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Effects of Skin Penetration Enhancers in Topical Antiaging Products Containing α-Hydroxyacids and Hyaluronic Acid

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Background: Transdermal drug delivery has several advantages and has been vastly investigated over the last decades. Chemical enhancers improve the quantity of drug penetration through the skin.
Objectives: In this study, some conventional solvents and surfactants were used as enhancers to promote dermal penetration of α-hydroxyacids (AHA) and hyaluronic acid (HA).
Materials and Methods: A total of 42 different formulations containing AHA or HA as the active ingredient and a solvent or surfactant as the enhancer were prepared. The experiments for determination of transdermal absorption of AHA or HA for each formulation were performed using a diffusion cell and a slice of chicken skin as model at 32℃. After 1.5 or 12 hours, samples from the medium were collected and analyzed for AHA or HA concentration.
Results: After 1.5 and 12 hours, the maximum permeated amount of AHA were 89.8 and 342.5 mg, respectively, which related to the formulations with liquid paraffin. After 1.5 and 12 hours, the maximum permeated amount of HA were 57.3 and 70.6 mg, respectively, which related to the formulations with glycerol.
Conclusions: The most effective enhancer for AHA and HA were liquid paraffin and glycerol, respectively. The most effective surfactant for both AHA and HA was Tween 80. The effects of the enhancers were increased by prolonging the exposure time.

Keywords: Acids; Hyaluronic Acid; Solvents; Surfactants

1. Background

Hyaluronic acid (HA) is a macromolecule used in topical pharmaceutical and cosmetic preparations. It is useful in wounds healing and wrinkles smoothing. The size of HA (approximately 200 kDa) may limit its free diffusion following topical administration. On the other hand, injections are painful and may need repeating (1). Advances in transdermal drug delivery offer a less invasive way of drug administration (1). Fortunately, studies suggest that efficient topical delivery of HA depends on the penetration enhancement strategies (2). One aim of the present study was to find the appropriate enhancers for topical delivery of HA. Transdermal delivery systems have been rather beneficial for the delivery of small drug molecules (1). Other widely used drugs in topical antiaging products are small molecules of α-Hydroxy acids (AHA). We aimed to find some enhancers that improve skin penetration of both AHA and HA. Development of transdermal products is restricted by the low permeability of the skin. To overcome this problem, numerous chemicals have been employed as skin permeation enhancers (3). Several groups of enhancers have been introduced including certain peptides, hydrophobic nanoparticles, surfactants, and solvents (3, 4). In the present study, 12 conventional hydrophilic and hydrophobic solvents, which have been widely investigated in the present literature, were selected to explore their effect on skin permeation of HA as well as AHA. In addition, four surfactants with completely different chemical structures were evaluated for their potentials as enhancers. It is known that hydrophilic molecules have difficulties for transdermal absorption in comparison with hydrophobic molecules. Therefore, the hydrophilic structure of both HA and AHA can highlight their need for using dermal absorption enhancers. For the skin penetration experiments, in vitro method was chosen. The use of in vitro models is also supported by the fact that stratum corneum (SC), the principle site of enhancer action, presents similar behavior in vivo and in vitro (5, 6).

2. Objectives

The objective of the study was to evaluate different chemical substances for their ability to enhance the skin delivery of two active ingredients including: Hydroxy Acids and Hyaluronic Acid.

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Table 1. Formulations 1 Through 17 and Their Constituents [a,b]

Formulation Number	Enhancer	Exposure Time, h	Permeated AHA, mg
1	DW, 2 mL	1.5	46.0
2	DW, 6 mL	12	298.2
3	Acetic acid (1M), 2 mL	1.5	48.0
4	Acetic acid (1M), 6 mL	12	108.1
5	Methanol, 2 mL	1.5	38.9
6	Ethanol, 2 mL	1.5	53.2
7	Ethanol, 6 mL	12	274.1
8	IPA, 2 mL	1.5	64.9
9	Glycerol, 2 mL	1.5	70.0
10	Glycerol, 6 mL	12	315.5
11	PG, 2 mL	1.5	54.9
12	EA, 2 mL	1.5	64.7
13	THF, 2 mL	1.5	56.4
14	DMSO, 2 mL	1.5	59.3
15	LP, 2 mL	1.5	89.8
16	LP, 6 mL	12	342.5
17	Olive oil, 2mL	1.5	51.6

[a] Abbreviations: AHA, α-hydroxyacids; DW, deinoized water; IPA, isopropyl alcohol; PG, propylene glycol; EA, ethyl acetate; THF, tetrahydrofurane; DMSO, dimethyl sulfoxide; and LP, liquid paraffin.
[b] All formulations contained 500 mg of α-hydroxyacids.

