

FORMULATION AND IN-VIVO EVALUATION OF A COSMETIC MULTIPLE EMULSION CONTAINING VITAMIN C AND WHEAT PROTEIN

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ABSTRACT

The purpose of the study was to see the effect of two antiaging agents in one stable multiple emulsion prepared using natural oil. Vitamin C, which is a very unstable ingredient and is decomposed in the presence of oxygen, active as an antioxidant, was entrapped in the inner aqueous phase of w/o/w multiple emulsion. In this way, slow release can be expected and the effect of vitamin C can be increased since it is protected from the external environment by entrapping it in the internal phase. The other ingredient which is a product of wheat proteins was also used as an antiaging agent in the oily phase. Both of the ingredients increase the synthesis of collagen fibers in the dermis. Therefore, a synergistic effect can be produced by using the two ingredients in one formulation.

In this study, multiple emulsions were prepared by the two-step method. Basic formulation containing no active material and a stable formulation containing vitamin C in the internal aqueous phase and wheat protein in the oily phase were prepared. The oil used was macadamia nut oil since it contains a high quantity of palmitoleic acid which is the natural ingredient of the young skin.

Basic formulation as well as the active formulation after confirming their stabilities, were applied to the cheeks of 11 human volunteers for four weeks. Different parameters of the skin were monitored every week to see any effect produced by these emulsions. The data obtained was evaluated statistically.

It was found that the active formulation as well as base increased the moisture of the skin as verified by statistical tests. However, there were no significant variations in other parameters like skin sebum, pH, elasticity, melanin and erythema, concerning the two formulations.

Keywords: Multiple emulsion, vitamin C, wheat proteins, dermatological evaluation.

INTRODUCTION

Multiple emulsions are defined as emulsions in which both types of emulsions exist simultaneously (Becher, 1965). They combine the properties of both w/o and o/w emulsions. In addition, they have the potential advantage of incorporating high capacity of drugs (Raynal *et al.*, 1993), prolonged release of active drug (Adeyeye and Price, 1991), incorporating incompatible materials (Semenzato *et al.*, 1994) and protecting active substances from environmental conditions (Dhams and Tagawa, 1996). The preparation of multiple emulsions with natural oils is a challenging work due to the stability problems. In this study, macadamia nut oil which is a natural oil has been used. This oil has been preferred because of its cosmetic benefits for the skin and non-toxic, non-allergenic and non-staining properties. It shows excellent absorbency by protective barrier and ideal slipperiness for massaging (Ken K. 1991). It is the only vegetable source of palmitoleic acid which is present up to 21% (Ako *et al.*, 1995). In addition, it also contains proteins, fats, carbohydrates, minerals and vitamins (Ricks, 1991).

The role of vitamin C as an antioxidant (Halliwell and Gutteridge, 1989) and in the protection of skin against

UVB light has been recognized by many workers (Padayatty and Levine, 2001, Eberlein-Konig *et al.*, 1998). Vitamin C also improves the synthesis of collagens resulting in the increase in suppleness of the skin (Hata and Senoo, 1989). Vitamin C plays a key role in the synthesis of *stratum corneum* barrier lipids (Ponec *et al.*, 1997). This vitamin has been incorporated into the internal phase of the multiple emulsions prepared, in this study.

Proteins from wheat are considered to be useful in cosmetic products (Challoner *et al.*, 1997). One such protein condensed with palmitic acid is also claimed to be antiaging by increasing human dermal fibroblasts in cultures (Handjani-vila *et al.* 1976). This protein (Lipacide PVB) is claimed to increase the synthesis of collagen at low concentrations and to act as moisturizer at high concentrations (Handjani-vila *et al.* 1976). This material was used in our study in the oil phase of multiple emulsion.

