Reduction of Skin Irritation by the Control of Skin Permeation of Methyl Paraben

***Seong-Hoon Jeong, Mun-Eok Park, Seh-Hoon Kang, Cheon-Koo Lee**
Cosmetics Research Center, LG Chemical Ltd. #84, Jang-dong, Yusong-gu, Taejon, 305-343, Korea

Abstract

The skin permeation study has two meanings in cosmetics. One is how to promote the skin permeation of active materials (e.g. skin whitening agents, anti-ageing agents) for improving their bioavailabilities and the other is how to decrease it of irritants (e.g. preservatives, perfumes, sunscreen agents) for reducing their skin side effects. In this study, we selected methyl paraben (MP), one of the preservatives, as a model irritant and tried to reduce the skin irritation by the decrease of skin permeation. Furthermore, the relationship between skin permeation and skin primary irritation was discussed.

For in vitro skin permeation experiments, Franz type diffusion cells (effective diffusional area: 1.766 cm^2) and the excised skin of female hairless mouse from 8 weeks old were used. The donor compartment was charged with oil only or O/W emulsion containing 0.3% MP. We selected 19 oils, including esters, triglycerides, plant oils, hydrocarbons, and alcohols, which are broadly used in cosmetics. We determined the oil/water partition coefficient of MP at 32+C. The skin primary irritation was evaluated with female guinea pig (8-10 weeks, 350-400 g).

The skin permeability of MP from the oils showed following order:

ester oils > triglycerides > plant oils > hydrocarbons > alcohols

We considered that this result was based on the different effect of each oil on the barrier function of stratum corneum. In O/W emulsion containing each oil, the skin permeability of MP decreased as the oil/water partition coefficient of MP increased. The skin primary irritation increased as the skin permeability of MP increased.

In conclusion, we suggest that the skin irritation could be reduced by the decrease of skin permeability of MP, which may be obtained by the good selection of oils in cosmetic preparations.

Introduction

As many of the cosmetic ingredients support the growth of bacteria, yeasts and fungi and microbial contamination can be happened during manufacture or use, most cosmetics require preservatives. The microbial contamination may cause discoloration, malordors and physical and chemical degradation of products, in addition to the potential adverse effects of pathogens on consumers.

All the preservatives used can be harmful to the consumer by their potency to induce skin irritation[1-4] and they are the second most common allergens in cosmetics, overtaken only by fragrance[5]. A lot of manufactures are eagerly looking for less irritating substances, but nevertheless the incidence of irritation by preservatives is increasing.

Esters of para-hydroxybenzoic acid (parabens) are still the most widely used preservatives in cosmetic formulations[1]. They form a group of different esters of benzoic acid, important ones are methyl, ethyl, propyl and butyl esters. As each of them is effective against a different range of microorganisms, usually a combination of esters is used. The most water-soluble one, the methyl paraben, is used in aqueous solution of 0.2-0.3%, less soluble higher esters are added in concentrations of 0.01-0.025%[1].

By the way, irritants can cause skin side effects by penetrating the stratum corneum and damaging viable cells in the skin. Therefore, if we can decrease the skin permeation of MP from cosmetics, we'll be able to reduce the skin irritation caused by MP.

In the present study, we selected methyl paraben (MP), one of the most widely used parabens, as a model irritant and evaluated the skin permeation of MP from various oils and O/W emulsions containing each oil. The

oils were including esters, triglycerides, plant oils, hydrocarbons and alcohols, which are broadly used in cosmetics. Furtheremore, we evaluated the skin primary irritation with female guinea pig and discussed the relationship between the skin permeation and the skin primary irritation.

Experimental

Materials: The skin permeant for this study was *p*-hydroxybenzoic acid methyl ester (methylparaben, MP) and it was purchased from Sigma Company (St. Louis, MO, USA). High performance liquid chromatography (HPLC) grade methanol, phosphoric acid and water were used to prepare HPLC mobile phase. All other ingredients were reagent or cosmetic grade.

Determination of Oil/Water Partition Coefficient: The partition coefficient experiment was conducted by adding 10 ml of each oil to 30 ml Teflon FEP centrifuge tubes (Nalgene, Nalge Company, NY, USA) containing 10 ml water with MP. Initial concentration of MP was measured and there's a list of oils used in this study in Table II. The tubes were sealed, shaken at 100 rpm at 32+C for 72 hours and centrifuged at 2000 rpm for 5 min. Then 0.1 ml aliquots of the aqueous phase were pipetted out and analyzed for MP by the HPLC method which will be described later in Skin Permeation Studies. The partition coefficients were calculated from the ratio of the counts in the oil phase and the aqueous phase. The experiment was run in triplicate.