Table 2. Formulations 18 Through 21 and Their Constituents [a,b]

Formulation Number	Enhancer	Exposure Time, h	Permeated AHA, mg
18	SLS, 500 mg	1.5	41.5
19	Tween 80, 500 mg	1.5	49.0
20	Glycine, 500 mg	1.5	46.3
21	Albumin, 500 mg	1.5	15.3

[a] Abbreviations: AHA, α-hydroxyacids; and SLS, sodium lauryl sulfate.
[b] All formulations contained 500 mg of α-hydroxyacids and 2 mL of DW.

Table 3. Formulations 22 through 38 and Their Constituents [a,b]

Formulation Number	Enhancer	Exposure Time, h	Permeated AHA, mg
22	DW, 2 mL	1.5	0.3
23	DW, 6 mL	12	26.9
24	Acetic acid (1M), 2 mL	1.5	5.8
25	Acetic acid (1M), 6 mL	12	34.1
26	Methanol, 2 mL	1.5	4.1
27	Ethanol, 2 mL	1.5	8.2
28	Ethanol, 6 mL	12	18.7
29	IPA, 2 mL	1.5	23.4
30	Glycerol, 2 mL	1.5	57.3
31	Glycerol, 6 mL	12	70.6
32	PG, 2 mL	1.5	11.5
33	EA, 2 mL	1.5	0.3
34	THF, 2 mL	1.5	0.2
35	DMSO, 2 mL	1.5	2.1
36	LP, 2 mL	1.5	23.2
37	LP, 6 mL	12	31.8
38	Olive oil, 2 mL	1.5	6.5

[a] Abbreviations: AHA, α-hydroxyacids, DW, deionized water, IPA, isopropyl alcohol; PG, propylene glycol; EA, ethyl acetate; THF, tetrahydrofurane; DMSO, dimethyl sulfoxide; and LP, liquid paraffin.
[b] All formulations contained 500 mg of α-hydroxyacids and 2 mL of DW.

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Table 4. Formulations 39 Through 42 and Their Constituents[a,b]

Formulation Number	Enhancer	Exposure Time, h	Permeated AHA, mg
39	SLS, 500 mg	1.5	7.7
40	Tween 80, 500 mg	1.5	8.1
41	Glycine, 500 mg	1.5	0.5
42	Albumin, 500 mg	1.5	0.4

[a] Abbreviations: AHA, α-hydroxyacids; and SLS, sodium lauryl sulfate.
[b] All formulations contained 500 mg of α-hydroxyacids and 2 mL of DW.

3. Materials and Methods

AHA (lactic acid), HA, liquid paraffin (LP), acetic acid, ethyl acetate (EA), methanol, ethanol, isopropyl alcohol (IPA), glycerol, propylene glycol (PG), tetrahydrofurane (THF), dimethyl sulfoxide (DMSO), olive oil, sodium lauryl sulfate (SLS), glycine, and human serum albumin were purchased from Sigma-Aldrich company, USA.

3.1. Preparation of Formulations

Every formulation, i.e. formulations 1 through 42, were prepared via mixing 500 mg of AHA or HA, as the active ingredient, with 2 mL of each solvent (listed in Tables 1 and 3) or 500 mg of each surfactant (listed in Tables 2 and 4) as the enhancer, using a mechanical overhead mixer (Heidolph, RZR 2020, Germany) at 3000 rpm for 20 minutes. Mixing was continued to make a lotion.

