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Influence of glycolic acid as a component of different formulations on skin penetration by vitamin A palmitate

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Synopsis

Among the many active agents for dermocosmetic purposes that have been described, marketed, and prescribed, vitamins (vitamin A palmitate among them) and alpha-hydroxy acids such as glycolic acid have been gaining scientific importance. When penetrating the skin, vitamin A palmitate contributes to leaving it soft and smooth, improving its properties as a water barrier. With the topical application of vitamin A palmitate, the skin is stimulated to produce more epidermal protein, making the epidermis thicker and covered with a better formed keratin layer. Glycolic acid is part of a new generation of cosmetics used for treatment. It is a fascinating active agent with a simple molecular structure that has yielded highly satisfactory results in terms of recovery of aged skin. The combination of low concentrations of glycolic acid with vitamin A palmitate has been extensively used in dermocosmetic formulations since it has been speculated that glycolic acid reduces the cohesion of corneocytes, stimulating natural skin desquamation, and also increases skin hydration, thus being considered to increase skin penetration by vitamin A palmitate and to potentiate its pharmacodynamic effects. The objective of the present study was to investigate the influence of the presence of glycolic acid on *in vivo* skin penetration by vitamin A palmitate. Non-ionic gel, gel cream, and cream formulations containing vitamin A palmitate combined or not combined with glycolic acid were studied. The formulations were applied to delimited areas on the depilated dorsum of 24 guinea pigs, and biopsies were collected one, two, and four hours later to determine the percentage of vitamin A palmitate that penetrated the skin during these time intervals. The results indicate that the presence of glycolic acid in the formulations containing vitamin A palmitate increases the behavior of skin penetration by vitamin A palmitate along time in the gel formulation.

INTRODUCTION

Among the numerous active compounds used for dermocosmetic purposes that have been described, marketed, and prescribed, vitamins and alpha-hydroxy acids are becoming increasingly popular, representing a challenge for many researchers (1–8). Vitamin A in its different forms has been widely used in topical preparations, and its esters are used as components of cosmetic formulations. When absorbed through the skin, vitamin A palmitate contributes to the maintenance of skin softness and smoothness, improving the water barrier properties of this tissue. This property has led to the use of vitamin A palmitate for the treatment of seasonal/environmental problems (dehydration, heating, pollution) and indicates its use in after-sun products because of its "normalizing" (corrective) properties (9–12).

Alpha-hydroxy acids, and glycolic acid in particular, are currently being used as actives to treat signs of skin aging (13,14). Glycolic acid at low concentrations in combination with dermocosmetic formulations containing vitamin A palmitate has been prescribed by many dermatologists, who believe that glycolic acid, by stimulating natural skin desquamation and increasing skin hydration, may promote a better bioavailability of vitamin A palmitate in the skin. This theory, however, is based only on clinical observations.

The objective of the present investigation was to study the effect of glycolic acid on *in vivo* skin penetration by vitamin A palmitate in gel, gel cream, and cream formulations containing vitamin A palmitate combined or not combined with glycolic acid.

EXPERIMENTAL PROCEDURE

SAMPLES OF COSMETIC PREPARATIONS

Three formulations (Table I) were prepared in a Fisatom shaker at approximately 2000 rpm and supplemented or not supplemented with 2% vitamin A palmitate (1,000,000 IU/g) combined or not combined with 10% glycolic acid at 70% concentration.

pH DETERMINATION

The pH of the formulations under study was measured with an Analion pH-meter using samples diluted 0.5:10 in distilled water.

