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# Preparation of quercetin and rutin-loaded ceramide liposomes and drug-releasing effect in liposome-in-hydrogel complex system

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ABSTRACT

In this study, we developed a 2-step delivery system to enhance transdermal permeation of quercetin and its glycoside rutin, an antioxidant. Liposome-in-hydrogel complex systems were prepared by incorporating ceramide liposomes, which consist of biocompatible lipid membranes, into cellulose hydrogel. We evaluated the encapsulation efficiency, *in vitro* release behavior, and skin permeability of formulations that remained stable for over 3 weeks. Rutin had greater encapsulation efficiency and better *in vitro* release properties than quercetin. However, quercetin demonstrated greater skin permeability than rutin. We also found that liposome-in-hydrogel complex systems (quercetin, 67.42%; rutin 59.82%) improved skin permeability of quercetin and rutin compared to control (phosphate buffer, pH 7.4) (quercetin, 2.48%; rutin, 1.89%) or single systems of hydrogel (quercetin, 31.77%; rutin, 26.35%) or liposome (quercetin, 48.35%; rutin, 37.41%). These results indicate that liposome-in-hydrogel systems can function as potential drug delivery systems to enhance transdermal permeation of the water-insoluble antioxidants quercetin and rutin.



## 1. Introduction

Stratum corneum is the outermost layer of the skin, which functions as primary barrier to protect the skin from potentially harmful environmental agents. In addition, by inhibiting the loss of moisture to the outside, intercellular lipids in the stratum corneum help maintain homeostasis of the skin, protecting the skin from dehydration. However, the skin barrier can be adversely affected when a drug is delivered via the transdermal route. Therefore, there has been an increase in research investigating a variety of drug delivery systems aimed at promoting better skin permeation of active materials [1–4].

Hydrogels are 3-dimensional networks that, consist of hydrophilic polymers that swell in aqueous solution and retain a large amount of water without dissolving. Recently, the development of cellulose-based hydrogels has been actively pursued. Hydrogels based on cellulose have biodegradable properties, high permeation of active materials, a high degree of swelling, and no associated toxicity or irritation. In particular, owing to high biocompatibility, hydrogels are widely used in the medical and pharmaceutical fields as drug delivery vehicles [5–7].

Liposomes have a phospholipid composition that is similar to the lipid bilayer of cell membranes in the body. Moreover, both hydrophilic and hydrophobic drugs can be loaded within the internal space of liposomes. Liposomes have low toxicity and are highly biocompatible. For these reasons, liposomes are generally used as a drug delivery system [8].

Quercetin and its glycoside, rutin are typical flavonoids that are reported to act as strong antioxidants [9]. These flavonoids, have been widely used as anti-oxidants in cosmetics, but their use is limited because of poorly water solubility Therefore, research has been focused on ways to improve their solubility in water.

In this study, a 2-step delivery system consisting of ceramide liposomes composed of biocompatible membranes and porous cellulose hydrogel, was designed to enhance transdermal permeation of quercetin and rutin. We also studied the effects of interactions between the 2 delivery systems by conducting a skin permeation test using a Franz diffusion cell.

## 2. Materials and methods

### 2.1. Materials

Cellulose (microcrystalline, powder), (±)-epichlorohydrin (⩾99.0%, ECH), L-α-phosphatidylcholine (from egg yolk, ⩾60%, egg PC), cholesterol (⩾99.0%, Chol), oleic acid (⩾99.0%, OA), quercetin and rutin were purchased from Sigma (USA). Ceramide-3 (DS-CERAMIDE Y30) was obtained from Doosan Co. (Korea). Solvents such as 1,3-butylene glycol, methanol, ethanol,

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chloroform, and acetone, were of analytical grade. Preparation of the hydrogel required the use of a stirrer (HSD 120-03P, Misung Scientific Co., Ltd., Korea) and a centrifuge (2236R, Gyrozen Co., Ltd., Korea). Preparation of liposomes required the use of a rotary evaporator (Buchi, Switzerland) and a probe sonicator (Branson, USA). UV Spectrometer (Cary 100, Agilent, USA) and HPLC (Shimadzu, Japan) were used to determine the concentration of quercetin and rutin.

### 2.2. Preparation of ceramide liposomes

Liposomes were prepared using the thin-film hydration method [10,11]. Egg PC, Cer, Chol, OA, quercetin, and rutin were dissolved in 25 mL of a chloroform–methanol mixture (4:1) using a fixed molar ratio (Table 1). The mixtures were evaporated in a rotary evaporator to remove traces of solvent and also to form a film. To prevent separation of phospholipid membrane by hydrolysis, the film was hydrated with phosphate buffer (pH 9.0) for 1 h at a temperature that was above the lipid transition temperature, and the product was maintained as a liposome solution at pH 7.0 ± 0.5. The vesicle suspension was then homogenized using a probe sonicator, passed through a 0.45 μm filter (Minisart CA 26 mm), and stored until use.

