# Effects on Skin Irritation and Turnover Rate by the Control of Skin Permeability of Alpha-hydroxyacids

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## Abstract

The effect of a novel delivery system, water in oil emulsion containing chitosan hydrogel as a inner phase (W/O-C) was evaluated, and the relationships between the skin permeation, the skin primary irritation and the skin turnover rate of AHAs were discussed. We selected glycolic acid (GA), lactic acid (LA), malic acid (MA), and tartaric acid (TA) as model AHAs.

The steady state fluxes of 4 AHAs across the excised hairless mouse skin increased as the molecular weights of the AHAs decreased (GA>LA>MA>TA). The skin turnover times were shortened in all AHAs, compared with control. The skin permeation and the skin primary irritation of the LA decreased and the skin turnover time increased, as the pH increased. The maximum therapeutic index was obtained with pH 3.8, 0.5 M LA. It was suggested that the skin permeability of LA might be a main factor for prediction of the skin irritation and the skin turnover time. On the other hand, the W/O-C containing pH 3.8, 0.5 M LA indicated a good sustained release property of LA, compared with water in oil emulsion without chitosan hydrogel (W/O) or oil in water emulsion (O/W).

The skin permeability and the skin irritation of AHAs from the W/O-C decreased, compared with W/O or O/W, however the skin turnover time showed almost the same value as W/O or O/W.

In conclusion, we suggest that the control of the skin permeation of AHAs would be an important tool for reducing the skin irritation and for maintaining the positive effect of AHAs, and the W/O-C system could be a potential candidate for future cosmetological application of AHAs.

## Introduction

Recently, alpha hydroxyacids (AHAs) have been gaining a lot of attention for their anti-aging effects. It has been reported that AHAs reduced the thickness of hyperkeratotic

stratum corneum by reducing corneocyte cohesion at lower levels of stratum corneum, and sustained application of AHA increased the thickness or firmness of dermis, resulting in reduction of wrinkles[1-4]). However, the high concentration of AHAs enough to obtain these effect might cause serious skin irritation. According to CTFA[5]), the rate of adverse reaction complaints for AHA products was about one and a half times higher than that for traditional moisturizers. This rate may be not so serious because the beneficial effects of AHAs are more appealing. However, our unpresented reports indicated that the skin irritation of AHAs for the Koreans was very serious, and this result led us to consider that the Korean's skin might be more sensitive than the Westerner's.

Therefore, we need more information about the dose, effectiveness or skin irritation for AHAs to develop AHA products which are suitable for the Korean.

On the other hand, irritants can cause the skin side effects by penetrating the stratum corneum and damaging viable cells in the skin. Therefore, the data of skin permeation could provide a significant clue to predict the skin irritation. We supposed that the skin permeation of AHAs be low because of their hydrophilic properties. However, the strong keratolytic effect of AHAs might reduce the barrier function of stratum corneum and then accelerate the skin permeability of them dramatically. This might be one of the reasons of side effects to the skin.

In the present study, we selected glycolic acid (GA), lactic acid (LA), malic acid (MA), and tartaric acid (TA) as model AHAs and then evaluated the skin permeability, the skin primary irritation and the skin turnover rate. Especially, we chosed LA as a representative of AHAs and tried to obtain an optimal condition for practical using with more studies on concentration and pH. Furthermore, a novel delivery system, water in oil emulsion containing chitosan hydrogel as a inner phase was developed and evaluated.

## Experimental

### *Materials*

Glycolic acid (GA), DL-lactic acid (LA), L(-)-malic acid (MA) and L(+)-tartaric acid (TA) were purchased from Sigma company (St. Louis, MO, USA). Chitosan (Flonac N) was kindly supplied from Kyowa Tecnos Company (Chiba, Japan). All other chemicals were of reagent or cosmetic grade.

### *Skin Permeation Studies*

Vertically assembled Franz type diffusion cells (Microette transdermal diffusion system, Hanson Research Corporation, Chatsworth, CA, USA) were used for in vitro skin permeation experiments. The system consisted of Franz type diffusion cells with an effective diffusional area of 1.77 $cm^2$ and receptor volume of 7.0 ml, autosampler, and cell drive system with rpm controller. The fundamental experiments were performed

according to the method given in our previous report[6]). Briefly, the excised skin of female hairless mice was obtained from 8-9 weeks old, 27-33 g animals. The above skin was mounted on the diffusion cell, and the receptor compartment was filled with 7.0 ml of 50 mM phosphate buffered saline (PBS) at pH 7.4 maintained at 32°C. The donor compartment was charged with 0.25 ml of 0.5 M AHA preparation at 32°C and capped.