STUDY OF *IN VIVO* SKIN PENETRATION BY VITAMIN A PALMITATE IN THE FORMULATIONS UNDER STUDY

To assess *in vivo* penetration we used 24 guinea pigs weighing on average 350 g. The animals' backs were depilated, and 0.05 g of the formulations listed in Table I supple-

Table I
Formulations

	Percentage of components in each formulation		
Components	F1	F2	F3
Hydroxyethyl cellulose	2.00	2.00	—
Glycerin	3.00	3.00	3.00
Propyleneglycol	2.00	2.00	2.00
Methyldibromo glutaronitrile and phenoxyethanol	0.20	0.20	0.20
DL-α-tocopherol	0.01	0.01	0.01
Squalene	—	2.00	2.00
Hydrogenated lecithin (powder)	—	1.00	1.00
Self-emulsifying base	—	—	4.00
Distilled water q.s.	1.00	1.00	1.00

mented with vitamin A palmitate and with vitamin A palmitate plus glycolic acid was added to paired areas of 2.0 cm^2 each. The formulations containing vitamin A palmitate were added to one of the paired areas, and the formulations containing vitamin A palmitate plus glycolic acid were added to the other. After observation for one, two, and four hours, the skin surface of the animals was cleaned with ethyl alcohol and biopsies were obtained with the aid of a dermatologic punch, with the removal of a 0.5 cm^2 area, as illustrated in Figure 1.

The biopsy material was fragmented with a forceps and surgical scissors and extracted with isopropylic alcohol for 24 minutes by ultrasound. After extraction, the material was filtered through a qualitative paper filter, the filtrate was made up to a defined volume, and samples of the filtrate were concentrated and submitted to spectrophotometry using a double-beam Beckman spectrophotometer at 326 nm.

The amount of vitamin A palmitate that penetrated the skin was calculated using a standard vitamin A palmitate curve, as shown in equation 1:

$$A/A_1 \times 100 = \% \text{ vitamin A palmitate present in the skin} \qquad (1)$$

where A is the amount of vitamin A palmitate at one, two, or four hours, and A_1 is the amount of vitamin A palmitate applied to the skin.

RESULTS

DETERMINATION OF THE pH OF THE FORMULATIONS UNDER STUDY

The pH values obtained for the formulations under study are listed in Table II, which

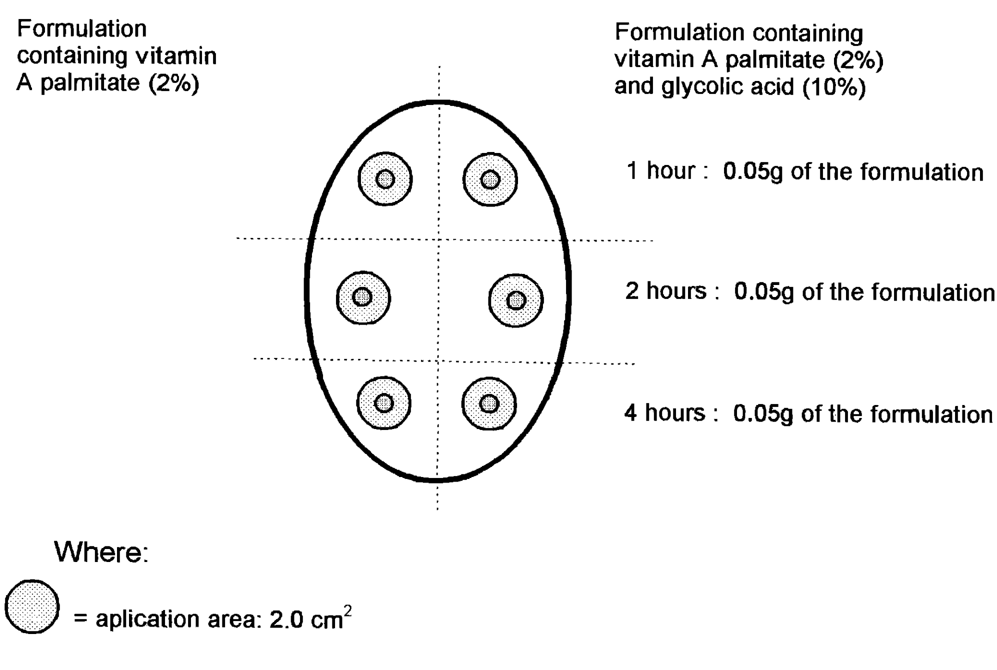

Figure 1 Schematic representation of the skin of the guinea pig dorsum with the areas used in the study of vitamin A palmitate penetration.

shows that the presence of glycolic acid significantly decreased the pH of all the dermocosmetic bases studied.