After the preparation of the multiple emulsions, accelerated stability studies were performed for six months. Following the assurance of their stabilities, one active formulation and one basic formulation containing

no active ingredient were applied to the skin of human volunteers for four weeks to see whether any synergistic effect can be produced against aging of skin and whether any improvements in the skin can be obtained. Dermatological tests including tests for irritation, moisturization, sebum and melanin contents, pH and elasticity of skin were performed every week to see any effect of active ingredients. These two formulations were compared with regard to their effects on the skin. Statistical tests were performed to evaluate the data obtained.

MATERIALS AND METHODS

Chemicals

Abil EM 90 (polysiloxane polyalkyl polyether copolymer) which is w/o emulsifying agent was supplied by Goldschmidt (Germany), L (+) ascorbic acid was purchased from E. Merck (Germany), Lipacide PVB was supplied by Givaudan-Lavirotte (France), macadamia nut oil from Alban Muller International (France), magnesium sulphate from E. Merck (Germany), Synperonic PE/F127 (ethylene oxide/propylene oxide block copolymers) from Uniqema (Belgium), and triethanolamine from Carlo Erba (Italy).

Preparation of multiple emulsion

In this study, multiple emulsions were prepared by the two-step process (Raynal *et al.*, 1993 and Yazan *et al.*, 1993).

Preparation of the basic formulation

Primary emulsion (PE) containing 26 % macadamia nut oil, 4 % lipophilic surfactant (Abil EM 90), 69% water and 0.7 % magnesium sulphate was prepared. 80% of the primary emulsion was added to the aqueous phase containing 0.8% hydrophilic surfactant (Synperonic PE/F 127) to obtain the basic multiple emulsion formulation.

Preparation of the active formulation

Oily phase consisting of 26 % macadamia nut oil, 6% Abil EM 90 and 0.5% Lipacide PVB was mixed with the aqueous phase consisting of water, 0.7 % magnesium sulphate and 1 % Vitamin C to form the primary emulsion. 75% of the primary emulsion was added to the aqueous phase containing 2% Synperonic and triethanolamine to obtain the multiple emulsion.

Skin testing

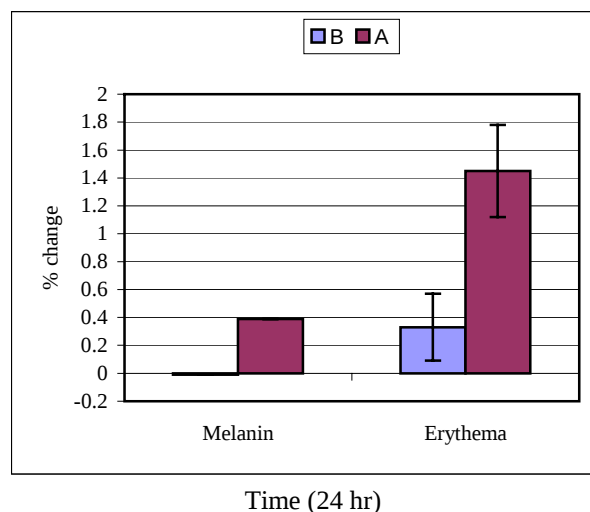
11 human volunteers were selected whose ages were between 40 and 65 years. Both male and female volunteers were included in this work. Prior to the tests, the volunteers were examined by a cosmetic expert for any serious skin disease or damage especially on cheeks and forearms. Before the study, every volunteer was provided with a volunteer protocol. This protocol stating the terms and conditions of the test was signed by each

volunteer individually. Volunteers were not informed about the contents of formulations. All the skin tests were done at 25°C and 40% relative humidity conditions. On the first day, patch test was performed on the forearms of each volunteer to determine any possible reactions to the multiple emulsions. In addition, basic values of skin moisture, sebum, pH and elasticity were noted for the right and left cheeks of each individual. On the second day, each volunteer was provided with two creams. One cream was the basic formulation and the other was the formulation containing the active ingredients. Each cream was marked with "right" or "left" indicating application of that cream to the respective cheek. The creams were applied by all the volunteers themselves as instructed for 30 days. Each individual was instructed to come on days 8, 15, 22 and 29 for the skin measurements and the skin testing was done and values were noted for different parameters of skin. Sensory evaluation test, score between 0 and 5 was filled in by each volunteer at the end of period.