Preparation of various Oils and O/W Emulsion containing MP: We divided 19 oils in five groups, including esters, triglycerides, plant oils, hydrocarbons and alcohols. Esters are cetearyl isononanoate (Cetiol SN-1, Henkel), decyl oleate (Cetiol V, Henkel), neopentyl glycol dicaprate (Estemol N-01), octyldodecyl myristate (Eutanol GM, Henkel), isocetyl myristate (ICM-R), isopropyl palmitate (IPP), isostearyl isostearate (ISO, Gatte fosse), isononyl isononanoate (Salacos 99) and trioctanoin (TIO), triglyceride is caprylic/capric triglyceride (Lexol GT-865, Inolex); plant oils are olive oil (Cropure, Croda), olive oil (Cropure OL, Croda), jojoba oil (Jojoba oil, PNJ), macademia nut oil (Macademia oil, Unione Marine) and meadowfoam seed oil (Meadowfoam oil, Croda); hydrocarbons are mineral oil (Drakeol 7, Penreco), squalane (Squalane, Kishimoto) and cyclomethicone (Silicone 344, Dow Corning) and alcohol is octyl dodecanol (Eutanol G, Henkel). Various oils containing 1.0%MP were prepared and Table I represents the formula of oil in water (O/W) emulsion. Each mixture of oil phase and aqueous phase was dissolved separately using a propeller agitator at 75+C. The oil phase was added to the aqueous phase at 5000 rpm with homomixer (Robomix, Tokushu Kika Kogyo Co., Japan) for 4 min and then cooled to room temperature.

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 1.766 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[8]. Briefly, the excised skin of female hairless mouse was obtained from 8 weeks old, 27-33 g animals. The above excised skin was mounted on the diffusion cell, epidermal side up, 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 each MP containing preparation at 32+C and capped. Seven hundred microliter aliquots were withdrawn from the receptor compartment at predetermined times for 24 h and replaced with an equal volume of fresh PBS maintained at 32+C.

The sample solution was filtered through a membrane filter (pore size, 0.2 μm; MFS-3; Micro Filtration Systems, CA, USA) and injected into HPLC. The HPLC consisted of solvent delivery pump (Waters 600 pump, Waters Co., MA, USA), C18 column (Waters Symmetry, 3.9150 mm), UV detector (Waters, 486 UV Detector) and data processing system (Waters Millennium). The mobile phase was 30% MeOH in 0.05% phosphoric acid and the flow rate was 1.0 ml/min. Wavelength of 254nm was selected and the temperature of the column was kept at 40+C. The retention time was 2.16 min for *p*-hydroxybenzoic acid and 5.90 min for MP. The correction of concentration against each sample point was also undertaken.

Calculation of Permeation Parameters

For each diffusion cell, the cumulative permeation amount of MP 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 Skin Primary Irritation

Female guinea pigs (8-10 weeks, 350-400 g) were shaved along the dorsal 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 0.1 ml of MP 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 evaluations) using the Draize method of scoring.

Results and Discussion

Skin Permeation of MP from Various Oils: Figure 1 represents the skin permeation profiles of MP dissolved in each oil across the excised hairless mouse skin. The corresponding permeation parameters and oil/water partition coefficients are listed in Table II.

The oil/water partition coefficients showed large differences among the oils, especially hydrocarbon oils like Drakeol 7, Squalane and Silicones have low values whereas ester oils have high values. We could suppose when we make an emulsion using hydrocarbons or silicone oils with low partition coefficients, that MP mainly be not in oil phase but in aqueous phase or interface between both.

In the permeation experiments, steady state fluxes ranging from 0.83%/h to 2.22%/h and lag times ranging from 0.70 h to 2.87 h were observed for the MP. The great steady state fluxes were obtained from ester oils like isopropyl palmitate and Cetiol V, which are known as having similar polarity with skin lipids, whereas octyl dodecanol showed the lowest value. The difference of cumulative permeation amount after 24 h between these two kinds of oils is more than two times. The skin permeability of MP from the oils showed the following order

Ester oils > triglycerides > plant oils > hydrocarbons > alcohols

We considered that this result was due to the different effect of each oil on the barrier function of stratum corneum and/or on the partition property of MP to the skin lipids.