STUDY OF *IN VIVO* SKIN PENETRATION BY VITAMIN A PALMITATE IN THE FORMULATIONS UNDER STUDY

After extracting vitamin A palmitate from the biopsy material, we determined its percentages of penetration as s function of time for the formulations studied. The results are presented in Table III.

A test based on the Tukey test indicated that seven animals are sufficient to detect statistically significant differences for the variation determined by analysis of variance.

ANALYSIS OF VARIANCE

To determine the possible presence of statistically significant differences among the formulations studied in terms of vitamin A palmitate penetration into the skin, the data were analyzed by analysis of variance, followed by the Tukey test, which was more adequate for the mathematical model used in the experiment. The results are summarized in Tables IV through VII.

Time factor. Analysis of variance revealed a significant difference at the 1% level with respect to the times studied in the present investigation, indicating that the time factor strongly affected vitamin A palmitate penetration into the guinea pig skin. The complementary Tukey test for the time factor demonstrated that at time one-hour, penetration was significantly lower than at the other two times studied, which, in turn, did not differ from one another. The results of the Tukey test for the time factor are shown in Table IV.

Glycolic acid factor. Analysis of variance showed that there was no significant difference in percent penetration between formulations containing or not containing glycolic acid.

Table II
pH Values Obtained

Formulations	pH
Gel	
No additive	5.00
Vitamin A palmitate added (2%)	5.10
70% glycolic acid added (10%)	2.70
Vitamin A palmitate (2%) + 70% glycolic acid (10%) added	2.70
Gel cream	
No additive	5.50
Vitamin A palmitate added (2%)	5.90
70% glycolic acid added (10%)	2.85
Vitamin A palmitate (2%) + 70% glycolic acid (10%) added	2.85
Cream	
No additive	6.00
Vitamin A palmitate added (2%)	5.50
70% glycolic acid added (10%)	2.70
Vitamin A palmitate (2%) + 70% glycolic acid (10%) added	2.70

Table III
Percentages of Applied Dose of Vitamin A Palmitate in the Skin of Guinea Pigs Exposed to the Various Formulations (IU/0.5 cm^2 Guinea Pig Skin)

	Time (h)					
Formulation	1		2		4	
Gel						
Without glycolic acid	3.93	5.79	5.02	7.83	5.30	9.12
	4.55	6.72	6.59	2.26	6.68	8.37
	10.07	5.46	2.88	8.83	6.83	9.39
	9.34	9.44	3.30	4.96	8.59	7.92
With glycolic acid	6.21	7.72	9.55	4.16	7.13	4.77
	4.09	5.76	9.91	13.26	4.82	8.07
	8.16	10.75	6.82	14.90	4.67	6.83
	6.08	4.03	13.23	6.38	6.60	6.12
Gel cream						
Without glycolic acid	4.44	5.08	7.41	12.09	6.41	5.74
	3.20	4.04	7.66	5.28	11.07	7.83
	3.30	4.18	3.24	8.39	5.91	10.11
	3.45	5.25	7.78	6.62	4.39	10.01
With glycolic acid	6.89	8.20	5.81	8.78	7.08	4.95
	4.19	5.28	7.53	6.62	5.26	9.22
	8.67	4.92	8.25	6.48	8.60	5.72
	3.35	3.48	7.57	11.74	5.40	3.41
Cream						
Without glycolic acid	2.95	7.55	5.51	6.09	7.66	5.69
	3.61	6.44	3.23	3.55	4.74	5.58
	4.86	3.22	4.72	4.09	3.57	5.26
	3.18	3.09	5.64	3.18	5.46	4.09
With glycolic acid	4.77	4.40	4.43	2.49	7.13	3.39
	4.17	2.55	4.03	3.27	6.37	5.47
	3.71	2.55	6.91	4.54	5.27	5.13
	3.50	3.34	5.62	2.25	6.49	3.38

It should be pointed out that, due to the depilation, there probably was a change in the thickness of the corneal layer of the guinea pig skin. An attempt was made to reduce this error to a minimum by depilating the skin 24 hours before the experiment.