### 2.3. Stability test of ceramide liposomes

Stability of unloaded ceramide liposomes and quercetin or rutin loaded ceramide liposomes was evaluated by measuring the average particle size and distribution. Size and distribution in a liposome solution was assessed 70 times, with 3 repeated measurements using a particle size analyzer (Otsuka ELS-Z2, Otsuka Electronics, Japan) at 25 °C, with a scattering angle of 165° using an Argon laser. The average particle size was determined by cumulative analysis, and distribution was resolved using the Contin method [12].

### 2.4. Encapsulation efficiency of quercetin or rutin in ceramide liposomes

Unloaded quercetin or rutin was recovered from a 2 mL volume of liposome solution by passing the liposome solution through a 0.45 μm filter (Minisart, CA 26 mm), and degrading the solution with 15 mL of ethanol. The ethanol was evaporated in a rotary evaporator and quercetin or rutin was then redissolved in 2 mL of ethanol. The concentration of quercetin and rutin was determined using HPLC, and the following Eq. (1) was used to calculate the encapsulation efficiency of quercetin or rutin into liposomes:

$$EE = \frac{C_e}{C_i} \times 100\ (\%) \quad (1)$$

$EE$ encapsulation efficiency (%)
$C_i$ initial concentration of drug (μM)
$C_e$ concentration of encapsulated drug (μM)

### 2.5. Preparation of cellulose hydrogel

We prepared cellulose hydrogel using a modification of the method used by Ciolacu et al. [13]. Cellulose powder (0.5 g) was dispersed into 13.45 mL of 9% NaOH solution at room temperature, and the sample was dissolved by freezing at −70 °C for 24 h. After thawing, 11.55 mL of distilled water was added to the cellulose solution, followed by the drop wise addition of 3 mL of ECH, with continuously stirring. The crosslinking reaction was subsequently performed at 80 °C for 8 h. When the reaction was completed, the cellulose hydrogel product was washed with distilled water to remove the NaOH and NaCl that had formed, followed by an acetone wash to remove excess ECH. Solvent was removed from the sample by centrifugation, and the sample was dried in a vacuum at room temperature prior to storage.

### 2.6. Preparation of a ceramide liposome-in-cellulose hydrogel complex

The complex was prepared by incorporating 5 mL of ceramide liposome solution into 0.1 g of dried cellulose hydrogel. The ceramide liposome penetrated into and was absorbed within the matrices of swollen hydrogel.

### 2.7. Incorporating efficiency of ceramide liposome into cellulose hydrogel and degree of water uptake of cellulose hydrogel

Dried cellulose hydrogel (0.1 g) was swollen in 5 mL of a ceramide liposome solution at 37 °C for 24 h in order to measure incorporation efficiency. To assess the amount of incorporated liposome, the concentration of quercetin or rutin in liposomes was determined, and for this, liposome solutions before and after incorporation were analyzed using HPLC.

The following Eq. (2) was used to calculate the concentration of quercetin or rutin in liposomes incorporated into matrices of cellulose hydrogel:

$$IE = \frac{v_1 c_1 - v_2 c_2}{v_1 c_1} \times 100\ (\%) \quad (2)$$

$IE$ incorporation efficiency (%); $v_1$ volume of initial liposome solution (mL); $c_1$ initial concentration of the drug (μg/mL); $v_2$ remaining

**Table 1**
Physical stability of unloaded liposomes assessed by particle size according to different formulations.

| Sample | | Liposome formulation (molar ratio) | | | | Mean particle size | Particle size distribution |
|---|---|---|---|---|---|---|---|
| | | PC[a] | Cer[b] | Chol[c] | OA[d] | | |
| Unloaded liposome | L-a | – | 1 | 1 | 1 | Aggregation (N.D.) | |
| | L-b | 1 | – | 1 | 1 | 200.57 ± 83.16[e] | 131.63 ± 47.67 |
| | L-c | 0.25 | 0.75 | 1 | 1 | 366.45 ± 5.75 | 206.15 ± 90.25 |
| | L-d | 0.5 | 0.5 | 1 | 1 | 517.49 ± 270.76 | 564.28 ± 367.98 |
| | L-e | 0.75 | 0.25 | 1 | 1 | 240.45 ± 36.12[e] | 187.98 ± 39.88 |
| | L-f | 0.75 | 0.25 | 0.5 | 1 | 140.48 ± 8.86[e] | 71.88 ± 16.77 |
| | L-g | 0.8 | 0.2 | 1 | 1 | 136.55 ± 12.40[e] | 69.71 ± 5.17 |
| | L-h | 0.8 | 0.2 | 0.5 | 1 | 139.49 ± 17.17[e] | 77.31 ± 9.29 |

