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The Effect of Vehicles and Pressure Sensitive Adhesives on the Percutaneous Absorption of Quercetin through the Hairless Mouse Skin

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To investigate the feasibility of developing a new quercetin transdermal system, a preformulation study was carried out. Therefore, the effects of vehicles and pressure-sensitive adhesives (PSA) on the *in vitro* permeation of quercetin across dorsal hairless mouse skin were studied. Among vehicles used, propylene glycol monocaprylate (PGMC) and propylene glycol monolaurate were found to have relatively high permeation flux from solution formulation (i.e., the permeation fluxes were 17.25±1.96 and 9.60±3.87 μg/cm^2/h, respectively). The release rate from PSA formulations followed a matrix-controlled diffusion model and was mainly affected by the amount of PSA and drug loaded. The overall permeation fluxes from PSA formulations were less than 0.30 μg/cm^2/h, which were significantly lower compared to those obtained from solution formulations. The lower permeation fluxes may be due to the decrease of solubility and diffusivity of quercetin in the PSA layer, considering the fact that the highest flux of 0.26 μg/cm^2/h was obtained with the addition of 0.2% butylated hydroxyanisole in PGMC-diethylene glycol monoethyl ether co-solvents (80-85 : 15-20, v/v). Taken together, these observations indicate that improvement in the solubility and diffusivity of quercetin is necessary to realize fully the clinically applicable transdermal delivery system for the drug.

Key words: Quercetin transdermal system, Vehicles, Pressure-sensitive adhesives, Release, Permeation

INTRODUCTION

Quercetin is a flavonoid (3,5,7,3',4'-pentahydroxyflavon) which is phenolic compound widely found in vegetable food (Hertog *et al.*, 1993; Stavric, 1994). Flavonoids are known to delay oxidant injury and cell death by scavenging reactive oxygen species such as superoxide anion (O_2·), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2) (Arora *et al.*, 1998; Silva *et al.*, 2002). Quercetin especially has been shown to scavenge O_2·, singlet oxygen ($^1O_2$) and ·OH radicals, to prevent lipid peroxidation, to inhibit cyclooxygenase and lipoxygenase enzymes, and to chelate transition metal ions (Formica and Regelson, 1995; Gordon and Roedig-Penman, 1998). The antioxidant activity of flavonoids, including quercetin, has paid attention because of their potential application in an anticancer therapy (Lamson and Brignall, 2000). In addition, this class of compounds was reported to prevent oxidative stress-related chronic diseases such as ischemic heart disease and diabetes (Hollman and Katan, 1997; Skibola and Smith, 2000), suggesting the applicability in the prevention of oxidative injuries. Despite these potentials, however, quercetin has poor bioavailability with oral administration due to its extensive metabolism and low absorption in the gastrointestinal tract, which limits the further development efforts. Consistent with the *in vivo* observations, we demonstrated that quercetin did not permeate through rabbit duodenal mucosa in spite of improving solubility and/or employing permeation enhancers (Chun and Suh, 1998). Currently, strategies for the improvement in the biopharmaceutical characteristics has not been devised for quercetin.

In general, transdermal delivery system has been paid attention as an alternative dosage form to oral delivery (Gwak and Chun, 2002; Gwak *et al.*, 2002; Nokhodchi *et al.*, 2003). In addition to the avoidance of metabolism in

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the gastrointestinal tract and the liver, the transdermal delivery system has several advantages including the maintenance of the blood level of drug for an extended period of time and a ready discontinuation of drug input thereby reducing side effects. Despite these advantages, however, only a limited number of drugs can be administered percutaneously due to low skin permeability of most drugs through skin. Furthermore, this route of flavonoid administration has not been studied in the literature. The objective of this study, therefore, was to assess the feasibility of development of the transdermal delivery system for quercetin, a model flavonoid, by examining the physicochemical properties relevant in the transdermal delivery of the drug. We were particularly interested in the examination of the effect of vehicles and pressure-sensitive adhesives on the *in vitro* permeation of the drug through dorsal hairless mouse skin.