3.2. Permeation Test

The transdermal penetration of AHA or HA was determined by a diffusion cell with an effective diffusion area of 10 cm^2 with a glass cap. Its 30 mL volume receiver chamber was filled with phosphate-buffered saline (PBS). An isolated piece of skin of a three-month-old chicken was fixed between two chambers as the diffusional membrane, making an almost stretched skin. Isolated skins were carefully selected in order to have low underlying fat tissue. They were accurately selected in order to be completely similar in terms of thickness of fat tissue and the number of hair follicles. Each formulation was placed and spread on the skin. Then the cap (donor chamber) was placed and fixed on that to avoid evaporation. The cell was placed in a shaker-incubator (Heidolph Incubator 1000, Heidolph Co., Germany) with a temperature of 32℃ for three hours (3, 21). The cap was taken away every 15 minutes and the lotion was rubbed evenly by a swab to help the active ingredient penetration. Three milliliters of PBS was taken after exposure time of 1.5 hours (or 12 hours for formulations 2, 4, 7, 10, 16, 23, 25, 28, 31, and 37) and analyzed for the concentration of AHA at 210 nm or for HA at 260 nm, using a UV-Vs spectrophotometer (Perkin-Elmer-Lambda25, USA). For the formulations containing 6 mL of the related solvent (formulations 2, 4, 7, 10, 16, 23, 25, 28, 31, and 37), 2 mL of the solvent was added during preparation of the formulation (like the other formulations with 2 mL of solvent); after three and six hours of incubation, 2 mL of the solvent was added to the formulation on the skin after each incubation time, i.e. a total of 6 mL for each mentioned formulation.

3.3. Statistical Analysis

Each formulation was tested three times for AHA or HA permeation and the data were reported as mean ± SD (n = 3). One-way analysis of variance (ANOVA) was used for comparing the mean differences. SPSS for Windows (version 11.5.0, SPSS Inc., Chicago, IL, USA) was employed for statistical analysis. $P < 0.05$ was considered significant.

4. Results

Permeability experiments resulted in permeation of about 40 to 90 mg AHA through the skin during 1.5 hours by formulations 1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 15, and 17, while each formulation contained 2 mL of the related solvent. During 12 hours, the AHA permeation of about 298, 108, 274, 315, and 342 mg by formulations 2, 4, 7, 10, and 16 was achieved, while each formulation contained 6 mL of the related solvent. Permeation of AHA was about 15 to 50 mg during 1.5 hours by formulations 18 through 21, while each formulation contained 500 mg of the related surfactant. In case of formulations containing HA, results showed HA permeation of 0.2 to 57.3 mg during 1.5 hours by formulations 22, 24, 26, 27, 29, 30, 32, 33, 34, 35, 36, and 38, each containing 2 mL of the related solvent. Results also showed HA permeation of 18.7 to 70.6 mg during 12 hours by formulations 23, 25, 28, 31, and 37, each containing 6 mL of the related solvent. Permeation of HA was 0.4 to 8.1 mg during 1.5 hours by formulations 39 through 42, each containing 500 mg of the related surfactant. Figures 1 and 2 show permeation of AHA through the skin from different formulations after 1.5 hours, and Figure 3 shows similar data after 12 hours. Using solvents as permeation enhancers (Figure 1), the maximum permeation during 1.5 hours belonged to the formulations 15 and 9 (89.8 mg with 2 mL of LP and 70 mg with glycerol, respectively) ($P < 0.05$). The minimum permeation during 1.5 hours belonged to the formulation 5 (about 39 mg) ($P < 0.05$) containing 2 mL of methanol. Other solvents lead to permeations between these amounts with a maximum of about 65 mg ($P < 0.05$). In case of using surfactants as permeation enhancers (Figure 2), the maximum AHA permeation was 49 mg for formulation 19 containing Tween 80 ($P < 0.05$) and the minimum permeation was 15.3 mg for formulation 21 containing albumin ($P < 0.05$). According

