the addition of *d*-limonene. Although we reported an impressive enhancement of *d*-limonene on the percutaneous absorption of KPF in gel ointment,[5] there was no promoting activity of *d*-limonene in the matrix (adhesive/KPF/*d*-limonene), suggesting that ethanol is an essential adjuvant to obtain the activity of *d*-limonene. Although ethanol is known to promote the percutaneous absorption of drugs,[9,10] even greater enahncement is required for the development of a transdermal drug delivery system because the absorption of many drugs through human skin is low.

We reported the combined effect of *d*-limonene with ethanol on the percutaneous absorption of indomethacin.[11] The absorption of indomethacin through rat skin greatly increased with increasing ethanol concentration (from 30 to 50%) in a gel ointment containing *d*-limonene. In the present study, therefore, we investigated the preparation of acrylic polymers in which sufficient amounts

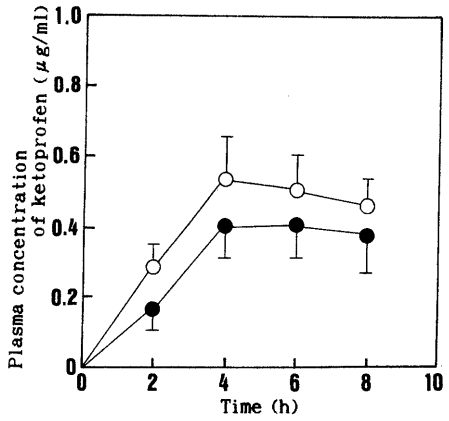

Fig. 2. Percutaneous Absorption of Ketoprofen from Acrylic Adhesive Tapes in Rats

Each point represents the mean ± S.E. of four determinations. ●, containing 4% ketoprofen and 2% *d*-limonene; ○, control (without *d*-limonene).

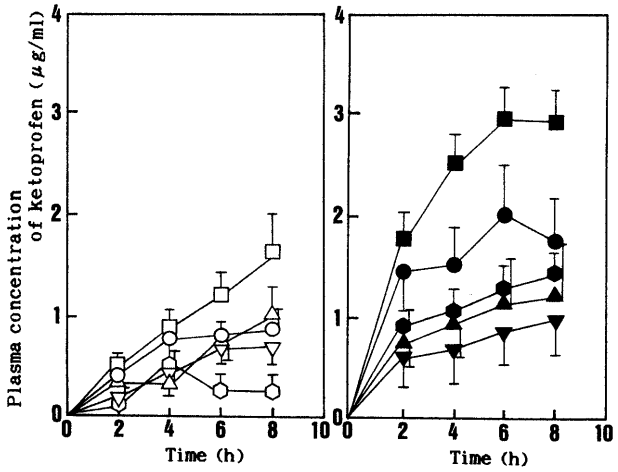

Fig. 3. Percutaneous Absorption of Ketoprofen from Gel Patches Consisting of 2EHA and Hydrophilic Monomers (6 : 4 in Weight) in Rats

Each point represents the mean ± S.E. of three to five determinations. Open symbols are control without *d*-limonene and closed symbols are the gel patch containing *d*-limonene. ○, ●, 2EHA–AA; ▽, ▼, 2EHA–DMAA; △, ▲, 2EHA–HEA; ⬡, ⬢, 2EHA–VP; □, ■, 2EHA–DEGMA.