MATERIALS AND METHODS

Materials

Quercetin, rutin, oleic acid, lauric acid, linoleic acid and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Propylene glycol monolaurate (PGML, Lauroglycol® 90), propylene glycol laurate (PGL, Lauroglycol® FCC), propylene glycol monocaprylate (PGMC, Capryol® 90), diethylene glycol monoethyl ether (DGME, Transcutol® P), caprylocaproyl macrogol-6 glycerides (LBS, Labrasol®), polyethylene glycol-8 glyceryl linoleate (LBF 2609, LBF WL 2609 BS) were obtained from Gattefossé (Gennevilliers Cedex, France). Propylene glycol (PG), oleyl alcohol and ethanol were of analytical grade. Methanol used was of HPLC grade. Other reagents were of analytical grade.

Acrylic pressure-sensitive adhesive (PSA) solutions in organic solvents which were Duro-Tak® 87-2196 (copolymer: acrylate-vinylacetate, functional group: -COOH, 45% solution of self-crosslinking acrylic copolymer, 3000 cps, solubility parameter 16), Duro-Tak® 87-2287 (copolymer: acrylate-vinylacetate, functional group: -OH, 50.5% solution of non-crosslinking acrylic copolymer, 18000 cps, solubility parameter 16) and Duro-Tak® 87-2510 (copolymer: acrylate, functional group: -OH, 40.5% solution of non-crosslinking acrylic copolymer, 4500 cps, solubility parameter 16) were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA).

Analysis

Samples from release and permeation studies were analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (Series 410, Perkin-Elmer, USA) with a detector (Model LC 90, Perkin-Elmer, U.S.A.) set at 375 nm and an integrator (Model 4290, Varian, USA). An ODS column (μ Bondapak C18, 3.9 ×300 mm, 10 μm, Waters, USA) equipped with a C18 Radial Pak insert was used. The mobile phase was composed of methanol, water and acetic acid (58 : 42 : 1, v/v) and delivered at a flow rate of 1.2 mL/min. The injection volume was 20 μL. The internal standard used was rutin (20 μg/mL). A calibration curve was constructed based on peak area measurements.

Preparation of quercetin solution formulation and transdermal system

To determine the effects of various vehicles and enhancers on the permeation of quercetin, appropriate amounts of quercetin were dissolved in pure solvent or co-solvents. The permeation experiment was then carried out (see below).

When it was necessary to study the permeation of quercetin across the skin in the presence of PSA, an experimental transdermal formulation was developed and permeation study was carried out. Therefore, an appropriate amount of quercetin was dissolved in various pure solvents or co-solvents and then mixed with three types of acrylic adhesive solutions: Duro-Tak® 87-2196, Duro-Tak® 87-2287 and Duro-Tak® 87-2510. Quercetin transdermal system was prepared by casting the above solutions on a polyester release liner coated with silicone (Gelroflex ALU-PET 100 μ-2S DR, 3M, USA) using a casting knife. The area of the cast solutions was 10 cm×7 cm. They were set at room temperature for 4 h to evaporate the solvents, and then dried overnight in an oven set at 37°C. The dried film was transferred onto a backing film (Scotchpak 1109, 3M, USA). Table I shows formulation compositions for the preparation of quercetin transdermal system.

Procedure for release studies from quercetin transdermal system

Quercetin transdermal system was mounted on a side-by-side permeation system (Valia-Chien Permeation System, Crown Bioscientific Inc., NJ, USA); the drug loaded-layer was in contact with the receptor compartment. In this set-up, the area of cell openings was 0.64 cm^2. Receptor compartment cells were filled with 40% PEG 400 in saline and the media were stirred by a Teflon-coated magnetic bar for an adequate mixing. The release media were maintained at 32°C. At predetermined time intervals, 100 μL of receptor solutions were collected, and mixed with 100 μL of internal standard solution. The amount of quercetin released from various PSA transdermal systems was, then, determined by HPLC.