(N.D.: No data).
[a] L-α-Phosphatidylcholine (from egg yolk, ⩾60%).
[b] Ceramide-3 (DS-CERAMIDE Y30).
[c] Cholesterol.
[d] Oleic acid (unsaturated fatty acid, C18).
[e] Formulation sustained stable condition for more than 3 weeks.

volume of liposome solution (mL); $c_2$ concentration of drug remaining (μg/mL).

The degree of water uptake into the cellulose hydrogel was evaluated for all ceramide liposome formulations. Degree of water uptake is known to depend on the swelling capacity of the hydrogel, and indicates the volume of absorbable water versus the weight of dried hydrogel. To investigate whether the degree of water uptake into the hydrogel was similar in all liposome formulations, 0.1 g of dried hydrogel was swollen in 5 mL of liposome solution at 37 °C for 24 h. Samples were periodically removed from the surface of the hydrogel solution and immediately weighed.

The following Eq. (3) was used to calculate degree of water uptake into the hydrogel:

$$WU = \frac{H_s - H_d}{H_d} \times 100\ (\%) \tag{3}$$

WU water uptake (%)
$H_s$ weight of swollen hydrogel (g)
$H_d$ weight of dried hydrogel (g)

### 2.8. Stability test for ceramide liposomes released from cellulose hydrogel

To investigate whether the stability of ceramide liposomes was sustained during the process in which liposomes were released from hydrogel after incorporation into matrices of swollen hydrogel, 0.1 g of dried hydrogel was swollen in 5 mL of liposome solution at 37 °C for 24 h. Then, hydrogel loaded with liposomes was added to 5 mL of phosphate buffer (pH 7.4); liposome was released again at 37 °C for 24 h. Particle size and distribution of released liposome was measured using a particle size analyzer.

### 2.9. Assessment of skin permeation and release behavior of quercetin and rutin via interactions between ceramide liposomes and cellulose hydrogel

A skin permeation study was performed using a 9 mm Franz diffusion cell (with a 5 mL receptor volume) and a V6A stirrer model (Permegear, USA) using different conditions of ceramide liposomes in a cellulose hydrogel system, where the liposome solution was loaded with 500 μM of quercetin or rutin and incorporated into hydrogel for 24 h. Control samples (500 μM of quercetin or rutin) were dissolved in phosphate buffer (pH 7.4), whereas the experimental groups were created by dissolving quercetin or rutin (500 μM) in ceramide liposomes and cellulose hydrogel, respectively.

Full-thickness skin was removed from the dorsal side of ICR hairy albino mice (8-week-old females). Subcutaneous fat and bristles were carefully removed from the skin, but not from the dermis. The skin (1.5 cm × 1.5 cm) was fixed between the donor and the receptor phase of the stratum corneum side facing upward into the donor compartment. A 2% solution of FANCOL® HCO-40 (40 mol ethylene oxide adduct of hydrogenated castor oil) in 20% ethanol solution was used as the receptor phase in the solubility tests for quercetin and rutin. The temperature of the cell was maintained at 37.0 ± 0.2 °C using a constant-temperature water bath. After adding 5 mL of receptor phase into the receptor chamber, receptor phase was continuously stirred at 150 rpm for 24 h. Samples (0.3 mL) were applied to the skin surface (0.6362 $cm^2$) of the donor. Next, 0.2 mL of receptor phase was withdrawn through the sampling port of the Franz diffusion cell at 2, 4, 8, 12, and 24 h (a total of 5 times). The receptor phase was immediately replenished with an equal volume of fresh receptor phase. The withdrawn sample was analyzed by HPLC, and the concentrations of quercetin or rutin were determined.

To determine the amount of quercetin and rutin remaining in skin, non-permeable samples were washed with phosphate buffer saline (pH 7.4). After washing, the stratum corneum was removed by tape stripping using 4 strips of scotch tape (3M, Korea) and the remaining skin was cut into small pieces. The quercetin and rutin in the tape strips and skin were dissolved in ethanol using a sonicator. The concentrations of extracted quercetin and rutin were determined using HPLC.