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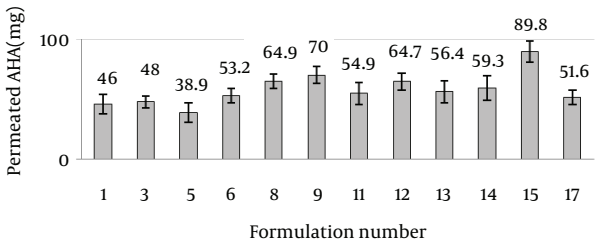
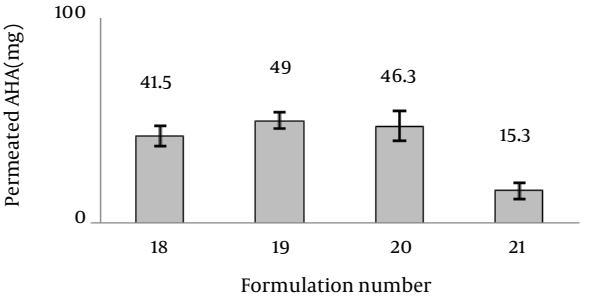
Figure 1. Permeated AHA for Formulations 1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 15, and 17



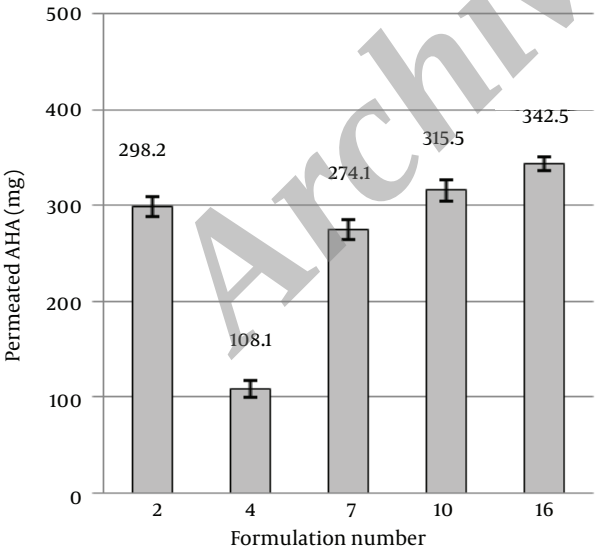
Permeated AHA (mg) for formulations 1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 15, and 17 containing 2 mL of DW (no solvent), acetic acid (1 M), methanol, ethanol, isopropyl alcohol (IPA), glycerol, propylene glycol (PG), ethyl acetate (EA), tetrahydrofurane (THF), dimethyl sulfoxide (DMSO), liquid paraffin (LP), and olive oil, respectively (exposure time, 1.5 hours) (n = 3).

Figure 2. Permeated AHA for Formulations 18, 19, 20, and 21



Permeated AHA (mg) for formulations 18, 19, 20, and 21 containing 500 mg of sodium lauryl sulfate (SLS), Tween 80, glycine, and albumin, respectively (exposure time, 1.5 hours) (n = 3).

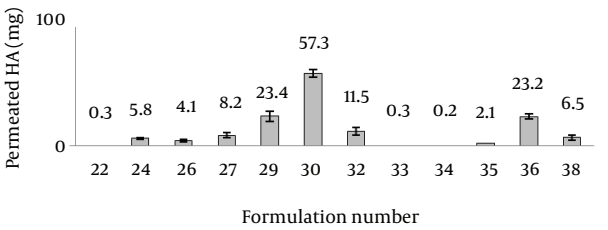
Figure 3. Permeated AHA for Formulations 2, 4, 7, 10, and 16



Permeated AHA (mg) for formulations 2, 4, 7, 10, and 16 containing 6 mL of DW, acetic acid (1M), ethanol, glycerol, and liquid paraffin (LP), respectively (exposure time, 12 hours) (n = 3).

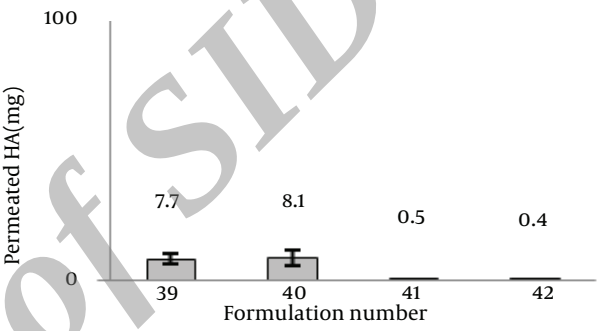
to Figure 3, the permeation of AHA after 12 hours showed a maximum of 342.5 mg for formulation 16 ($P < 0.05$) and a minimum of 108.1 mg for formulation 4 ($P < 0.05$) containing LP and acetic acid (1 M), respectively.