Procedure for skin permeation *in vitro*

Male hairless mice aged 6~8 weeks were purchased from Samtako Bio Korea Co., Ltd. (Osan, Korea). After

Table I. Formulation compositions for the preparation of quercetin transdermal delivery system

FN	Amount loaded (mg)	Vehicles (v/v)	PSA (Duro-Tak®)	Additives
01	15	PGL:DGME = 7:3	87-2196	-
02	30	PGL:DGME = 7:3	87-2196	-
03	60	PGL:DGME = 7:3	87-2196	-
04	30	PGL:DGME = 7:3	87-2196[a)]	-
05	30	PGL:DGME = 7:3	87-2287	-
06	30	PGL:DGME = 7:3	87-2510	-
07	30	PGL:DGME = 8:2	87-2196	-
08	30	PGMC:DGME = 8:2	87-2196	-
09	30	PGMC:DGME = 8:2	87-2196	3% linoleic acid
10	30	PGMC	87-2196	0.2% BHA
11	30	PGMC:DGME = 95:5	87-2196	0.2% BHA
12	30	PGMC:DGME = 90:10	87-2196	0.2% BHA
13	30	PGMC:DGME = 85:15	87-2196	0.2% BHA
14	30	PGMC:DGME = 80:20	87-2196	0.2% BHA

One milliliter of vehicle containing quercetin was mixed with 4 g of each PSA and spread at the thickness of 0.51 mm except for [a)] which 1 mL of vehicle was added to 6 g of PSA and spread at 0.71 mm. FN: Formulation number.

sacrificing hairless mouse with ether, the dorsal skin was carefully excised. Quercetin solution formulation or transdermal system of an appropriate size was applied to the epidermal side of the skin, and mounted on a side-by-side permeation system; the dermal side was in contact with the receptor compartment. Receptor compartment cells were filled with 40% PEG 400 in saline and the media were stirred by a Teflon-coated magnetic bar for an adequate mixing. The permeation media were maintained at 32°C. At predetermined time intervals, 100 μL of receptor solutions were collected, and mixed with 100 μL of internal standard solution. The amount of quercetin permeated was, then, determined by HPLC.

Data analysis

The steady-state flux (J_s), lag time (T_L), diffusion coefficient (D), skin/vehicle partition coefficient (K), and apparent permeation coefficient (P_{app}) can be described by equations 1-3 (Barry, 1983).

$$J_s = (dQ/dt)_{ss} \cdot 1/A = DKC/h \quad (1)$$

$$D = h^2/6T_L \quad (2)$$

$$P_{app} = dQ/dt \cdot 1/A \cdot 1/C_s \quad (3)$$

A : the effective diffusion area
h : the thickness of skin
C : the constant concentration of the donor solution
C_s : the drug concentration in the saturated solution
$(dQ/dt)_{ss}$: the steady-state slope

RESULTS AND DISCUSSION

Effect of vehicles

The permeation parameters of quercetin from different vehicles across the excised hairless mouse skin were listed in Table II. Among various types of vehicles, ester-type vehicles showed relatively high enhancing effects. Especially, PGMC resulted in the highest enhancing effect when the drug concentration was fixed at 30 mg/mL. Using equations 1-3, diffusivity, apparent permeation coefficient and skin/vehicle partition coefficient were evaluated. Compared to PGML, PGMC had a higher solubilizing property for quercetin, which was reported to be 2.87 and 8.24 mg/mL in our earlier study, respectively (Gwak *et al.*, 2004). Also, PGMC had a relatively short lag time compared to PGML, which PGMC had a higher diffusivity (equation 2). In contrast, the ether-type vehicle, DGME, was found to have a very low permeation flux even though the lag time was smaller than that for PGMC. The extremely low permeability was attributable to its very high solubility (291 mg/mL), and, thus, the thermodynamic activity is expected to be very low at the fixed concentration of 30 mg/ml (i.e., the concentration used in the permeation experiment). In the literature, DGME has been used as a penetration enhancer due to its ability to ease the partitioning by increasing the solubility of a compound in the skin (Cho and Choi, 1998). When DGME was added to PGL, the solubility of quercetin was found to be 4.60 mg/mL. As expected, the permeability was also enhanced by the addition.