The following Eq. (4), representing Korsmeyer–Peppas model, was used to investigate the mechanism of diffusion of quercetin or rutin into the skin following release from liposomes in the hydrogel system [14–16]

$$F = \left(\frac{M_t}{M}\right) = k_r \times t^{n_r} \tag{4}$$

$F$ fractional drug release into the dissolution medium; $M_t$ amount of drug released at time $t$; $M$ amount of total drug; $k_r$ rate constant of the drug delivery system; $n_r$ diffusional exponent related drug transport mechanism.

We determined the diffusion rates for quercetin and rutin from liposomes in the hydrogel system using $k_r$ values, and we examined the transport mechanisms of quercetin and rutin into skin using $n_r$ values.

### 2.10. Statistical analysis

All experiments were repeated 3 times and statistical analysis was performed using the Student's $t$-test at a significance level of 5%.

## 3. Results and discussion

### 3.1. Stability of unloaded ceramide liposomes

We prepared ceramide liposomes by varying the molar ratio of components and measured particle sizes and distribution of these liposomes (Table 1). When liposomes were prepared with Cer:Chol:OA = 1:1:1 (L-a), which was similar to the composition of intercellular lipid, the aggregation of liposomes was observed immediately. Therefore, egg PC was added in order to improve the problem. When ceramide was completely replaced by egg PC (L-b), the average particle size of the liposomes remaind constant at 200 nm for over 3 weeks. Liposomes were prepared with the following molar ratios:egg PC:Cer = 1:3(L-c), 1:1(L-d), 3:1(L-e), and 4:1(L-g). Among these ratios, we confirmed that the optimum molar ratios were 3:1(L-e) and 4:1(L-g) which were stable for over 3 weeks.

Cholesterol can influence stability of vesicles, encapsulation efficiency or skin permeability of active materials [17]. Therefore, in order to investigate the effects of Chol, we prepared ceramide liposomes by decreasing the molar ratio of Chol from 1 to 0.5. As a result, when the molar ratios of egg PC:Cer:Chol:OA were 0.75:0.25:0.5:1 (L-f) and 0.8:0.2:0.5:1 (L-h), the particle sizes and distribution of liposomes decreased.

### 3.2. Stability of quercetin and rutin loaded ceramide liposomes

We measured particle sizes and distribution of quercetin and rutin loaded ceramide liposomes (Table 1) over a 3 weeks (Table 2). The particle sizes and distribution of quercetin and rutin loaded ceramide liposomes showed no significant differences, the only exception being L-e. The particle sizes and distribution of L-e (egg PC:Cer:Chol:OA = 0.75:0.25:1:1) decreased significantly. Therefore, we concluded that liposomes loaded with quercetin or rutin were stable, because there were negligible changes in particle

**Table 2**
Physical stability of quercetin (Q) or rutin (R) loaded liposomes assessed by particle size according to different formulations.

| Sample | | Liposome formulation (molar ratio) | | | | | | Mean particle size | Particle size distribution |
|---|---|---|---|---|---|---|---|---|---|
| | | PC[a] | Cer[b] | Chol[c] | OA[d] | Q | R | | |
| Quercetin loaded liposome | L-e | 0.75 | 0.25 | 1 | 1 | 0.5 | - | 162.09 ± 8.85[e] | 85.70 ± 10.97 |
| | L-f | 0.75 | 0.25 | 0.5 | 1 | 0.5 | - | 131.48 ± 9.31[e] | 70.78 ± 6.00 |
| | L-g | 0.8 | 0.2 | 1 | 1 | 0.5 | - | 161.31 ± 4.40[e] | 68.71 ± 11.29 |
| | L-h | 0.8 | 0.2 | 0.5 | 1 | 0.5 | - | 130.56 ± 2.09[e] | 70.00 ± 2.61 |
| Rutin loaded liposome | L-e | 0.75 | 0.25 | 1 | 1 | - | 0.5 | 152.76 ± 3.89[e] | 87.93 ± 8.60 |
| | L-f | 0.75 | 0.25 | 0.5 | 1 | - | 0.5 | 134.08 ± 4.60[e] | 84.79 ± 4.20 |
| | L-g | 0.8 | 0.2 | 1 | 1 | - | 0.5 | 151.99 ± 5.64[e] | 90.00 ± 9.02 |
| | L-h | 0.8 | 0.2 | 0.5 | 1 | - | 0.5 | 131.48 ± 7.32[e] | 75.68 ± 8.20 |

(N.D.: No data).
[a] L-α-Phosphatidylcholine (from egg yolk, ⩾60%).
[b] Ceramide-3 (DS-CERAMIDE Y30).
[c] Cholesterol.
[d] Oleic acid (unsaturated fatty acid, C18).
[e] Formulation was stable for more than 3 weeks.

size and distribution over the 3-week study periods (Table 2). Therefore, we investigated the skin permeability and release mechanisms under these stable conditions.