Seven hundred microliter aliquots were withdrawn from the receptor compartment periodically for 24 h and replaced with equal volume of fresh PBS maintained at 32°C.

The sample solution was filtered through a membrane filter (pore size, 0.2 um; MFS-13; Micro Filtration Systems, CA, USA) and injected onto the HPLC. The HPLC consisted of a solvent delivery pump (Waters 600 Pump, Waters Co., MA, USA), a cation exchange column (Shodex Ionpak KC-811, 8*300 mm, Showa Denko K.K., Tokyo, Japan), a UV detector (Waters 486 UV Detector), and a data processing system (Waters Millennium). The mobile phase was 0.1 % phosphoric acid, and the flow rate was 1.0 ml/min. A wavelength of 210 nm was selected, and the temperature of the column was maintained at 50°C. For each AHA, the retention times were 8.67 min for GA, 8.90 min for LA, 7.28 min for MA, and 6.74 min for TA. The correction of concentration against eash sample point was also undertaken.

### *Calculation of Permeation Parameters*

For each diffusion cell, the cumulative permeation amount of AHAs versus time was plotted. The steady state flux (%/h) and the lag time (h) could be calculated from the slopes and the intercepts, respectively, on the time axis of the linear portion of the plots.

### *Determination of n-Octanol/Water Partition Coefficient*

Each AHAs was dissolved in 100 ml of water or each buffer solution (pH 3.8, 5.0, 7.0) and initial concentrations of AHAs were measured. Each of these solutions was added to 100 ml of n-octanol in a tightly closed conical tube and the mixtures were shaken for 24 h at 32°C and centrifuged at 2000 rpm for 5 min. Then 0.1 ml aliquots of the water phase were pipetted out and analyzed for AHAs by the HPLC method described above.

### *Determination of Turnover Time*

The experiment was performed by our dihydroxy acetone (DHA) method. Briefly, the Hill top chamber (Hill top Research, USA) containing 0.4 ml of 10 % DHA solution was applied to two test sites of upper forearm for 1 h. To increase the color intensity, this process was repeated at 6 h after first application. The stratum corneum of the test sites was colored to dark brown. After 24 h, a fixed amount of the AHAs preparations was applied to one test site. The other site remained for control. Prior to each application of the AHAs preparations, the skin color (as L, a, b values) of the test site was measured with colorimeter (Minolta, Japan) and average color difference, delta E, was calculated between each treated site and the control. The decolorization percentage of

each test site versus time (day) was plotted. The turnover time (day) could be calculated from the slopes and the intercepts obtained from regression analysis, on the time axis of the plots.

### *Determination of Skin Primary Irritation*

Three male and three female guinea pigs (8-10 weeks, 350-400g) were shaved along the dosal surface of the back, 24 h in advance of the procedure. Two test sites in the shaven area were then delineated with picric acid. One test site remained intact. Approximately 0.5 ml of AHA preparation was placed onto a gauze pad and then applied directly to the animal's back. The gauze pad was covered with an occlusive bandage and tightly secured to the animal. The patches were removed following 48 h of exposure and each test site was wiped with dry disposable paper towels. The test sites were evaluated for dermal irritation approximately 1 h and 48 h after patch removal (48 h and 96 h evalutions) using the Draize method of scoring.

### *Preparation of Water in Oil Emulsion with Chitosan Gel*

Table III represents the formulas of oil in water (O/W), water in oil (W/O) and W/O containing chitosan hydrogel as a inner phase (W/O-C). Each mixture of oil phase and aqueous phase was dissolved seperately using propeller agitator at 60°C. The aqueous phase was added slowly to the oil phase at 8000 rpm with homomixer (Robomix, Tokushu Kika Kogyo Co., Japan), maitaining 60°C for 10 min. Thereafter, tripolyphosphate was added to the above at 8000 rpm for 4 min and cooled to room temperature. The other formulations (O/W, W/O) were prepared with general method.

## Results and Discussion

### *Skin Permeation, Skin Turnover Time, Skin Primary Irritation of AHAs by Water Solution Systems.*

Figure 1 represents the skin permeation profiles of GA, LA, MA, and TA across the excised hairless mouse skin from (a) 0.5 M AHAs water solutions and (b) 0.5 M LA buffered solutions of pH 3.8, 5.0, and 7.0. The corresponding permeation parameters, molecular weight and n-octanol/water partition coefficients are listed in Table I.