RESULTS AND DISCUSSIONS

Patch Test

Before the application of the formulations to human volunteers, patch test was performed to test any reaction to the formulations. *Erythema and melanin* values were measured by Mexameter (Courage & Khazaka). Percentages of change after 24 hrs in *melanin/erythema* are shown in fig. 1 which shows that there was a decrease in melanin level and erythema level after application of both active formulation as well as base but statistically these changes were insignificant.

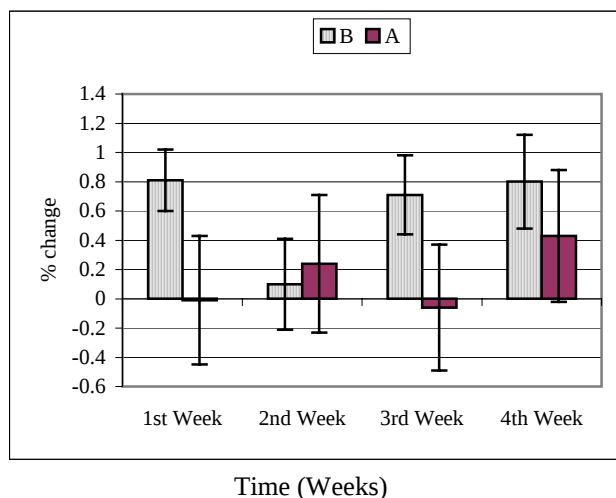


■ B = Basic Formulation ■ A = Active Formulation
Fig. 1: Percentages of change in *melanin/erythema* after 24 hours.

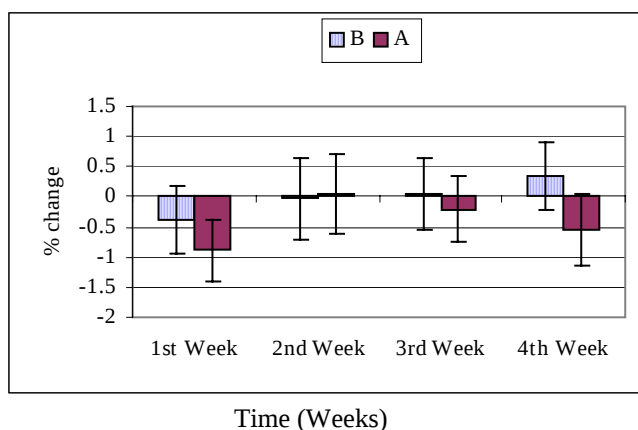
Melanin and Erythema

The percentages of change in the amounts of *melanin and erythema* following the applications of the formulations

for 28 days on the cheeks of human volunteers are demonstrated in figs. 2 and 3.



■ B = Basic Formulation ■ A = Active Formulation
Fig. 2: Percentages of change in *melanin* after application of preparations.



■ B = Basic Formulation ■ A = Active Formulation
Fig. 3: Percentages of change in *erythema* after application of preparations.

Melanocytes are present in the basal layer of the epidermis. These melanocytes manufacture a special pigment called *melanin* which is responsible for the color of the skin.

In this study, the effect of the active formulation on the production of *melanin* was examined. It was found that active formulation reduced melanin less than the base but there was no significant effect of the formulations on reduction of *melanin* as shown by ANOVA test and that there was no significant variation between the effects of two creams using paired sample t-test. This showed that none of the two ingredients had any significant effect on *melanin* during the application period of 28 days.