Skin Permeation of MP from O/W Emulsions: Before the skin permeation experiments of MP using O/W emulsion, we compared the skin permeability of MP between water and TIO as a typical oil containing 0.2% MP. As in Figure 2, the skin permeability of MP from water was much higher than that from TIO. It might be considered that this result was due to the large different partition property of MP from the vehicles to the lipids of stratum corneum and it could provide an important information to predict the skin permeation of MP in emulsion.

Table I shows the formulation of O/W emulsion containing MP prepared by the use of each oil. The skin permeation profiles and the corresponding permeation parameters are represented in Figure 3 and Table III.

Some oils were found to have similar tendency of skin permeation of MP between single oils and O/W emulsions. Particularly, Eutanol G, TIO and Estemol N-01 showed a low skin permeability of MP from single oils and O/W emulsions. We considered that MP could be easily dissolved in these oils because they have high O/W partition coefficients. So in the O/W emulsion, the concentration of MP is low in aqueous phase but high in oil phase. In consequence of that, we considered that the skin permeation of MP from the O/W emulsions showed a similar tendency as that from the single oils.

In this study, the skin permeability of MP showed that it increased as the oil/water partition coefficient of MP decreased. Hydrocarbon oils have low partition coefficients and it means that most of MP exists in aqueous phase or interface between the oil and water rather than in the oil phase. As a result, the concentration of MP became higher in aqueous phase and the skin permeability increased. This result was in good agreement with the result already mentioned in Figure 2.

Skin Primary Irritation: Figure 4 showed the skin primary irritation of 4 oils and 4 O/W emulsions, with or without MP. Cetiol V and Lexol GT-865 with a high permeability of MP, as mentioned above, showed the highest increase of skin irritation by the containing of MP when compared with other oils. Drakeol 7 and Eutanol G with a low permeability of MP showed little increase of the skin irritation by MP. Also, the O/W emulsion prepared with Drakeol 7 showed a large difference of skin irritation between with and without MP, due to the high permeability of MP.

These results suggest that the skin primary irritation of MP increases with an increase of skin permeability of MP.

Figure 5 showed the relationship between the skin permeation and the skin primsry irritation of MP, where the increase of irritation by MP are plotted against the skin permeation amount for 24 h. The relationship between the skin permeation and the skin irritation yielded a good linearity ($r = 0.9043$, $p = 0.01$).

Conclusion

In conclusion, it was suggested that the skin irritation reduces by the decrease of skin permeability of MP and the skin permeation studies of irritants could be an excellent tool to control the skin primary irritation. Furthermore we found that the decrease of skin irritation of MP could be obtained by the good selection of oils in cosmetic products.

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Table I. Formula of O/W Emulsion System Used in This Study

Raw materials	*Amount (w/w%)*
Oil phase	
Cetostearyl alcohol	1.50
Emollient (oil)	15.00
POE (20) sorbitan monostearate	1.00
Sorbitan stearate	0.50
Aqueous phase	
Glycerin	5.00
Methylparaben	0.30
Carboxyvinyl polymer	0.05
Triethanolamine	0.05
Water	to 100

Table 2. O/W partition coefficients of MP and skin permeation parameters of MP from various oils.

Oils	O/W Partition coefficient	Permeation over 24 h (%)	Lag time (h)	Steady state flux (%/h)
Cetiol SN-1	8.42	33.71	1.23	1.86
Cetiol V	8.37	37.74	1.63	2.22
Cropure	4.33	28.22	1.61	1.43
Cropure OL	4.30	27.20	1.57	1.38
Drakeol 7	0.02	19.50	2.87	1.06
Estemol N-01	19.99	18.53	0.98	0.90
Eutanol G	16.61	16.11	2.57	0.83
Eutanol GM	5.24	32.13	1.91	1.70
ICM-R	6.53	30.90	1.21	1.51
IPP	13.46	33.40	1.23	1.87
ISO	6.70	31.49	1.32	1.57
Jojoba Oil	4.57	29.64	1.84	1.70
Lexol GT-865	21.29	27.61	2.14	1.56
Macademia Oil	7.27	19.73	0.86	0.88
Meadowfoam	4.30	25.23	1.44	1.27
Squalane	0.02	16.20	0.70	0.87
Salacos 99	11.36	31.18	0.82	1.66
Silicone 344	0.10	19.32	1.28	1.14
TIO	16.63	16.86	2.02	0.88

Table III. Skin permeation parameters of MP from o/w emulsions.