With respect to AHAs water solutions, steady state fluxes ranging from 0.02 to 0.76 %/h and lag times ranging from 3.2 to 9.1 h were observed for the 4 AHAs. The greatest steady state flux was obtained for GA (0.76 %/h), which had the smallest molecular weight of all AHAs examined in this study, whereas TA (0.02 %/h), with the largest molecular weight and the lowest partition coefficient, showed the lowest value. In the case of LA (pKa=3.86) buffered solutions with different pH, the skin permeability of LA decreased remarkably as the pH increased. It was considered that this result was due to a decrease of n-octanol/water partition coefficient with an increase of ionic form of LA

according to the pH partition theory of Henderson-Hasselbalch's. It has already been reported that the skin permeation coefficients of nine steroidal drugs with almost the same molecular weight showed a good linear relationship to the lipophilicities of the drugs7), and when the physicochemical properties of drugs are similar, the skin permeation of the drugs will be improved as the molecular weights become smaller. The present results were in good agreement with these reports.

Table II shows the skin turnover time using volunteers and skin primary irritation using guinea pigs and therapeutic index from 0.5 M AHAs water solutions, 0.5 M LA buffered solutions and LA water solutions with various concentrations. Figure 2 represents the skin turnover time profiles of 4 AHAs obtained by regression analysis as a typical example.

The skin turnover time was measured with our dihydroxy acetone (DHA) method. The results obtained with the DHA method was consistent with the well-known method of using dansyl chloride[8]), however we considered that this method was more practical because the dansyl chloride might cause skin side effects.

The skin turnover times of 4 AHAs (11.7-13.6 day) were remarkably shortened compared with control (17.1 day) which had no treatment and TA (11.7 day) showed the shortest value. In the different pH buffered LA solutions, the turnover time increased with an increase in pH, especially it increased rapidly above pKa. With the respect to the different concentrations of LA, the skin turnover times were shortened as the concentration increased, however the difference among them was small. It was interesting to note that the skin turnover time was shortened with a decrease of the skin permeability of 4 AHAs. This result may be due to the increase of keratolytic effect by unpenetrated

AHA remained on the skin surface. However, in the case of LA with different pH, the skin turnover time was shortened with an increase of the steady state flux. Further detailed investigations are necessary to elucidate the mechanism of this result related to the sustained effects of AHA on the stratum corneum as well as the dermis and the different keratolytic efficacy of each AHA.

The score of skin primary irritation caused by different kinds of AHA showed serious values above 1.1. Especially, GA caused the highest skin irritation. In different pHs, the skin irritation of LA was in inverse propotion to the pH and in propotion to the skin permeability. The score of skin irritation was depend on the concentration of LA. On the other hand, the therapeutic index of LA calculated from the decreasing percentages of the skin turnover rate against the control and the skin primary irritation showed that the highest value (29.2) was obtained with 0.5 M, pH 3.8 buffered solution.

In these studies, we concluded that the skin permeability of AHA may be the main factor for prediction of the skin turnover time or the skin primary irritation. Furthermore, it was suggested that the optimum for cosmetological application of LA was 0.5 M of dose and 3.8 of pH, for the Koreans.

***Skin Permeation, Skin Turnover Time and Skin Primary Irritation of LA by Water in Oil Emulsion with Chitosan Gel System***

Table III shows the formulations of oil in water (O/W), water in oil (O/W) and W/O emulsion with chitosan gel (W/O-C), containing pH 3.8, 0.5 M LA. The parameters on the skin permeation, skin turnover time, skin primary irritation and therapeutic index are listed in Table IV. In all formulations, the skin permeability of LA showed a little value compared with the above results obtained from the water solution systems. The O/W showed essencially same skin permeability as the corresponding water solution system, and the greatest steady-state flux was observed. The W/O reduced slightly in both of the steady-state flux and the lag time, compared with the O/W. The skin permeability of LA from W/O-C indicated the futher decrease of steady-state flux and the shorter lag time. However, the present results suggested that the W/O-C might be a good sustained release preparation with a low initial flux and a long-term activity. On the other hand, the skin primary irritation of LA was reduced as following order: O/W>W/O>W/O-C. The skin turnover time showed about the same value in three formulations, therefore, the highest therapeutic index was obtained with the W/O-C.