As depicted in Fig. 1, permeation flux vs. DGME content curve was bell-shaped. The solubility of quercetin in the binary co-solvent system increased with the concentration of DGME [i.e., 0% DGME (4.60 mg/mL), 10% DGME (11.6 mg/mL), 20% DGME (32.7 mg/mL), 30% DGME (70.7 mg/mL), 40% DGME (74.3 mg/mL), 50% DGME (129.8 mg/mL), 60% DGME (159.3 mg/mL), 70% DGME (188.9 mg/mL) and 100% DGME (291.0 mg/mL)]. The

Table II. Permeation flux and lag time of quercetin through excised hairless mouse skin from various vehicles

Vehicles	J_s (μg/cm²/h)	T_L (h)
Ethanol	1.09 ± 0.47	1.97 ± 0.22
PG	0.07 ± 0.05	3.38 ± 0.23
PGL	2.01 ± 0.47	6.32 ± 0.87
DGME	0.86 ± 0.19	5.96 ± 0.63
PGML	9.60 ± 3.87	11.08 ± 1.96
PGMC	17.25 ± 1.96	4.16 ± 0.77
LBF 2609	1.42 ± 0.34	10.87 ± 2.36
LBS	0.02 ± 0.01	NA

Data were expressed as the mean ± S.D. (n = 3).
NA: not available

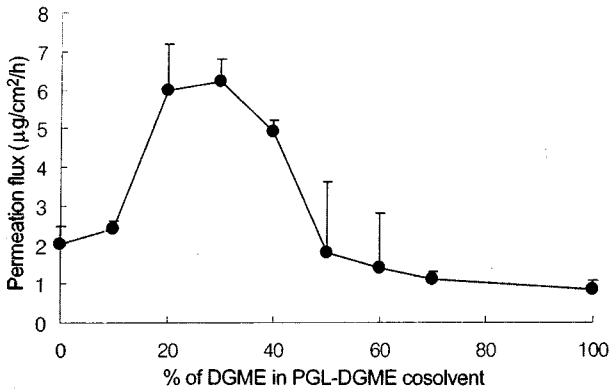

Fig. 1. Effect of DGME content (v/v %) in PGL-DGME co-solvents on the permeation of quercetin from solution formulation (n = 3).

permeation flux increased with the drug solubility up to 30% of DGME content, and the flux decreased thereafter, indicating an optimum concentration of DGME exists for the permeation of the drug. One of the plausible explanations for the subsequent decrease in the flux may be the decreased thermodynamic activity of the drug in such a high content of DGME in the binary system. Therefore, the suitable solubilization and the optimal thermodynamic activity have to be balanced for the vehicle for the drug. In addition to the change in driving force, permeation profiles can be affected by the alteration in the skin barrier property. In the literature, several mechanisms have been suggested for the modification of the skin barrier property: the reduction of skin resistance as a permeability barrier by disruption of tightly packed lipid regions of stratum corneum (Barry, 1987); increased skin/vehicle partitioning of the drug (Green *et al.*, 1988); increased solvent transport into or across the skin (Yamada *et al.*, 1987). Therefore, one of the mechanisms may also involved in the enhanced flux of the drug in the presence of DGME.

Fatty acids and fatty alcohols, compounds that are believed to affect the skin barrier function, have been employed as enhancers for a number of compounds including salicylic acid (Cooper, 1984), acyclovir (Cooper *et al.*, 1985), naloxone (Aungst *et al.*, 1986) and tegafur (Lee *et al.*, 1993). To study the impact on the skin permeation of quercetin in the presence of these enhancers, three fatty acids [one saturated fatty acid, viz, lauric acid (C12) and two unsaturated fatty acids, viz, oleic acid (C18 with one double bond) and linoleic acid (C18 with two double bonds), and one fatty alcohol (oleyl alcohol)] were selected and tested for the permeation enhancing activity. Thus, these fatty acids were respectively added to 40% DGME in saline at the concentration of 1%. In this study, the drug concentration in the donor compartment was set at 2 mg/mL. As described in Table III, without the enhancer, quercetin was not readily permeable across the skin as evidenced by the fact that the drug was not detectable in the receptor compartment for 24 h. With addition of enhancers, quercetin permeability was apparently enhanced since the drug was readily detected in the receptor compartment. Among the enhancers studied, linoleic acid showed the highest enhancing effect; the cumulative amount permeated after 24 h was about 75 and 5 times compared that of oleyl alcohol and oleic acid, respectively.

Table III. The effect of fatty acids and a fatty alcohol on the cumulative amount of quercetin permeated after 24 h through hairless mouse skin from 40% DGME in saline

Enhancers	Q_{24} (μg/cm²)	Detectable time (h)
None	–[a)]	–
Oleyl alcohol	4.3 ± 0.5	12
Oleic acid	57.6 ± 12.6	6
Lauric acid	83.4 ± 9.4	12
Linoleic acid	297.4 ± 12.0	2

Data were expressed as the mean ± S.D. (n = 3).
Q_{24}: Amount of quercetin permeated after 24 h.
[a)] Not detected.