Figure 4. Permeated HA for Formulations 22, 24, 26, 27, 29, 30, 32, 33, 34, 35, 36, and 38



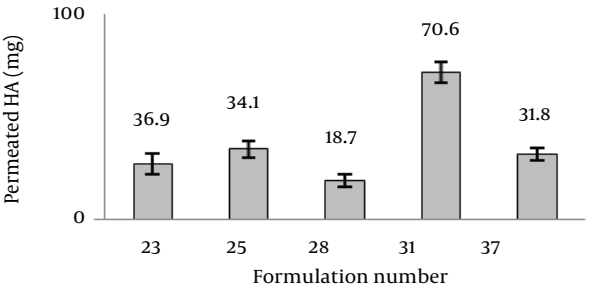
Permeated HA (mg) for formulations 22, 24, 26, 27, 29, 30, 32, 33, 34, 35, 36, and 38 containing 2 mL of DW (no solvent), acetic acid (1 M), methanol, ethanol, isopropyl alcohol (IPA), glycerol, propylene glycol (PG), ethyl acetate (EA), tetrahydrofurane (THF), dimethyl sulfoxide (DMSO), liquid paraffin (LP), and olive oil, respectively (exposure time, 1.5 hours) (n = 3).

Figure 5. Permeated HA for Formulations 39, 40, 41, and 42



Permeated HA (mg) for formulations 39, 40, 41, and 42 containing 500 mg of sodium lauryl sulfate (SLS), Tween 80, glycine, and albumin, respectively (exposure time, 1.5 hours) (n = 3).

Figure 6. Permeated HA for Formulations 23, 25, 28, 31, and 37



Permeated HA (mg) for formulations 23, 25, 28, 31, and 37 containing 6 mL of DW, acetic acid (1M), ethanol, glycerol, and liquid paraffin (LP), respectively (exposure time, 12 hours) (n = 3).

Figures 4 to 6 show permeation of HA through the skin from different formulations. Figures 4 and 5 show permeation of HA through the skin from different formulations after 1.5 hours, and Figure 6 shows similar data after 12 hours. Using solvents as permeation enhancers (Figure 4), the maximum permeation after 1.5 hours belonged to the formulations 30, 29, and 36 containing 2 mL of glycerol (57.3), IPA (23.4), and LP (23.2), respectively ($P < 0.05$). The minimum permeation after 1.5 hours belonged to the formulation 34, 33, and 22 (each about 0.3 mg) ($P < 0.05$) containing 2 mL of THF, EA, and DW, respectively (Figure 4). Other solvents lead to permeations between

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these amounts with a maximum of about 11.5 mg ($P < 0.05$). Using surfactants as permeation enhancers (Figure 5), the maximum and minimum HA permeation were 8.1 and 0.4 mg for formulation 40 (containing Tween 80; $P < 0.05$) and 42 (containing albumin; $P < 0.05$), respectively. Figure 6 presents permeated HA after 12 hours with a maximum of 70.6 mg for formulation 31 ($P < 0.05$) and a minimum of 18.7 mg for formulation 28 ($P < 0.05$) containing glycerol and ethanol, respectively.