## Conclusion

In conclusion, it was suggested that the skin permeability of AHA might be an excellent tool to control the skin turnover time or the skin primary irritation. Furthermore, we found that the optimal condition to use LA in cosmetics was 3.8 of pH and 0.5 M of dose for the Koreans and W/O-C which reduced the skin primary irritation and sustained the positive effects of LA, could be a good candidate for cosmeceutical use of AHA.

## References


1) E.Berardesca, H.Maibach, Cosmetics & Toiletries, 110(6), 30, 1995.
2) E.J.Van Scott, "V World Congress Int. Soc. Cosmet. Dermatol." 7, 1995.
3) W.P.Smith, Cosmetics & Toiletries, 109(9), 41, 1994.
4) E.J.Van Scott, R.J.Yu, Cutis, 43(3), 222, 1989.
5) FDC Reports "The Rose Sheet" Toiletries, Fragrances and Skin Care, 17(11), 1996.
6) C.K.Lee, T.Uchida, S.Goto, et al., J. Pharm. Sci., 83(4), 562, 1994.
7) C.K.Lee, S.Goto, et al., Drug Delivery System, 8(4), 283, 1993.
8) L.H.Jansen, M.T.Hojiyo Tomoko, A.M.Kligman, Br. J. Dermatol, 90, 9, (1974).

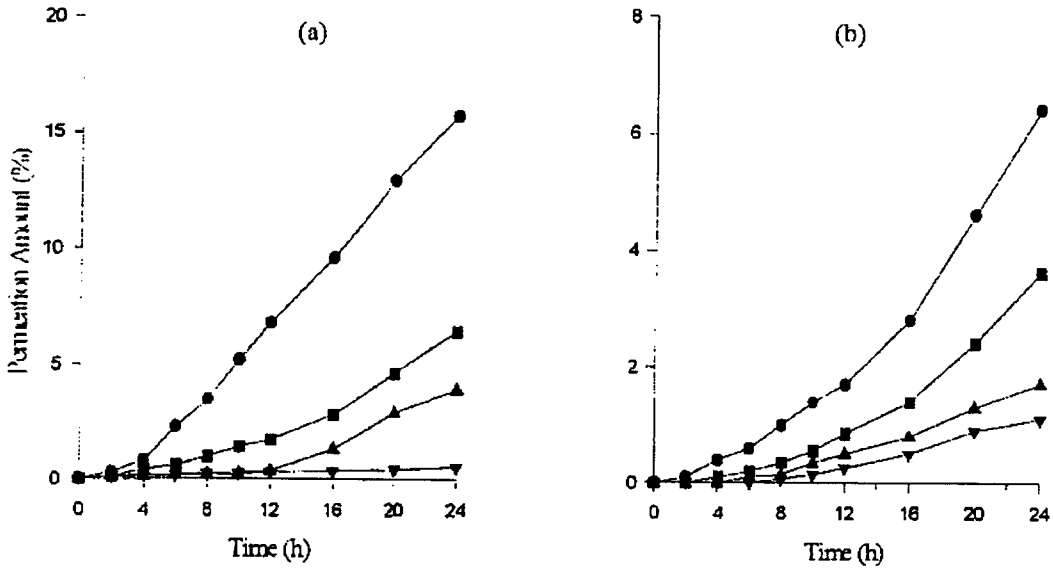


**Figure 1** - Skin permeation profiles of AHAs across the excised hairless mouse skin from (a) 0.5 M water solutions and (b) 0.5 M LA buffered solutions.
a: (●) GA, (■) LA, (▲) MA, (▼) TA
b: (●) LA, (■) pH 3.8, (▲) pH 5.0, (▼) 7.0

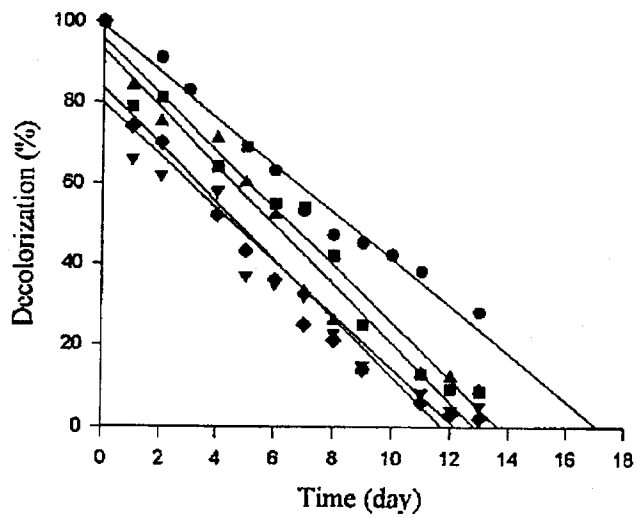


**Figure 2** - Skin turnover rate profile of 4 AHAs obtained by regression analysis.
(●) Control: y = -5.79 x + 98.93, r = 0.979
(■) GA: y = -7.00 + 95.51, r = 0.964
(▲) LA: y = -7.24 + 92.80, r = 0.944
(▼) MA: y = -6.55 + 79.70, r = 0.915
(◆) TA: y = -7.14 + 83.56, r = 0.944