Formulation factor. Statistical analysis showed a highly significant difference at the 1% level between formulations in terms of vitamin A palmitate penetration into the skin of

Table IV
Tukey Test Applied to the Time Factor Data

Time (h)	Means	Critical Tukey value (5%)
1	5.20#	
2	6.47*	1,0524
3	6.39*	

Means followed by identical symbols are not significantly different.

Table V
Tukey Test Applied to the Formulation Data

Formulations	Means	Critical Tukey value (5%)
Gel	7.06*	
Gel cream	6.46*	0.9257
Cream	4.54#	

Means followed by identical symbols are not significantly different.

the guinea pigs studied. Analysis of variance, however, although stating that this difference exists, does not specify which means differ from one another, so that a complementary Tukey test is needed to define identical and different means. The Tukey test for the formulation factor is presented in Table V.

Glycolic acid × time interaction. The interaction between these two factors was also statistically significant at the 5% level, with glycolic acid having a certain effect after two hours of contact with the skin. The Tukey test for this interaction is presented in Table VI.

Time × glycolic acid × formulation interaction. The interaction between these three factors was also significant at the 5% level. Graphs were constructed with the results obtained to better determine the variation of formulations containing or not containing glycolic acid as a function of time. The Tukey test for this interaction is shown in Table VII.

Figures 2 through 4 graphically present the data concerning the percentage of vitamin A palmitate penetration into the guinea pig skin as a function of these interactions. The interactions were analyzed separately since the interpretation of a triple interaction was inadequate.

DISCUSSION

Studies of skin penetration have been gaining importance in the scientific community. The combination of glycolic acid at low concentrations with vitamin A palmitate has been extensively discussed. On the basis of clinical observations, dermatologists have concluded that this combination provides excellent results in the treatment of skin aging.

Table VI
Tukey Test Applied for Comparision of the Mean Data Obtained for the Glycolic Acid × Time Interaction

Interaction	Means	Critical Tukey value (5%)
Without	5.13*	
Without	5.67*	
Without	6.90*	1.8292
With	5.28*	
With	7.27#	
With	5.87*	

Means followed by identical symbols are not significant different.

Table VII
Tukey Test Applied to the Time × Glycolic Acid × Formulation Interaction

Interaction	Means	Critical Tukey value (5%)
Gel × without × 1 h	6.91	
Gel × without × 2 h	5.21	
Gel × without × 4 h	7.77	
Gel × with × 1 h	6.59	
Gel × with × 2 h	9.78	
Gel × with × 4 h	6.13	
Gel cream × without × 1 h	4.12	
Gel cream × without × 2 h	7.31	
Gel cream × without × 4 h	7.68	3.4214
Gel cream × with × 1 h	5.62	
Gel cream × with × 2 h	7.85	
Gel cream × with × 4 h	6.20	
Cream × without × 1 h	4.36	
Cream × without × 2 h	4.50	
Cream × without × 4 h	5.26	
Cream × with × 1 h	3.62	
Cream × with × 2 h	4.19	
Cream × with × 4 h	5.33	

The cornified layer of the human skin is the most important barrier against penetration by several substances. Disarrangement of this barrier using chemical substances is the most rapid method to reach the dermis (15). It has also been observed that glycolic acid promotes an increase in epidermal thickness as well as an increased epidermal hydration (16). These factors indicate that vitamin A palmitate, when combined with glycolic acid, may tend to reach deeper layers of the epidermis or even to remain longer in it since some of the factors that may increase skin absorption may also increase the amount of substance retained in the skin (15).

In view of the many empirical conclusions reached with the use of vitamin A palmitate in combination with glycolic acid, in the present study we evaluated this combination on the basis of a parameter judged to be of importance, i.e., skin penetration. The study was based on the use of three different formulations containing vitamin A palmitate combined or not combined with glycolic acid.