### 3.3. Encapsulation efficiency of quercetin and rutin in ceramide liposomes

The encapsulation efficiency of quercetin and rutin in ceramide liposomes of different formulations is shown in Fig. 1. In PC:Cer = 3:1 (L-e, f), encapsulation efficiency of rutin was greater than quercetin. However, in PC:Cer = 4:1 (L-g, h), there were no significant differences between quercetin and rutin. The encapsulation efficiency of quercetin and rutin was greater when using a molar ratio of 0.5 of Chol (L-f, h) compared to 1 (L-e, g). In terms of encapsulation efficiency, the optimal formation of quercetin was L-h, while rutin was L-f. Furthermore, the encapsulating efficiency of rutin was typically greater than quercetin. This result demonstrated that rutin was more effective for penetration between the lipid membranes of the liposome than was quercetin, because rutin has hydrophilic and hydrophobic properties attributed to its disaccharide content or aglycone structure.

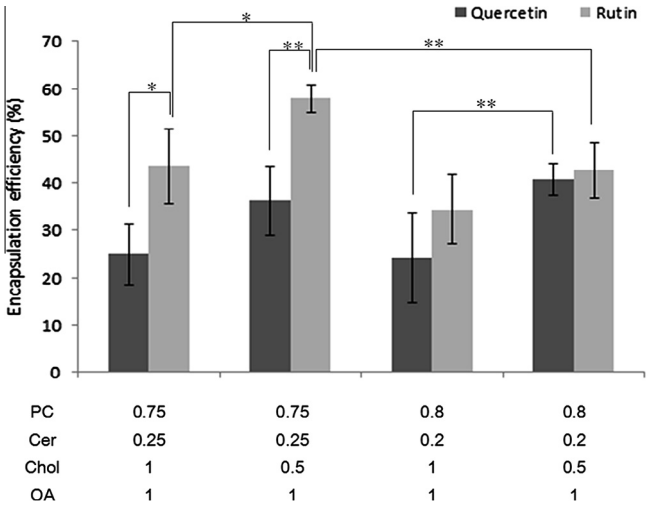



**Fig. 1.** Encapsulation efficiency (EE) of quercetin (■) or rutin (■) in ceramide liposomes of different formulations.*$p < 0.05$, **$p < 0.005$.

### 3.4. Incorporating efficiency of ceramide liposomes into cellulose hydrogel, and degree of water uptake by cellulose hydrogel

In a previous study, we revealed that hydrogel consisting of 2% cellulose with 12% ECH as a crosslinker had the greatest extent of water uptake (684.57%) [18]. Here we evaluated incorporation efficiency into cellulose hydrogels, and degree of water uptake of cellulose hydrogel when liposome solutions with different formulations (L-e, f, g, h) were incorporated into the hydrogel (2% cellulose/12% ECH) (Table 3). Consequently, the average water uptake and incorporating efficiency was 656.36 ± 5.35% and 65.03 ± 0.70%, respectively. The cellulose hydrogel-liposome formulations investigated in this study had a similar extent of water uptake, compared to the original hydrogel we described in our previous study.

### 3.5. Stability test of ceramide liposomes released from cellulose hydrogel

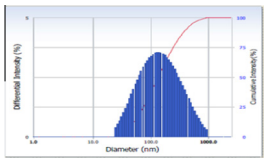
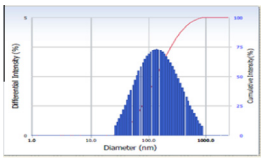
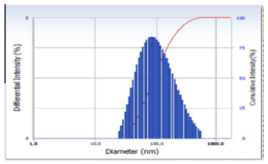
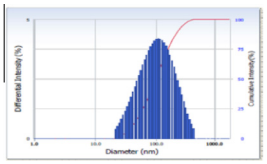
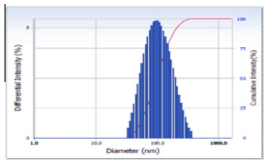
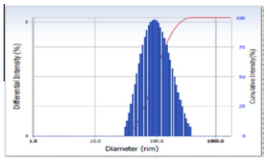
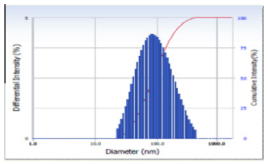
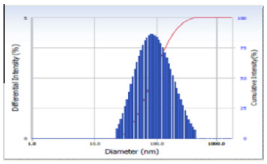
In order to confirm stability of liposomes following incorporation into cellulose hydrogel, we analyzed liposome solutions before incorporation into hydrogel and after release from hydrogel, using a particle size analyzer. The average particle sizes and distribution

