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Effects of Dimethylformamide and L-Menthol Permeation Enhancers on Transdermal Delivery of Quercetin

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In this work a feasibility study of transdermal delivery system for quercertin (Q) in carbopol gel through abdominal hairless pig skin in vitro was performed. Dimethylformamide (DMF) and L-menthol (M) were selected as enhancers. Permeation experiences were carried out by using Franz-type diffusion cells. Phosphate saline buffer (pH 7.4) was used in the receptor compartments. All the system was maintained at 32 ± 0.5°C with a circulating water jacket and magnetic stirring (180 rpm). Samples were analysed by UV-VIS spectrophotometer at 255 nm. Flux (J_m) values, permeation (P) and diffusion (D) coefficients were obtained. Results of Q in CG permeation experiences with different percentages of DMF and M showed that 16.7% DMF and 1.95% L-menthol enhancers were the best quantities for the system tested. Enhancer effect can be attributed to direct action on membrane structure by promoting its distension. Therefore, enhancer substitutes for water in pores, improving active principal permeation through pig skin. M significantly increases Q permeation about 17 times higher than control. The results of permeation experiments with M and DMF using the same enhancer concentration (1.42%) conclude that M action is 9 times higher than DMF, approximately, indicating that M is an effective enhancer for a transdermal therapeutic system of Q in CG as vehicle.

Keywords quercetin, transdermal delivery, hairless pig skin, permeation enhancers

INTRODUCTION

Drug release through a transdermal route is limited by skin permeability. Owing to its anatomical structure, biochemical composition, and molecular organization, stratum corneum, the outermost skin layer, is generally regarded as the primary barrier to drug penetration following topical applications.[1] This barrier can be more permeable to solutes, including skin permeation enhancers in the topical drug formulation. These enhancers can be added to pharmaceutical formulations reduce diffusional penetration barrier. Ideally, they are pharmacologically inert, and they interact with skin constituents, inducing a temporary reversible increase on its permeability.[2–4] Particularly, the alterations observed in model stratum corneous lipid system after the inclusion of terpenes suggest that they disrupt the interlamellar hydrogen-bonding network at the polar head group region. Further, terpenes also increased the hydration levels of the lipid system probably by forming new aqueous channels. They transform the stratum corneous lipids from a highly ordered orthorhombic perpendicular subcellular packing to a less ordered hexagonal subcell packing.[5] On the other hand, aprotic solvent dimethylformamide is less potent penetration enhancing chemical alternatives to dimethylsulfoxide. At low concentrations, their activity as enhancers is a result of partitioning into the keratin regions. At higher concentrations, they increase lipid fluidity by disruption of lipid packing as a result of solvation shell formation around the polar head groups of the lipids.[6]

Pig skin has been widely studied, and it is more comparable to human skin than any other animal tissues.[7] Because pig and human skin have similar lipidic surface, barrier thickness, and morphological aspects, it has been suggested that using pig skin can be useful for estimation of in vitro human skin permeation behavior.[8]

Flavonoids are a group of phenolic compounds widely distributed in nature. They are well known because of their antimicrobial, antiviral, antineoplasic, anti-inflammatory, antioxidant, and antiplatelet activities.[9–13] Particularly, quercetin, an important dietary flavonoid, reduces arterial pressure and endotelial disfunction and shows anti-inflammatory and antioxidant activity, among

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other properties.[12,14] However, quercetin has poor bioavailability with oral administration due to its extensive metabolism and low absorption in the gastrointestinal tract. In vivo studies showed that this flavonoid did not permeate through rabbit duodenal mucous in spite of improving solubility and using permeation enhancers.[15] Strategies for the improvement in the biopharmaceutical characteristics has not been devised for quercetin.

With use of in vitro methods, one can choose the best vehicle–enhancer combination for each dermatologically active agent.

In the present study, the effect of dimethylformamide (DMF) and L-menthol (M) on the in vitro quercetin (Q) permeability through abdominal hairless pig skin was investigated to select an enhancer as the first step toward developing a transdermal therapeutic system.

MATERIALS AND METHOD

Materials

Materials used were as follows: quercetin (Q) (Figure 1) (Sigma-Aldrich); carbopol gel (CG); phosphate buffer saline pH=7.4 (PBS); dimethylformamide (DMF); L-menthol (M); and abdominal hairless pig skin.

Skin Permeation Studies

Permeation experiments were performed by using automatic sampler Microette System (Hanson- Research) with 1.767 cm^2 area Franz-type diffusion cells. The hairless skin section was assembled on a receptor compartment of the diffusion cells with stratum corneum facing donor phase. Skin was pretreated with PBS for 6 hr. Then 0.246 g of gel formulation containing 0.02225 g of quercetin were uniformly spread on skin stratum corneum. PBS, pH=7.4, was used as receptor phase. All the system was maintained at 32 ± 0.5°C with a circulating water jacket and magnetic stirring (180 rpm). At predetermined intervals, 100 μL of receptor phase were removed and replaced with an equal volume of fresh receptor solution, keeping the sink conditions. The samples were analyzed by UV-VIS spectrophotometry at 255 nm. Experiments were performed in quadruplicate. Cumulative corrections were made to determine total amount of drug permeated at each time interval. In vitro quercetin transdermal permeation experiments in carbopol gel using dimethylformamide and menthol as enhancers were performed by using identical procedure.