The experimental data obtained were analyzed statistically to determine differences in percentages of skin penetration (17). The statistical study conducted to determine the effect of glycolic acid and vehicle on the skin penetration of vitamin A palmitate showed that the presence of glycolic acid in the formulation alters the penetration of vitamin A palmitate into the guinea pig skin. The penetrating behavior of vitamin A palmitate in formulations with and without glycolic acid along time differed at the 5% level of probability. The penetration of vitamin A palmitate in formulations containing glycolic acid was accelerated between one and two hours but rapidly fell between two and four hours. In contrast, the formulation without glycolic acid maintained a more or less uniform rate of penetration throughout the experimental period (one to four hours). In summary, the amount of vitamin A palmitate that penetrated the skin in the absence of the acid took four hours to do so, whereas with the addition of glycolic acid it took only two hours.

The interaction among the three sources of variation (times × glycolic acid × formulations) was also significant at the 5% level. These interactions were analyzed separately in view of the inadequate interpretation of a triple interaction. Thus, the behavior of formulations along time was studied individually as a function of the presence or absence of glycolic acid. The study of skin penetration by vitamin A palmitate along time in the different formulations and in the absence of glycolic acid showed that the gel cream and the cream virtually presented the same behavior after one hour, whereas the gel favored a much higher vitamin A palmitate penetration. After two hours, however, the penetration of vitamin A palmitate in the gel formulation fell abruptly, whereas it increased abruptly in the gel cream formulation, with the two formulations thus presenting opposite behaviors. In the cream formulation, penetration of vitamin A palmitate was practically constant.

In contrast, skin penetration by vitamin A palmitate along time in the different formulations and in the presence of glycolic acid was higher in the gel formulation within one hour, and this difference was more marked compared to the cream formulation. After two hours, all formulations presented some increase in penetration compared to the previous time, but the gel and gel cream formulations showed identical behavior, i.e., they favored a marked increase in vitamin A palmitate penetration, whereas the cream formulation showed a very small increase in vitamin A palmitate penetration during this time interval. After four hours, vitamin A palmitate penetration was similar for all formulations, although the curves showed a fall in penetration for the gel and gel cream formulations along time, and a continuous increase in vitamin A palmitate penetration for the cream formulation was observed.

The study of the effect of glycolic acid on skin penetration by vitamin A palmitate along time in the gel formulation showed that the glycolic acid factor did not alter vitamin A palmitate penetration during the first hours. After two hours, the gel formulation containing glycolic acid favored a much higher vitamin A palmitate penetration compared to the gel formulation without glycolic acid. Between two and four hours, the formulation with glycolic acid caused a fall in vitamin A palmitate penetration probably due to the metabolism of vitamin A palmitate into the skin (18), whereas the formulation without glycolic acid favored increased penetration. Thus, the gel formulation containing glycolic acid promoted a faster vitamin A palmitate penetration than the formulation without glycolic acid (Figure 2).

The gel cream formulation containing glycolic acid promoted a slight increase in vitamin A palmitate penetration during the first hour. After two hours, the formula with glycolic acid and the formula with no glycolic acid behaved in a similar manner, both of them favoring increased vitamin A palmitate penetration. In contrast, after four hours the formula with glycolic acid caused a fall in vitamin A palmitate penetration, whereas the gel cream formula without glycolic acid caused increased penetration (Figure 3).

Thus, the behavior of the gel cream was generally similar to that of the gel formula since in the gel cream glycolic acid also promoted a more rapid penetration of vitamin A palmitate along time. However, the gel cream formula without glycolic acid did not show a fall in vitamin A palmitate penetration after two hours, as was the case for the gel formula.