Effect of PSAs

In designing a transdermal drug delivery system, it is essential to find an appropriate vehicle which solubilizes the target drug, mixes well with PSA, and/or possesses a good permeation profile. From our previous study using solution formulations of quercetin, PGMC and PGML showed a relatively high permeation fluxes. For the case of PGML, however, the lag time of permeation was somewhat prolonged. Thus, PGMC and PGL were selected to examine the effect of PSAs on the permeation of quercetin. DGME was also used as the co-solvent at the concentration of 5-30% since the addition was found to increase the drug solubility.

To evaluate the peeling property, quercetin transdermal systems were prepared by mixing drug solutions in vehicles such as PGMC alone, PGL-DGME and PGMC-DGME co-solvents with three types of PSAs: Duro-Tak® 87-2196, Duro-Tak® 87-2287, and Duro-Tak® 87-2510. With increasing proportions of DGME and quercetin in the co-solvents, the cohesive property was reduced and became excessively sticky. Good peeling and adhesive properties were obtained by using combinations of Duro-Tak® 87-2196 and PGMC containing not more than 20% of DGME (FN 10~14). Other PSAs did not found to have a good peeling property due to reduced cohesive property.

Before a drug permeates across the skin, the drug should be appropriately released from matrix. In addition to the release, the drug has to be properly diffused out across the PSA layer and released. To study this aspect, we studied the release of quercetin from PSA matrices.

Fig. 2 depicts the release profile of quercetin from PSA matrices containing PGL-DGME binary systems. A matrix-controlled diffusion model ($Q' = k't^{1/2}$, Q': amount released, k': release rate constant) (Chien and Lambert, 1974) was established for all the PSA amounts studied; the releases from all transdermal systems tested were proportional to the square root of time. The release rate of the drug increased (i.e., 16 to 32.21 μg/cm^2/h$^{1/2}$ for 15 and 30 mg of the dose, respectively) with the loading dose of the drug. In addition, the release rate decreased with the increase in PSA amount (i.e., 32.21 vs. 5.07 μg/cm^2/h$^{1/2}$ release rates for 4 and 6 g of PSA, respectively) even though the thickness of the matrix increased. It is possible that the decreased release of the drug by the large amount of PSA may be associated with the decreased diffusion and decreased drug solubility in the PSA. The ratio of DGME to PGL did not influence the quercetin release significantly as shown in Fig. 2 (FN 2 vs. FN 7).

In the transdermal system containing PGMC-DGME co-solvent, the release rate was not significantly affected by the addition of an unsaturated fatty acid (linoleic acid, FN 9) as an enhancer or BHA as an antioxidant (FN 14), as evidence by the fact that the release rates from these test formulations were similar to that of FN 2 (i.e., the release rates of FN2, FN 9 and FN 14 were 32.21, 26.03 and 28.78 μg/cm^2/h$^{1/2}$, respectively). These observations indicate that the release rate is not affected by the solvent composition or ratio, or the presence of additives. In contrast, the release rate was influenced by the relative ratio of loaded drug to PSA (Fig. 2).

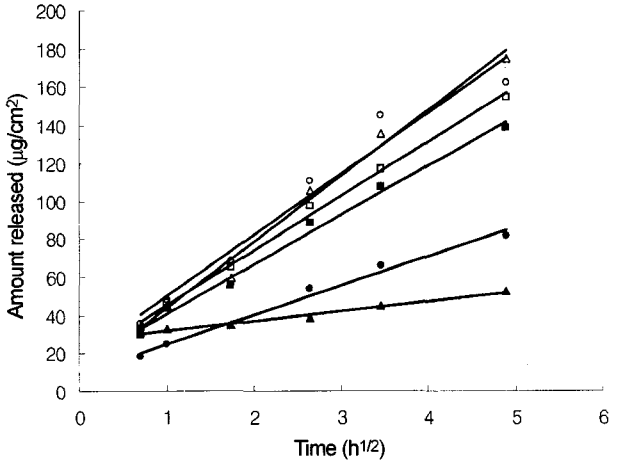

Fig. 2. Release profiles of quercetin from PSA transdermal system (n = 3). Key: ●, FN 1; ○, FN 2; ▲, FN 4; △, FN 7; ■, FN 9; □, FN 14