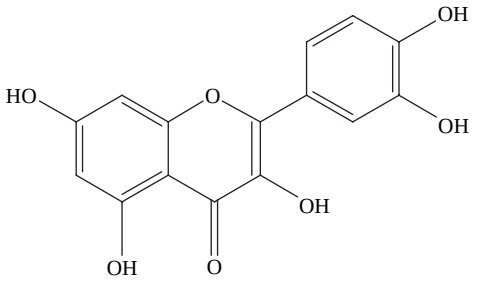

Figure 1. Structure of quercetin (3,5,7,3',4'-pentahydroxyflavone).

RESULTS

Most substances are absorbed through skin by passive diffusion according to Fick´s law.[16] Drug flux (J_m) through stratum corneum under stationary conditions (i.e., without variations of diffusion coefficient or solute concentration throughout) can be described by first Fick´s law:

$$J_m = D.\ \Delta C / \Delta X = Q_m/(t.A) \quad (1)$$

where J_m is the amount of mass diffusing across a plane of area unit per time unit, D is a proportionality constant known as diffusion coefficient, and ($\triangle C/\triangle X$) is the concentration increment through membrane.

Expression 1 can be written as:

$$Q_m/A = J_m \cdot t \quad (2)$$

This equation determines the J_m value from the slope of the linear graph Q_m /A versus t.

Permeation coefficient P (cm/s) can be calculated as the quotient between flux and substance concentration in the donor compartment (g/cm^3), assuming negligible concentration in the receptor compartment, according to the following equation, applied to quercetin:

$$P=J_m/[Q]_{donor} \quad (3)$$

Furthermore, Eq. (1) determines an estimation of the diffusion coefficient D (cm^2/s).

These physicochemical parameters were evaluated by means of permeation experiences using abdomen hairless pig skin and Q in CG with DMF as enhancer in concentrations between 0 and 25%, approximately. Table 1 summarizes flux values (J_m) and permeation (P) and diffusion (D) coefficients for the studied system.

On the other hand, similar experiments using L-menthol (0–5% approximately) were performed (Table 2).

Figure 2 shows three permeation profiles: without enhancer, with 16.7% DMF, and 1.42% L-menthol.

Figure 3 illustrates diffusion coefficient dependence with DMF percentage.

Table 1
Physicochemical parameters of quercetin permeation in carbopol gel with dimethylformamide as enhancer

% DMF	$J_m \times 10^7$ ($g.cm^{-2}.s^{-1}$)	$P \times 10^6$ ($cm.s^{-1}$)	$D \times 10^7$ ($cm^2.s^{-1}$)
0*	1.03 ± 0.452	4.63 ± 2.01	3.32 ± 1.44
4.76	1.10 ± 0.18	4.94 ± 0.799	4.07 ± 0.659
16.7	4.37 ± 0.449	19.6 ± 2.02	16.2 ± 1.66
23.1	2.63 ± 0.240	11.8 ± 1.11	9.75 ± 0.914

Membrane thickness: 8.247×10^{-2} cm; (*) 7.170×10^{-2} cm; J_m: flux; P and D: permeation and diffusion coefficients.

Table 2
Physicochemical parameters of quercetin permeation in carbopol gel with L-menthol as enhancer

% L-menthol	$J_m \times 10^7$ ($g.cm^{-2}.s^{-1}$)	$P \times 10^5$ ($cm.s^{-1}$)	$D \times 10^7$ ($cm^2.s^{-1}$)
0	1.03 ± 0.452	0.463 ± 0.201	3.32 ± 1.44
1.42*	6.25 ± 1.76	2.81 ± 0.790	25.2 ± 7.09
2.52*	5.09 ± 0.433	2.29 ± 0.195	20.5 ± 1.75
4.59	1.16 ± 0.192	0.521 ± 0.0865	3.74 ± 0.619

Membrane thickness: 7.170×10^{-2} cm; (*) 8.973×10^{-2} cm; J_m: flux; P and D: permeation and diffusion coefficients.