Oils	Permeation over 24 h (%)	O/W Partition coefficient	Lag time (h)	Steady state flux (%/h)
Cetiol SN-1	42.23	8.42	1.80	2.37
Cetiol V	49.93	8.3	2.027	3.25
Cropure	43.26	4.33	0.62	2.22
Cropure OL	44.63	4.30	1.41	2.06
Drakeol 7	78.20	0.02	1.10	6.36
Estemol N-01	30.00	19.99	1.78	1.73
Eutanol G	28.73	16.61	2.61	1.36
Eutanol GM	44.61	5.24	2.23	2.53
ICM-R	44.10	6.53	0.77	2.65
IPP	45.48	13.46	1.64	2.19
ISO	45.43	6.70	2.19	2.87
Jojoba Oil	50.00	4.57	2.03	3.13
Lexol GT-865	39.84	21.29	1.76	1.85
Macademia Oil	39.60	7.27	2.56	2.22
Meadowfoam	43.24	4.30	2.14	2.43
Squalane	72.71	0.02	1.81	6.27
Salacos 99	37.86	11.36	1.35	2.23
Silicone 344	70.62	0.10	0.78	6.39
TIO	31.78	16.63	2.96	1.61

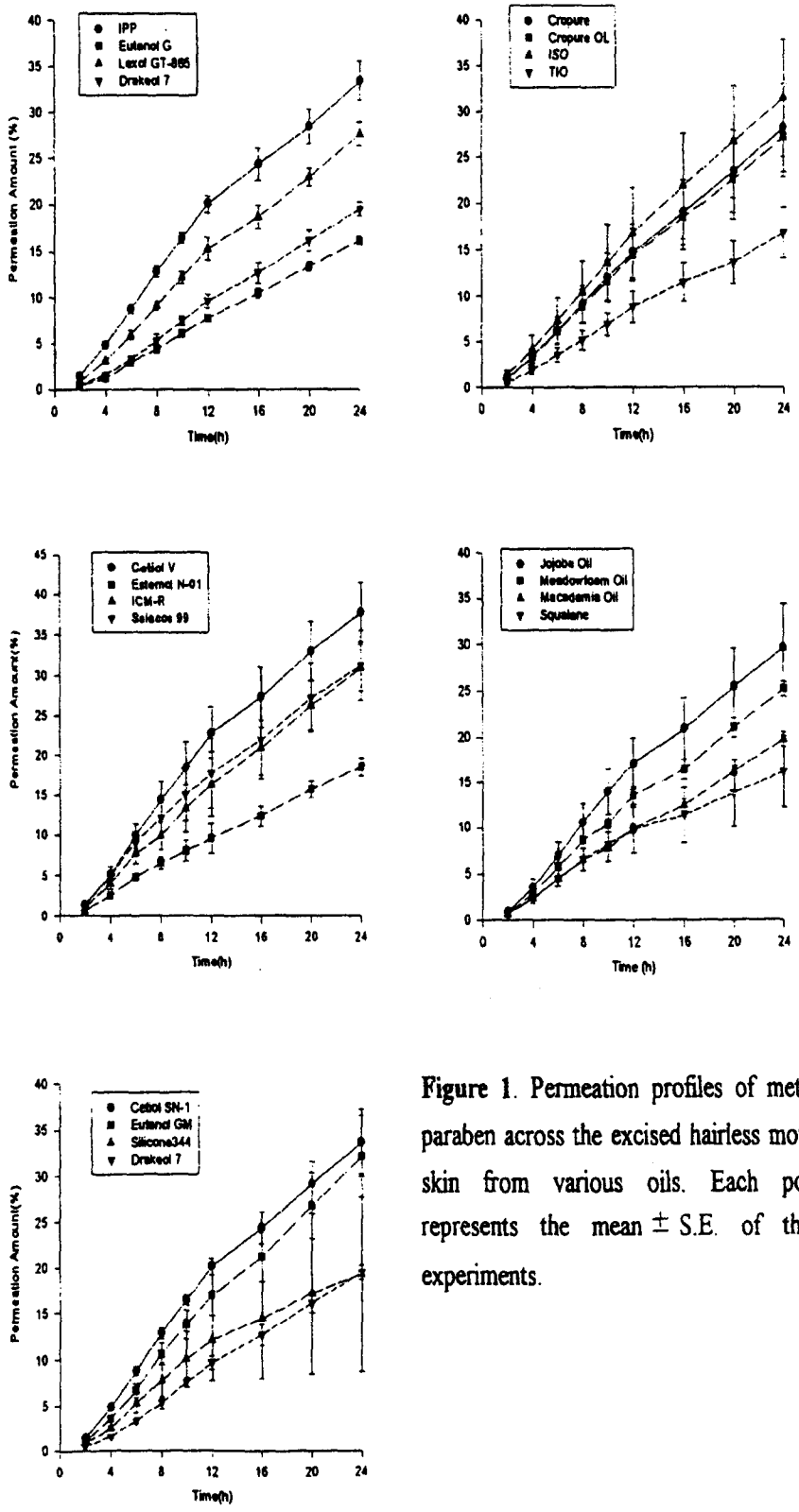

Figure 1. Permeation profiles of methyl paraben across the excised hairless mouse skin from various oils. Each point represents the mean ± S.E. of three experiments.

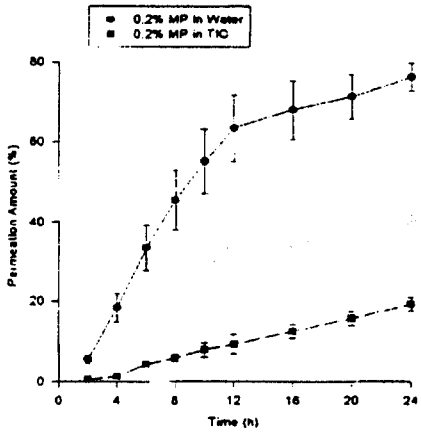

Figure 2. Permeation profiles of methyl paraben across the excised hairless mouse skin from oil and water vehicles. Each point represents the mean ± S.E. of three experiments.

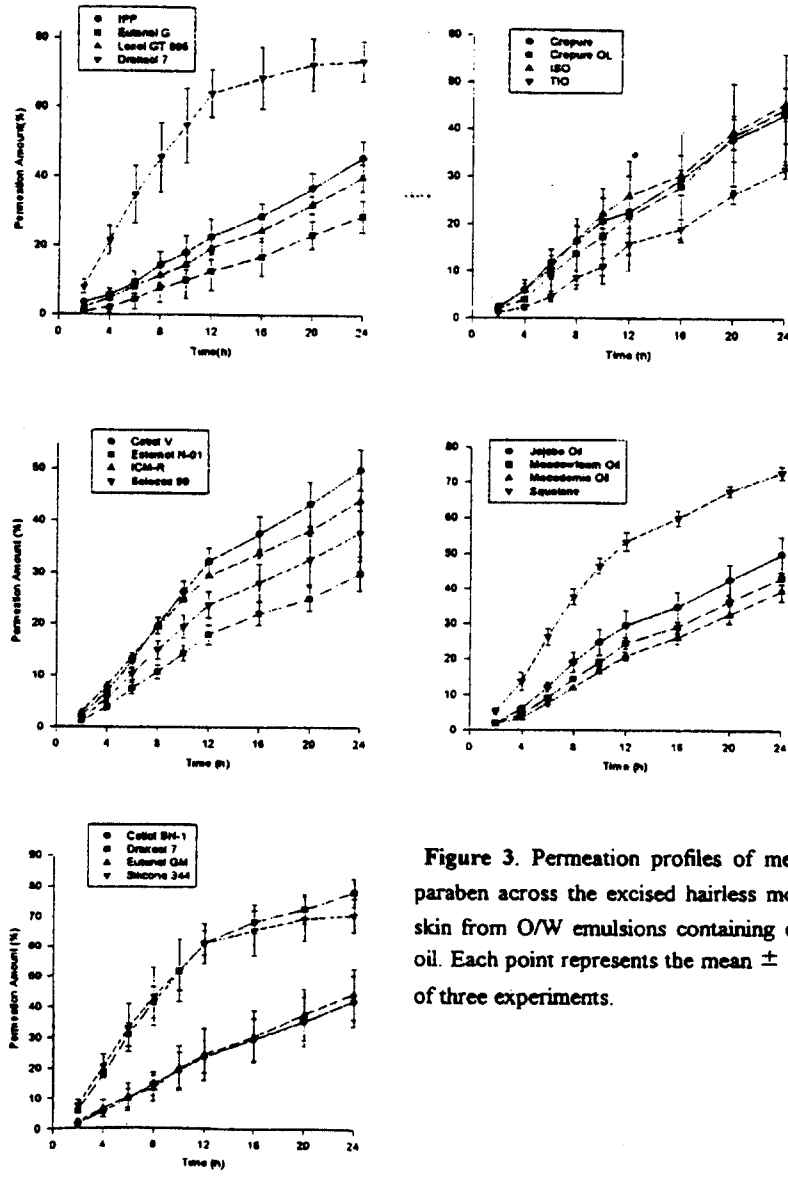

Figure 3. Permeation profiles of methyl paraben across the excised hairless mouse skin from O/W emulsions containing each oil. Each point represents the mean ± S.E. of three experiments.