TABLE III. The Concentration of Additives in the Hydrogel Prepared by Impregnating Method

	Without *d*-limonene				With *d*-limonene				
	Concentration of additives (w/w%)			Total weight of hydrogel (g)	Concentration of additives (w/w%)				Total weight of hydrogel (g)
	Ketoprofen	Ethanol	Water		Ketoprofen	Ethanol	Water	*d*-Limonene	
2EHA–AA	6.2±0.9	13.8±3.2	20.4±3.8	0.73±0.01	3.5±0.2	13.5±1.0	13.6±1.0	9.8±0.4	0.74±0.02
2EHA–HEA	7.0±0.6	13.0±1.7	19.8±1.9	0.78±0.02	4.4±1.0	11.7±0.3	7.4±0.9	16.3±1.4	0.79±0.04
2EHA–DMAA	9.4±1.4	23.5±3.0	7.6±4.3	0.73±0.02	5.1±1.1	12.2±1.8	7.5±2.0	15.6±0.9	0.74±0.02
2EHA–VP	10.0±0.7	14.3±0.7	16.0±1.2	0.75±0.02	5.0±0.4	11.7±0.5	2.7±0.6	20.8±1.6	0.76±0.05
EA–DEGMA	5.4±0.3	22.6±3.1	12.0±3.1	0.75±0.02	5.1±0.7	14.1±1.4	15.2±2.8	5.5±0.8	0.72±0.03
BA–DEGMA	6.1±0.6	19.5±1.4	14.3±2.1	0.76±0.02	4.4±0.5	12.0±0.6	13.8±0.5	10.2±1.1	0.77±0.05
2EHA–DEGMA	6.0±0.3	14.6±1.8	19.5±2.3	0.71±0.01	3.9±0.2	12.8±1.2	5.2±2.4	18.4±1.9	0.67±0.06
LA–DEGMA	5.6±0.6	14.1±2.0	20.5±2.5	0.70±0.03	3.6±0.6	12.1±0.2	1.2±0.8	23.5±0.6	0.74±0.05

Values are the mean±S.D. of three determinations.

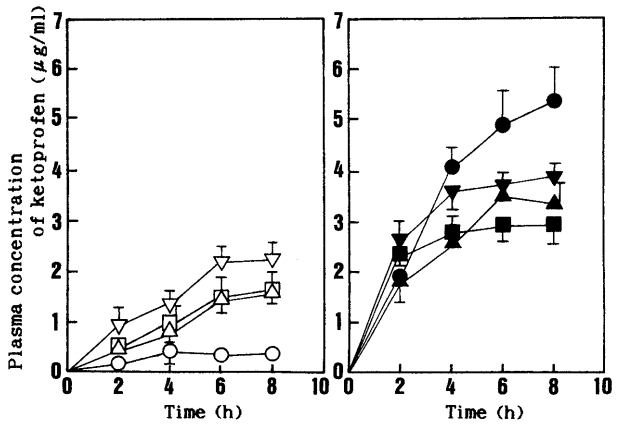

Fig. 4. Percutaneous Absorption of Ketoprofen from Gel Patches Consisting of Hydrophilic Monomers and DEGMA (6:4 in Weight) in Rats

Each point represents the mean±S.E. of three to five determinations. Open symbols are control without *d*-limonene and closed symbols are the gel patch containing *d*-limonene. ○, ●, EA–DEGMA; □, ■, 2EHA-DEGMA; △, ▲, BA–DEGMA; ▽, ▼, LA–DEGMA.

of ethanol and *d*-limonene can be loaded.

It is difficult for most acrylic adhesives to hold ethanol or water because of their high lipophilicities. In order to keep such hydrophilic additives in the matrix, we prepared an acrylic polymer which was composed of a large amount of hydrophilic component. Percutaneous absorption of KPF from gel patches synthesized by the combination of 2 EHA (hydrophobic component) and various hydrophilic monomers is shown in Fig. 3. Pronounced enhancement was observed in the presence of *d*-limonene, though there was wide variation in the amount of each compoment impregnated in the hydrogels (Table III). The highest plasma concentration was observed with the gel patch using DEGMA as a hydrophilic component. Even though the amount of KPF loaded was high in hydrogel using DMAA or VP as a hydrophilic component, the plasma level of KPF was rather low. This may be due to the interaction between the amino group of the hydrophilic component and the carboxylic group of KPF. Percutaneous absorption of KPF from gel patches using DEGMA (hydrophilic component) with various hydrophobic compounds is shown in Fig. 4. Without *d*-limonene, the plasma concentration of KPF increased with increasing lipophilicity of the polymers. Highest plasma concentration was observed when the copolymer with the lowest lipophilic property was used as a vehicle of the gel patch containing *d*-limonene, suggesting that the percutaneous absorption of drug and the activity of the enhancer depend on the physicochemical nature of the polymer used.