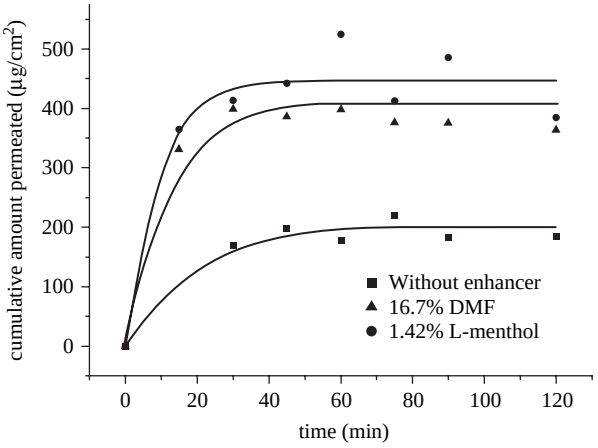

Figure 2. Permeation profiles of quercetin in carbopol gel through abdominal hairless pig skin without enhancer, dimethylformamide, and L-menthol as enhancers.

There are particularly interesting cases that show a linear ratio between permeation rate of active principle and square root of time. To analyse these situations, the fraction of permeation active principle is frequently

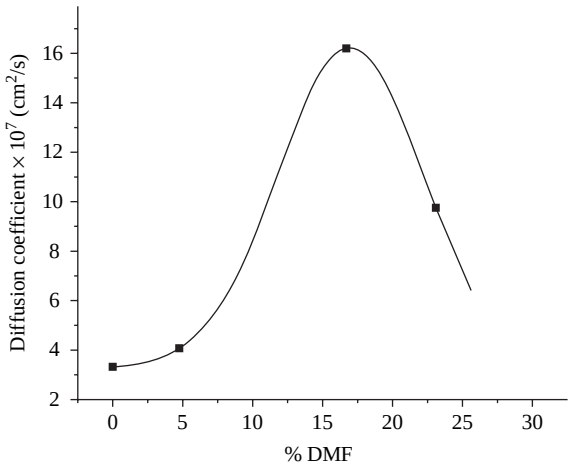

Figure 3. Quercetin diffusion coefficient dependence with dimethylformamide percentage.

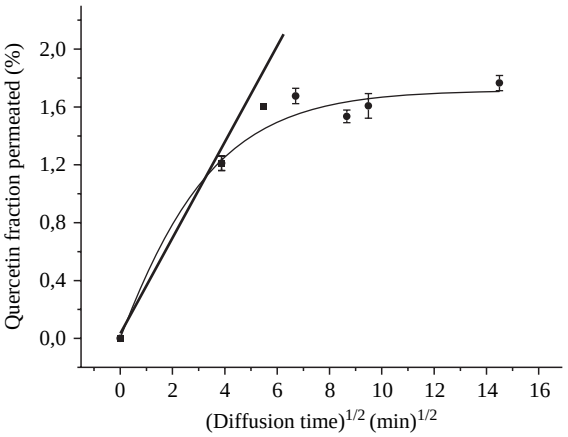

Figure 4. Linear form of permeated quercetin as a function of diffusion time according to Higuchi's equation.

expressed by Higuchi´s equation $Q_t/Q_o = k \cdot t^{1/2}$, where the constant k expresses drug diffusion rate, also related to the interaction between drug and vehicle.[17] Values of fraction of permeation active principle (Q/Qo × 100) versus the square root of time were plotted for both systems. Figure 4 shows the linear relation for Q-CG–DMF system.

DISCUSSION AND CONCLUSIONS

In vitro methods control laboratory conditions and elucidate particular factors that made modify drug penetration, although permeation may show variations in vivo tissue.

Flux value, permeation and diffusion coefficients estimated in the present study of quercetin permeation using carbopol gel as vehicle across abdominal hairless pig skin were higher in the presence of dimethylformamide and L-menthol enhancers (Tables 1 and 2).

From experimental results (Figure 3), the diffusion coefficient for the Q–CG–DMF transdermal system increase markedly with promotor percentages, being 16.7% the best concentration. On the other hand, the release rate obtained from Q-CG–M formulations with the different enhancer percentages assayed through pig skin established that 1.95% ($D = 5.19 \times 10^{-6}$ $cm^2.s^{-1}$) was the best L-menthol concentration; the value was obtained from the trend line.

L-menthol significantly increased the quercetin permeation about 16 times more than those of control. The study of quercetin permeation through skin with the same concentration of enhancers (1.42%) concluded that the action of L-menthol ($D = 2.52 \times 10^{-6}$ $cm^2.s^{-1}$) is approximately 8 times higher than dimethyl formamide ($D = 3.05 \times 10^{-7}$ $cm^2.s^{-1}$). This may be due to varying influence of enhancers on the biophysical properties of the stratum corneum. Enhancer effect can be attributed to direct action on membrane structure by promoting its distension. Therefore, enhancer substitutes for water in pores, improving active principle permeation through pig skin.

The linear relation between fraction of permeated active principle values and time square root indicates first-order kinetics, corresponding to a Fickian mechanism.

These results indicate that L-menthol is an effective enhancer for a transdermal therapeutic system of quercetin in carbopol gel as vehicle.

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