**Table 3**
Incorporation efficiency (IE) of ceramide liposomes of different formulations in cellulose hydrogel matrix and maximum degree of water uptake ($S_{max}$) of cellulose hydrogel.

| Liposome formulation (molar ratio) | | | | | | IE (%) | $S_{max}$[a] (%) |
|---|---|---|---|---|---|---|---|
| Egg | Cer | Chol | OA | Q | R | | |
| 0.75 | 0.25 | 1 | 1 | 0.5 | – | 64.53 ± 2.15 | 658.58 ± 8.04 |
| 0.75 | 0.25 | 0.5 | 1 | 0.5 | – | 64.74 ± 4.11 | 656.35 ± 5.11 |
| 0.8 | 0.2 | 1 | 1 | 0.5 | – | 65.11 ± 3.93 | 664.53 ± 5.38 |
| 0.8 | 0.2 | 0.5 | 1 | 0.5 | – | 65.44 ± 3.08 | 659.05 ± 6.97 |
| 0.75 | 0.25 | 1 | 1 | – | 0.5 | 65.84 ± 3.82 | 656.37 ± 6.35 |
| 0.75 | 0.25 | 0.5 | 1 | – | 0.5 | 63.60 ± 5.54 | 645.56 ± 9.42 |
| 0.8 | 0.2 | 1 | 1 | – | 0.5 | 65.85 ± 4.00 | 659.16 ± 7.54 |
| 0.8 | 0.2 | 0.5 | 1 | – | 0.5 | 65.16 ± 1.97 | 651.27 ± 4.10 |

[a] Maximum water uptake (degree of swelling).

**Table 4**
Stability of different formulations of ceramide-liposomes prior to incorporation into hydrogel and after release from hydrogel.

| Liposome formulation | | | | | Before incorporation into hydrogel | After release from hydrogel |
|---|---|---|---|---|---|---|
| | PC | Cer | Chol | OA | | |
| L-e | 0.75 | 0.25 | 1 | 1 |  202.55 ± 0.35 |  202.90 ± 0.72 |
| L-f | 0.75 | 0.25 | 0.5 | 1 |  128.55 ± 0.49 |  128.90 ± 1.23 |
| L-g | 0.8 | 0.2 | 1 | 1 |  117.65 ± 1.77 |  118.9 ± 0.52 |
| L-h | 0.8 | 0.2 | 0.5 | 1 |  117.50 ± 1.84 |  118.8 ± 0.97 |

of ceramide liposome of different formulations are shown in Table 4. In all conditions, the characteristics of both liposome solutions prior to incorporation into hydrogel and after release from hydrogel were similar. Therefore, it appeared that stability of the liposomes was well-maintained following release from hydrogel.

### *3.6. Examination of skin permeation and release behavior of quercetin and rutin resulting from interactions between ceramide liposomes and cellulose hydrogel*

We developed a complex liposome/hydrogel system in order to enhance transdermal permeation of quercetin and rutin, which are difficult to formulate because of their water-insoluble properties. In order to determine the skin permeability of this complex system, we prepared a control group used phosphate buffer (PB) (pH 7.4), and the experimental groups used a hydrogel single system (H) or a liposome single system (L-e, f, g, h). Our approach facilitated comparisons with a liposome-in-hydrogel complex system (HL-e, f, g, h) using Franz diffusion cell. Skin permeability was evaluated by quantitating the amount of drug in the stratum corneum (Tape) in the epidermis, and dermis (Skin), and amount of drug that had penetrated the skin (Transdermal). These results are shown in Fig. 2.

In terms of skin permeability, the liposome/hydrogel complex system (Q 67.42%, R 59.82%) was superior to PB (pH 7.4)(Q 2.48%, R 1.89%), the hydrogel single system (Q 31.77%, R 26.35%) or the liposome single system (Q 48.35%, R 37.41%).

Liposome/hydrogel complex system could also be characterized by its diffusion rate and diffusion exponent, which were calculated using Eq. (4), invoking the Korsmeyer–Peppas model (Table 5). Equation (4) (also using the Korsmeyer–Peppas model) was