The study of skin penetration by vitamin A palmitate along time in the cream formu-

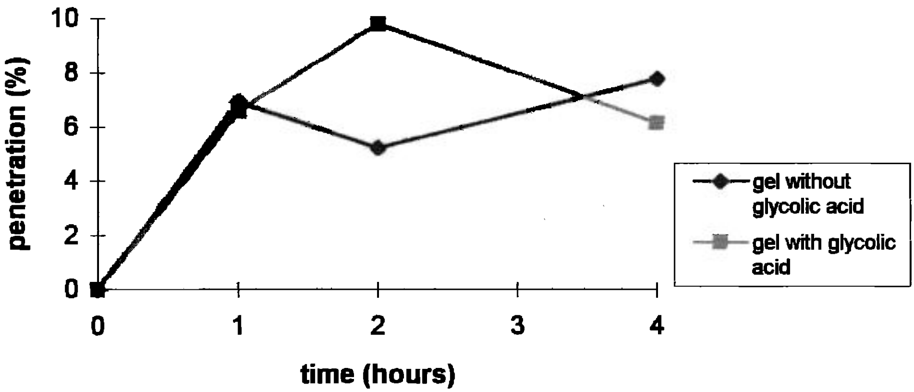

Figure 2. Skin penetration by vitamin A palmitate along time in a gel formulation.

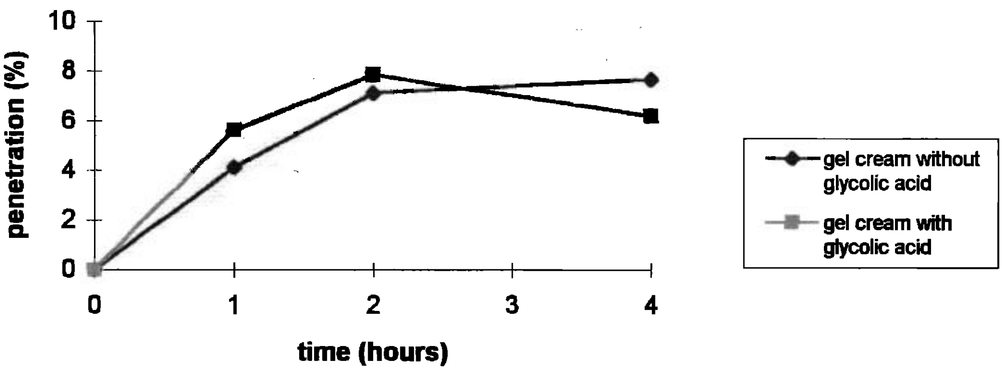

Figure 3. Skin penetration by vitamin A palmitate along time in a gel cream formulation.

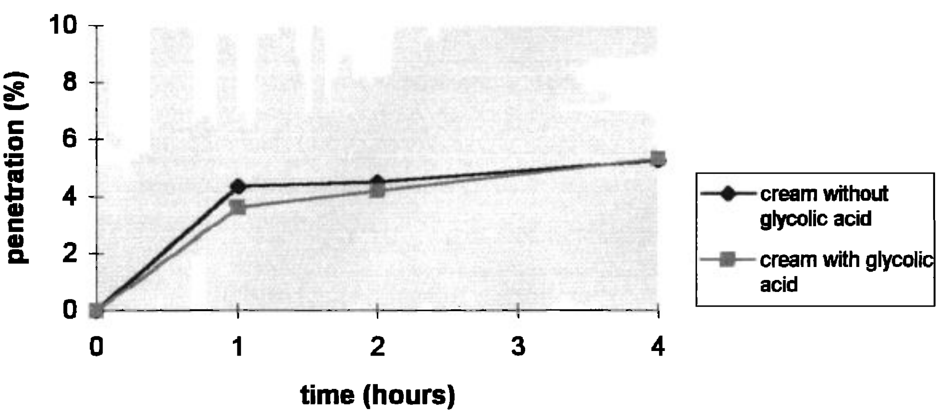

Figure 4. Skin penetration by vitamin A palmitate along time in a cream formulation.

lation showed that the behavior of the latter did not differ significantly in the presence or absence of glycolic acid (Figure 4).

CONCLUSION

Skin penetration tests showed that, under the present experimental conditions, the gel formula behaved differently in terms of vitamin A palmitate penetration when the vitamin was combined with glycolic acid, whereas the behavior of the cream and gel cream formulas were not affected, also showing the well known fact that vehicle interferes with the skin penetration of active principles.

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