TABLE IV. Permeation of Ketoprofen and Ethanol through the Rat Skin and Accumulation of Ethanol and *d*-Limonene in the Tissue

	Amount of ketoprofen permeated (μg/cm²)	Amount of ethanol permeated (mg/cm²)	Concentration in the skin tissue (mg/g rat skin)	
			Ethanol	*d*-Limonene
2EHA–AA	352.4± 17.6	29.6±3.4	14.8±1.5	5.9±1.7
2EHA–HEA	556.9± 27.8	36.7±2.6	12.6±4.2	5.6±2.0
2EHA–DMAA	287.5± 42.0	23.9±1.0	13.4±0.3	3.2±0.2
2EHA–VP	215.0±102.3	26.2±1.7	11.5±3.0	3.5±1.2
EA–DEGMA	596.3± 79.5	45.5±1.5	17.0±2.9	14.3±2.6
BA–DEGMA	440.2± 46.6	34.2±9.6	11.0±0.8	4.3±1.3
2EHA–DEGMA	419.7±132.4	33.3±2.7	12.3±1.7	2.2±0.3
LA–DEGMA	240.7± 24.3	19.6±2.1	10.4±2.3	1.5±0.3

Values are the mean±S.D. of three determinations.

Skin Permeation Experiment and Partitioning of *d*-Limonene into the Skin The effect of the physicochemical properties of polymers on the percutaneous absorption of KPF were elucidated in permeation experiments of KPF and ethanol from the gel patches through rat skin and partitioning of *d*-limonene into the skin. The amount of KPF and ethanol permeated, and the concentration of ethanol and *d*-limonene in the skin tissue are summarized in Table IV. Values varied with the polymer formulation, and the highest permeation of KPF and ethanol was observed with the EA–DEGMA copolymer. *d*-Limonene permeation was not determined because of the very low solubility of *d*-limonene in buffer solution (<1.763 μg/ml), suggesting that *d*-limonene could not penetrate the skin, though it did accumulate in the lipophilic part of the skin. The highest accumulation of ethanol and *d*-limonene in the skin tissue was seen with EA–DEGMA copolymer. The amount of KPF permeated correlated well with that of ethanol as shown in Fig. 5 ($r=0.935$). If the interaction between the polymer and KPF is negligible, the percutaneous absorption of KPF is thought to be primarily dependent on ethanol. Figure 6 shows the relationship between the amount of *d*-limonene accumulated in the rat skin tissue and the amount of permeating ethanol. Ethanol

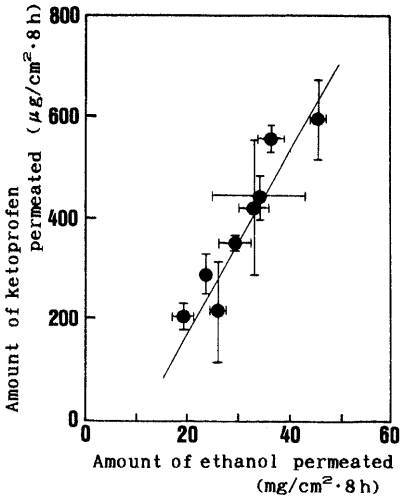

Fig. 5. Relationship between Amount of Ethanol and of Ketoprofen Permeated through Rat Abdominal Skin

Each point represents the mean±S.D. of four determinations at 8 h after the experiment began.