5. Discussion

Results presented in the previous section revealed that generally, the enhancers used in this study positively affected the dermal permeation of both AHA and HA. Most of the enhancers increased the AHA permeation in comparison with that of formulation 1 (without enhancer). LP and glycerol strongly enhanced the AHA permeation, which would be due to the low volatility and strong solvent characteristics for AHA. Therefore, they neither evaporate nor dissolved the AHA, which led to the AHA transfer across the skin. On the other hand, they could dissolve the lipids of skin layers resulting in a decrease in the lipid viscosity and therefore, enhanced AHA transfer through the skin. Although IPA and EA had strongly enhanced the AHA permeation, it was not as effective as LP and glycerol. In fact, although IPA and EA are almost volatile, they have the ability to dissolve both AHA and skin lipids. DMSO, THF, PG, ethanol, and olive oil moderately enhanced the AHA permeation. Most of these solvents can dissolve both hydrophilic and hydrophobic substances and therefore, dissolve AHA and skin lipids. Since these solvents possess strong ability to dissolve hydrophobic substances, they also create new pathways in skin layers for the transfer of AHA molecules. Among these solvents, olive oil showed weaker effects than others because of its disability to dissolve AHA. As the last group, acetic acid (1 M), DW, and methanol could weakly enhance the AHA permeation. Although they could dissolve AHA, they lacked the ability to dissolve skin lipids. Despite other solvents, methanol decreased the AHA permeation. Therefore, LP and glycerol were the most effective enhancers for AHA permeation to the skin.

In case of using surfactants as enhancers, only Tween 80 increased the AHA permeation. Glycine showed no change in AHA permeation, while SLS and albumin decreased the AHA permeation. Amphiphilic molecules of Tween 80 could deposit between amphiphilic molecules in the skin layers. Such a mechanism can disrupt the lipid structure of the skin and therefore, can enhance the AHA permeation. Glycine could not affect the permeation, because of its weak surfactant characteristic. In case of SLS, its molecules can form an impermeable complex with AHA molecules. This can be the reason that AHA permeation was less in the presence of SLS. Albumin with a much larger molecular weight than other enhancers could hardly pass the skin. Little amounts of penetrated albumin deposited in the skin layers and because of its large molecule, it inhibited the AHA permeation. Therefore, Tween 80 was the most effective surfactant enhancer for the AHA permeation. Increasing the used volume of each solvent including DW, acetic acid, ethanol, glycerol, and LP (formulations 2, 4, 7, 10, and 16, respectively) from 2 to 6 mL, and prolonging the exposure time from 1.5 to 12 hours resulted in dramatic improve in AHA permeation in comparison with those for same solvents with the volume of 2 mL and the exposure time of 1.5 hours. Such elevations in concentration and exposure time were more effective for LP and glycerol as well as DW, which gave the highest AHA permeations of 342.5, 315.5, and 298.2 mg, respectively. Such a method of using the enhancer can be recommended when a long-term application of the formulation is possible.

Okuda et al. reported that AHA did not considerably penetrate the human skin within one to three minutes. They used shampoos containing AHA followed by rinsing of the shampoo after one to three minutes. They concluded that the main determinants of AHA penetration into the human skin were pH, concentration, and time (7). Our results confirm these findings since the primary concentration of AHA or HA and the exposure time in our study were respectively larger and longer than those of Okuda et al. (7). Most of the formulations increased the HA permeation in comparison with the formulation 22, which had no enhancer. EA, THF, albumin, and almost glycine (formulations 33, 34, 42, and 41, respectively) were the exceptions that did not increase the HA permeation. These effects were related to the volatility of EA and THF, high molecular weight of albumin, and weak surfactant characteristics of glycine. The solvents, glycerol, IPA, LP, and PG (formulations 30, 29, 36, and 32, respectively) considerably enhanced the HA permeation. The reason was that they not only were good vehicles for HA molecules but also could strongly dissolve the skin lipids that led to decreased lipid viscosity and enhanced HA permeation. Considering such a mechanism, glycerol was the most potent solvent and enhancer for HA. Solvents including DMSO, methanol, acetic acid, olive oil, and ethanol as well as surfactants including SLS and Tween 80 moderately enhanced the HA permeation (formulations 35, 26, 24, 38, 27, 39, and 40, respectively). DMSO is more volatile than others and therefore, did not have enough time for its action. Methanol, acetic acid, and ethanol could dissolve both HA and skin lipids. Although olive oil did not dissolve the hydrophilic HA, it showed its potency to dissolve the skin lipids by its slight improvement in HA permeation. EA and THF enhanced the AHA permeation more than HA. This could be due to the small molecules of AHA were mixable with EA and THF that led to a slight decrease in EA and THF melting points and therefore, inhibited the rapid evaporation of EA and THF. This phenomenon gave the EA and THF enough time to act as enhancers. Generally, HA needed more time for permeation and also could not make a homogeneous solution or mixture with EA and THF. Therefore, EA and THF evaporated more rapidly witha lack of enough time for their enhancement action.