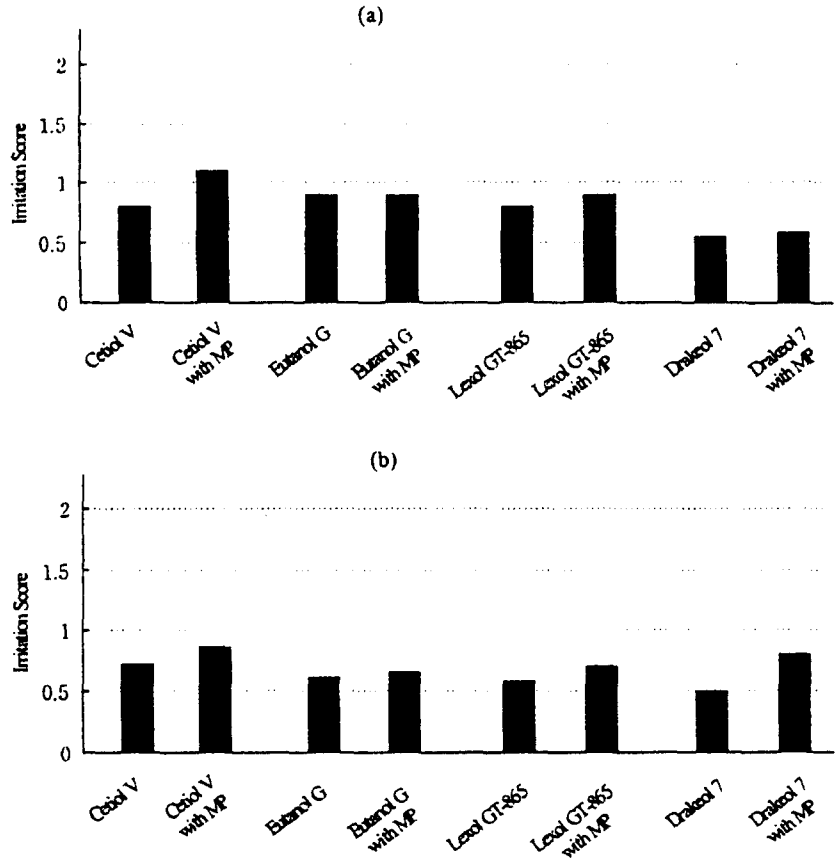

Figure 4. Skin primary irritation of various oils(a) and O/W emulsion(b) with or without MP.

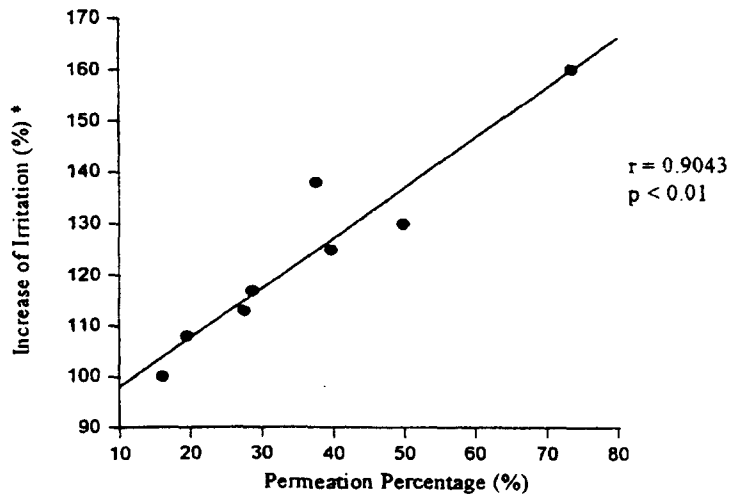

Figure 5. Relationship between Skin Permeation and Skin Primary Irritation of M.P.

* Oil with M.P. / Oil without M.P. × 100