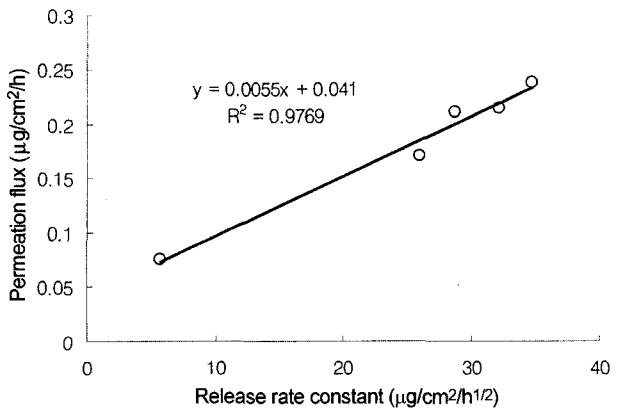

Fig. 3. Relationship between release rates and permeation fluxes of quercetin from transdermal systems.

The amount or type of PSAs appeared to influence the permeation rate of quercetin. As shown in Table IV, FN 2 (containing 4 g of PSA) resulted in a three fold higher flux, compared with that of FN 4 (containing 6 g of PSA). When the Duro-Tak 2510 was used instead of Duro-Tak 2196, the permeation rate decreased from 0.21 to 0.10 μg/cm^2/h. However, the amount of DGME mixed with PGL did not affect the permeation rate of quercetin as shown in FN 2 and FN 7. In the solvent system containing PGMC, the existence or ratios of DGME at the lower percentages than 20% did not affect the permeation rate of quercetin. The lag time was within 3 h except for FN 4 and FN 7, which high amount of PSA (6 g) was used and reduced amount of DGME was added to PGL at 2 : 8 v/v compared to other PGL-DGME (3 : 7 v/v) system. The prolonged lag time may be related to the reduced diffusivity.

The permeation flux of quercetin was affected by the addition of antioxidant. As reported in our earlier study (Gwak *et al.*, 2004), quercetin degraded in PGMC-DGME (8 : 2, v/v) co-solvent very rapidly; the remaining drug in the system was found to be 78.9% after 5 days. By the addition of 0.1% BHA, an antioxidant, 99.4% of the drug remained in the system after 5 days. Therefore, we reason

Table IV. Permeation flux and lag time of quercetin from various PSA formulations

FN	J_s (μg/cm^2/h)	T_L (h)
02	0.21 ± 0.03	NA
04	0.07 ± 0.002	5.93 ± 0.60
06	0.10 ± 0.02	2.03 ± 0.52
07	0.24 ± 0.07	5.92 ± 1.02
08	0.16 ± 0.05	NA
09	0.17 ±0.08	NA
10	0.25 ± 0.10	2.44 ± 1.15
11	0.24 ± 0.04	1.72 ± 0.98
12	0.25 ± 0.08	2.63 ± 0.47
13	0.26 ± 0.02	2.97 ± 0.36
14	0.26 ± 0.05	2.03 ± 0.58

Data were expressed as the mean ± S.D. (n = 3).
FN: formulation number
NA: Not available

that the relatively high permeation rate is attainable if the antioxidant is added to the test formulation. By the comparison of permeation fluxes for FN 8 and FN 14 (i.e., identical composition except for the BHA addition in FN 14, Table I), the flux was apparently higher for FN 14 (i.e., containing BHA, Table IV), indicating that the improvement of stability of the drug is associated with the enhanced flux of the drug across the skin.

The overall permeation fluxes were less than 0.30 μg/cm^2/h, which were significantly lower, compared to those obtained from solution formulations. The reduction in the permeation flux is associated with the reduced release rate in the presence of the PSA layer (Fig. 2), indicating the reduction is due to the decrease of solubility and diffusivity of quercetin in the PSA layer. Therefore, it was concluded that to enhance the permeation of quercetin through the skin from the PSA matrix, it was necessary to increase the release rate from the system and/or to adopt a reservoir-type controlled transdermal system.

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