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SLS and Tween 80, the well-known pharmaceutical surfactants, acted with the mechanism mentioned above for the permeation of AHA. The noticeable point was that the enhancing effect of SLS or Tween 80 for permeation of HA was significantly higher than that of AHA, which proved the more effectiveness of surfactants for permeation of larger molecules. Albumin and glycine did not enhance the HA permeation. Although peptide or protein structures are one group of surfactant compounds, this ineffectiveness was referred to the large molecular weight of albumin and weak surfactant structure of glycine. Since the molecular weight of HA was high, they needed enough space for passing the skin layers that was occupied by the large molecules of albumin.

Similar to the results for AHA, increasing the volume of each solvent including DW, acetic acid, ethanol, glycerol, and LP (formulations 23, 25, 28, 31, and 37, respectively) from 2 mL to 6 mL, and prolonging their exposure time from 1.5 hours to 12 hours led to a dramatic improvement in HA permeation in comparison with those for same solvents with the volume of 2 mL and exposure time of 1.5 hours. Such increase in concentration and prolongation of exposure time were more effective for glycerol, acetic acid, and then for LP, which presented the largest permeations of HA in this study with the average HA permeations of 70.6, 34.1, and 31.8 mg, respectively. This method of using extended exposure times could be performed when the related formulation can be applied and remain on the skin for a long time. Overall, results showed that the enhancement of HA permeation was less than that of AHA. Since both molecules have hydrophilic structures, such results were attributed to the higher molecular weight of HA than AHA.

There are several reports indicating the need for an enhancement mechanism to increase the permeation of HA through the skin. Yang et al. reported that considerable amount of HA could pass across mouse skin and enter the blood stream using its conjugation with human growth hormone (HA-hGH) via a receptor-mediated transdermal delivery method (8). Chen et al. also could deliver considerable amount of HA through the porcine skin using skin permeating and cell entering (SPACE) peptide carriers (2). Lim et al. synthesized HA and polyethylene glycol (PEG) hydrogel nanoparticles (37 nm) and used them as effective carriers in transdermal delivery systems. They obtained considerable nanoparticle penetration into a skin of albino guinea pig (9). All these researches suggested ways for improvement of HA permeation. Our results presented another way for such improvement via using suitable solvents and surfactants. This recommended way is significantly easier and more accessible for such a purpose, although it had slightly lower potency than suggested ways in the aforementioned studies. Most of the transdermal absorption enhancers used in this study elevated the AHA or HA skin permeation. Negligible amounts of HA permeated the skin without any enhancer or in the presence of DW. Enhancers were more effective for HA than AHA. The most potent enhancers for increasing the permeation of AHA after 1.5 hours were LP, glycerol, and EA, consecutively, and the most potent enhancers for increasing the permeation of HA after 1.5 hours were glycerol, IPA, and LP, consecutively. Permeations were considerably improved by increasing volume of enhancer and prolonging exposure time, which gave the maximum AHA or HA permeations in this study. The most potent enhancers for increasing the AHA permeation after 12 hours were LP, glycerol, and DW, consecutively, and the most potent enhancers for increasing the HA permeation after 12 hours were glycerol, acetic acid (1 M), and LP, consecutively. In this order, the maximum permeation of AHA was 342.5 mg obtained by the formulation containing 6 mL of LP with exposure time of 12 hours. The maximum permeation of HA was 70.6 mg obtained by the formulation containing 6 mL of glycerol with exposure time of 12 hours. The effective enhancers for AHA permeation, which were also effective for HA permeation, were glycerol and LP either in short-term (1.5 hours) or in long-term use (12 hours). The latter enhancers are recommended for using in topical cosmetic or pharmaceutical products containing both AHA and HA. If surfactants are to be used in such products, Tween 80, which was effective for increasing the permeation of both AHA and HA, is recommended. Ultimately, the use of albumin (or other proteins), methanol, or SLS in topical preparations containing AHA should be evaluated and optimized, because these enhancers decreased the amounts of permeated AHA.

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