**Table I** - Molecular Weight, n-Octanol/Water Partition Coefficient and Skin Permeation Parameters from 0.5 M AHAs Water Solutions and 0.5 M LA Buffered Solutions.

| Solutions | M.W. | Partition Cefficient | Lag time (h) | Steady State Flux (%/h) |
|---|---|---|---|---|
| GA | 76.05 | 0.202 | 3.2 | 0.76 |
| LA | 90.08 | 0.213 | 6.2 | 0.34 |
| MA | 134.09 | 0.077 | 9.1 | 0.25 |
| TA | 150.09 | 0.056 | 5.6 | 0.02 |
| pH 3.8 LA | | 0.090 | 7.4 | 0.20 |
| pH 5.0 LA | | 0.015 | 6.5 | 0.10 |
| pH 7.0 LA | | 0.001 | 7.4 | 0.07 |

**Table II** - Skin Turnover Time, Skin Primary Irritation and Therapeutic Index of AHAs from 0.5 M AHAs Water Solutions, 0.5 M LA Buffered Solutions and LA Water Solutions with Various Concentrations.

| Solutions | Skin Turnover Time (day) | Skin Primary Irritation | Therapeutic Index |
|---|---|---|---|
| GA | 13.6 | 1.4 | 14.6 |
| LA | 12.8 | 1.1 | 22.9 |
| MA | 12.2 | 1.3 | 22.0 |
| TA | 11.7 | 1.1 | 28.7 |
| pH 3.8 LA | 13.1 | 0.8 | 29.2 |
| pH 5.0 LA | 14.9 | 0.6 | 26.3 |
| pH 7.0 LA | 17.0 | 0.4 | 1.5 |
| 0.1 M LA | 14.9 | 0.9 | 14.3 |
| 0.3 M LA | 13.2 | 1.1 | 20.7 |
| 0.7 M LA | 12.2 | 1.5 | 19.1 |
| Control | 17.1 | | |

* Therapeutic Index = Decreasing rate (%) of Turnover Time against Control / Skin Irritation

**Table III** - Formulas of O/W, W/O and W/O-C Fomulation Systems Used in This Study

| Raw Materials | O/W | W/O | W/O-C |
|---|---|---|---|
| Oil Phase | | | |
| Cyclomethicone | 11.0 | 11.0 | 11.0 |
| Octyl dodecanol | 1.0 | 1.0 | 1.0 |
| Cetyl octanoate | 2.0 | 2.0 | 2.0 |
| Squalane | 1.0 | 1.0 | 1.0 |
| Caprylic/capric triglyceride | 2.0 | 2.0 | 2.0 |
| PEG-40 stearate | 2.0 | | |
| Sorbitan sesquioleate | 1.0 | | |
| Cetyl dimethicone copolyol | | 2.0 | 2.0 |
| Dimethicone copolyol | | 1.0 | 1.0 |
| Aqueous Phase | | | |
| Chitosan | 0.3 | | 0.3 |
| DL-Lactic acid (85%) | 5.3 | 5.3 | 5.3 |
| NaOH (10%) | 11.1 | 11.1 | 11.1 |
| Tripolyphosphate | 3.0 | 3.0 | 3.0 |
| Purified water | to 100 | to 100 | to 100 |

**Table IV** - Skin Permeation Parameters, Skin Turnover Time, Skin Primary Irritation and Therapeutic Index of LA from O/W, W/O and W/O-C Formulation Systems Containing pH 3.8, 0.5 M LA.

| Formulations | Lag time (h) | Flux (%/h) | Turnover Time (day) | Irritation | Therapeutic Index |
|---|---|---|---|---|---|
| O/W | 6.3 | 0.18 | 12.9 | 0.8 | 30.7 |
| W/O | 5.5 | 0.14 | 13.1 | 0.7 | 33.4 |
| W/O-C | 5.1 | 0.11 | 13.4 | 0.6 | 36.1 |