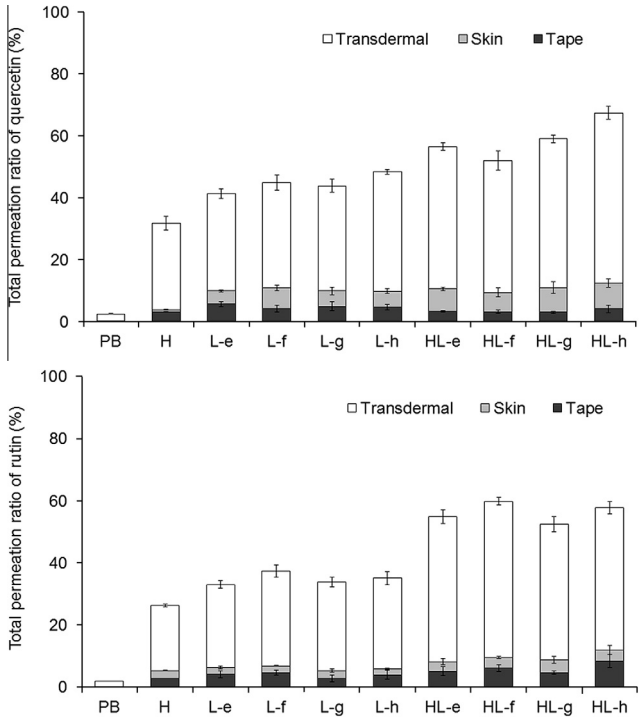



**Fig. 2.** Profiles of quercetin and rutin permeation through the dorsal skin of ICR mice for 24 h using phosphate buffer (pH 7.4) (PB), cellulose hydrogel (H), ceramide liposomes (L-e, f, g, h), or liposome in hydrogel systems (HL-e, f, g, h), Tape (■): stratum corneum, Skin (■): epidermis without stratum corneum and dermis, Transdermal (□): permeated through skin.

**Table 5**
Kinetic parameters of skin penetration with the correlation coefficient for cellulose hydrogel and liposomes in hydrogel containing quercetin (Q) and rutin (R).

| Sample | Korsmeyer–Peppas model | | | Drug release mechanism |
|---|---|---|---|---|
| | $k_r$[a] | $n_r$[b] | $R^2$ | |
| H-Q | 0.53 | 0.60 | 0.95 | Anomalous transport |
| HL-e-Q | 0.79 | 0.46 | 0.97 | Quasi-Fickian diffusion |
| HL-f-Q | 0.62 | 0.55 | 0.99 | Anomalous transport |
| HL-g-Q | 0.66 | 0.50 | 0.98 | Anomalous transport |
| HL-h-Q | 0.84 | 0.41 | 0.99 | Quasi-Fickian diffusion |
| H-R | 0.13 | 0.88 | 0.98 | Anomalous transport |
| HL-e-R | 0.40 | 0.79 | 0.99 | Anomalous transport |
| HL-f-R | 0.44 | 0.73 | 0.97 | Anomalous transport |
| HL-g-R | 0.35 | 0.82 | 0.98 | Anomalous transport |
| HL-h-R | 0.38 | 0.79 | 0.94 | Anomalous transport |

[a] The rate constant related to the properties of the drug delivery system.
[b] The diffusional exponent related to the drug transport mechanism. $n_r < 0.5$, quasi-Fickian diffusion; $n_r = 0.5$, Fickian diffusion; $0.5 < n_r < 1$, anomalous transport; $n_r = 1$, non-Fickian Case-II transport (zero-order release); $n_r > 1$, non-Fickian special case-II transport.

employed to analyze the transport mechanisms of drug into the skin by a diffusion exponent, $n_r$ When $0.5 < n_r < 1$ an anomalous or non-Fickian behavior was indicated: the drug is released by both diffusion of drug and erosion of hydrogel. When $n_r = 0.5$, a Fickian diffusion mechanism was indicated, and release by diffusion of drug was the predominant activity. When $n_r = 1$, a case II transport mechanism was indicated, and when $n > 1$, a special case II transport mechanism was indicated, and release by erosion of hydrogel was the prodominant mechanism. In a liposome-in-hydrogel complex system (HL-e, f, g, h), the diffusion rate of quercetin and rutin increased and the release of drugs depended on the diffusion of drug from the system. These results suggested that use of the liposome/hydrogel complex system as a drug delivery system has the potential to enhance transdermal permeation of quercetin and rutin.