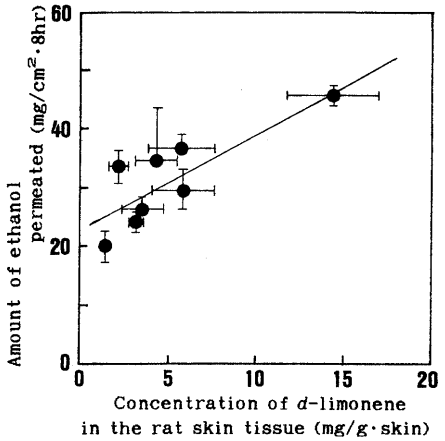

Fig. 6. Relationship between Concentration of *d*-Limonene in Tissue and Amount of Ethanol Permeated through Rat Abdominal Skin at 8 h after the Experiment Began

Each point represents the mean±S.D. of four determinations.

permeation increased with increasing concentration of *d*-limonene in the skin ($r=0.820$). Therefore, the partitioning of *d*-limonene and ethanol from a gel patch to the skin is one of the most important factors in the enhancement of drug absorption. This also suggests that the affinity between *d*-limonene and the polymer, and also between ethanol and polymer, has considerable effect on the percutaneous absorption of a drug. We then measured the swelling of the polymers in a sufficient amount of *d*-limonene and ethanol, respectively, to learn the affinity of *d*-limonene and ethanol to each polymer. Figure 7 shows the relationship between the amount of *d*-limonene in rat skin tissue and the swelling ratio of each polymer in *d*-limonene. The partitioning of *d*-linomene into the skin decreased with increase in the affinity of this substance to the polymer. No correlation was observed, however, between ethanol concentration in the skin tissue and swelling ratio of a polymer in ethanol (data not shown). These results strongly imply the importance of employing an appropriate polymer capable of distributing an adequate amount of *d*-limonene to skin tissue in a successful transdermal drug delivery system. In the design of the gel patch containing *d*-limonene and ethanol as absorption enhancers, a hydrophilic polymer such as EA–DEGMA is advantageous to enhance percutaneous absorption of a drug.

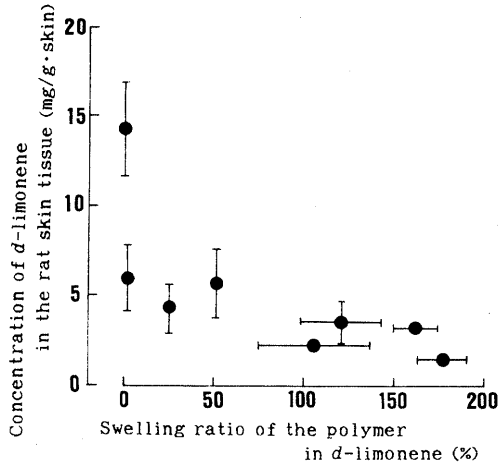

Fig. 7. Relationship between Swelling Property of the Polymer in *d*-Limonene and Concentration of *d*-Limonene in the Rat Skin Tissue

Each point represents the mean±S.D. of four determinations.

Conclusion

Ethanol and *d*-limonene are important additives and are indispensable for enhancing the percutaneous absorption of KPF. The partitioning of *d*-limonene, a lipophilic molecule, into the skin is strongly affected by the physicochemical property of the polymer used. A more hydrophilic polymer proved beneficial in distributing *d*-limonene in the skin. In fact, the highest plasma level and highest enhancing effect were observed with the EA–DEGMA copolymer, which has the lowest affinity to *d*-limonene among those tested; absorbed amounts of ethanol and *d*-linonene were also greatest with this copolymer.

In practice, however, the adhesiveness to the skin as a viscoelastic property is significant property required for materials used in a transdermal therapeutic system, since the area of effective drug diffusion depends on this to some extent. Unfortunately, the EA–DEGMA copolymer lacks this property of adhesiveness, and future work must focus on this.

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