## 4. Conclusions

In this study, we developed a 2-step delivery system, which was prepared by incorporating ceramide liposome into a porous cellulose hydrogel in order to enhance transdermal permeation of quercetin and rutin. We also studied drug release effects resulting from interactions between the systems. The encapsulation efficiency of rutin in ceramide liposomes was greater than quercetin. However, with regard to skin permeation, quercetin produced better results than rutin. In addition, the liposome-in-hydrogel system demonstrated greater skin permeation than phosphate buffered saline (pH 7.4), hydrogel, or liposomes. The diffusion rates ($k_r$) and diffusion exponents ($n_r$) of the test agents were calculated using the Korsmeyer–Peppas model. Quercetin had a faster diffusion rate than rutin, which reflected skin permeability. In addition, in the liposome/hydrogel complex system, the diffusion rate of both quercetin and rutin increased, and release of drugs also increased as the diffusion exponent decreased. Hydrogels temporarily disrupt the skin barrier by hydrating the skin, but they may also protect the skin from the external environment and water evaporation from the epithelial cells via a shielding function. Liposomes may act to reinforce the intercellular lipid, which disappears when the skin barrier is disrupted. Liposomes may also support the antioxidative defense system by sequestering quercetin and rutin within the stratum corneum. The results of this study suggest that the liposome/hydrogel complex system may be an effective drug carrier, enhancing transdermal permeation of the water-insoluble antioxidants.

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## References

[1] P. Garidel, B. Folting, I. Schaller, A. Kerth, The microstructure of the stratum corneum lipid barrier: mid-infrared spectroscopic studies of hydrated ceramide:palmitic acid:cholesterol model systems, Biophysical Chemistry 150 (2010) 144–156.
[2] G.S.K. Pilgram, A.M.E. Pelt, J.A. Bouwstra, H.K. Koerten, Electron diffraction provides new information on human stratum corneum lipid organization studied in relation to depth and temperature, J. Invest. Dermatol 113 (1999) 403–409.
[3] R.H. Wildnauer, J.W. Bothwell, A.B. Douglass, Stratum corneum biomechanical properties I. Influence of relative humidity on normal and extracted human stratum corneum, J. Invest. Dermatol 50 (1) (1971) 72–78.
[4] S.N. Park, M.S. Lim, M.A. Park, S.S. Kwon, S.B. Han, Transdermal delivery of quercetin using elastic liposomes: preparation, Characterization and *in vitro* skin permeation study, Korea Polym. J 36 (6) (2012) 705–711.
[5] A. Sannino, C. Demitri, M. Madaghiele, Biodegradable cellulose-based hydrogels: design and applications, Materials 2 (2) (2009) 353–373.
[6] N.A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, Hydrogels in pharmaceutical formulations, Eur. J. Pram. Biopharm 50 (2000) 27–46.
[7] C. Chang, L. Zhang, Cellulose-based hydrogels: present status and application prospects, Carbohydrate Polymers 84 (2011) 40–53.
[8] G.E. El Maghraby, B.W. Carry, A.C. Williams, Liposome and skin: from drug delivery to model membrane, Eur. J. Parm. Sci 34 (4–5) (2008) 203–222.
[9] C.A. Rice-Evans, N.J. Miller, G. Paganga, Antioxidant properties of phenolic compounds, Trends plant Sci 2 (1997) 152–159.
[10] Y. Maitani, M. Nakagaki, T. Nagai, Surface potential of liposomes with entrapped insulin, Int. J. Pharm 64 (1990) 89–98.
[11] K. Muramatsu, Y. Maitani, Y. Machida, T. Nagai, Effect of soybean-derived sterol and its glucoside mixtures on the stability of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylcholine/cholesterol liposomes, Int. J. Pharm 107 (1994) 1–8.
[12] B.S. Jin, S.M. Lee, K.H. Lee, A study on the factors affectin entrapment efficiency and particle size of ethosomes, J. Kor. Ind. Eng. Chem 17 (2006) 138–143.
[13] D. Ciolacu, A.M. Oprea, N. Anghel, G. Cazacu, M. Cazacu, New cellulose-lignin hydrogels and their application in controlled release of polyphenols, Mat. Sci. Eng. C 32 (3) (2012) 452–463.
[14] G.M. Khan, J.B. Zhu, Studies on drug release kinetics from ibuprofen-carbomer hydrophilic matrix tablets: influence of co-excipients on release rate of the drug, J. Control. Release 57 (1999) 197–203.
[15] J.H. Cha, J.K. Hong, S.W. Lee, J.U. Cha, W.H. Ko, H.H. Baek, H.J. Park, Dissolution profile analysis of hydroxypropyl methylcellulose-based vitamin C tablets, Kor. J. Food Sci. Technol 44 (3) (2012) 274–279.
[16] J. Siepmann, N.A. Peppas, Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC), Adv. Drug Deliv. Rev 48 (2001) 139–157.
[17] D. Sadava, D.M. Hillis, H.C. Heller, M.R. Berenbaum, Life: The Science of Biology, 9th ed., Freeman, San Francisco, 2011.
[18] M.H. Lee, S.J. Kim, S.N. Park, Development of porous cellulose-hydrogel system for enhanced transdermal delivery of quercetin and rutin, Korea Polym. J. 37 (3) (2013) 347–355.