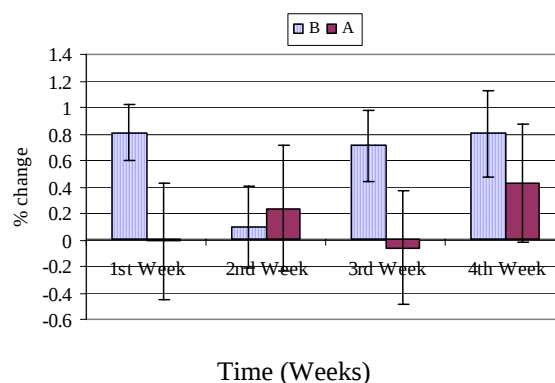
For confirming the safety of cosmetics, the important point is that cosmetics must not cause any reaction when

applied to the skin. The cause of reaction is not always due to cosmetic ingredients. Even if the safety of cosmetics is verified, it is known that environmental conditions such as temperature and humidity, misuse by the consumer, and the physical conditions may all cause reactions. Skin irritation is caused by the direct toxicity of chemicals on cells or blood vessels in the skin and is different from contact allergy which is caused by immune response.

In this study, patch tests were performed on the forearms of volunteers for 24 hours for both the active and the basic formulation. The two creams produced mild irritation but there was no significant change in the effects of two creams on irritation. In addition to this, irritation was constantly monitored every week for both the active and the basic formulations for 4 weeks, throughout the period of application. It was found that the two creams produced mild change in the level of irritation as shown by -ve values in figure 3 but with the help of ANOVA test, it was found that neither the active nor the basic formulation produced any significant irritation which is in accordance with reports already presented (Dreher *et al.*, 1999, Alster and West, 1998 and Farriol *et al.*, 1994). With the help of paired sample t-test it was evident that there was no significant variation in irritation with respect to the basic formulation in any volunteers for a period of 4 weeks. This showed that the formulations prepared are safe and can be used without any side effects.

Skin Moisture

The percentages of change in the skin moisture values before and after applications of formulations were measured by Corneometer (Courage & Khazaka) and are demonstrated in fig. 4.



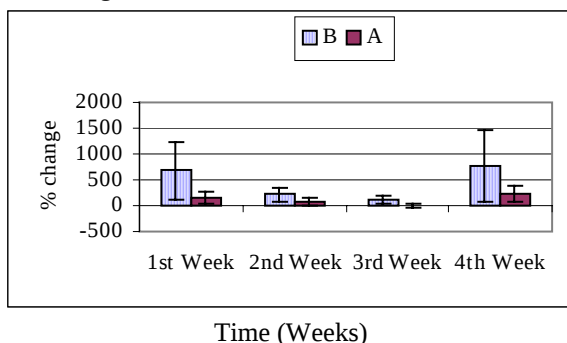
■ B = Basic Formulation ■ A = Active Formulation
Fig. 4: Percentages of change in values of skin moisture.

In the formulation studies, vitamin C has been used which is known to increase collagen fibers in the dermis (Hata and Senoo, 1989, Murad *et al.*, 1981, Geesin *et al.*, 1988, Pinnel *et al.*, 1987 and Kumano *et al.*, 1998). With the increase in collagen, the hydration conditions in the dermis are improved (Padayatty and Levine, 2001). In

addition to this, vitamin C improves the barrier functions of the *Stratum corneum* thus improving the moisture in the skin (Ponec *et al.*, 1997). In our study, it was found that there was an increase in moisture values in the 4th week after the application of the active formulation as well as the base and ANOVA test showed significant effect on skin moisture level with respect to the basic values ($p < 0.05$). There was no significant difference in the moisture values when the the values of basic formulation were compared with the values of active formulation ($p > 0.05$). This is not in accordance with the reports already presented (Padayatty and Levine, 2001 and Ponec *et al.*, 1997). It can be concluded that this increase in skin moisture values of the active formulation is not due to vitamin C and Lipacide[®] since Lipacide[®] does not act as moisturizer at this concentration (Biopredic Report). Vitamin C was incorporated in the inner most layer of multiple emulsion and it might be possible that its effect on skin moisture level was reduced due to this reason or this effect could not be initiated within 28 days of application.

Skin Sebum

The percentages of change in the skin sebum values before and after applications of formulations have been measured by Sebumeter (Courage & Khazaka) and are shown in fig. 5.



■ Basic Form = Basic Formulation ■ Active Form = Active Formulation

Fig. 5: Percentages of change in values of skin sebum.

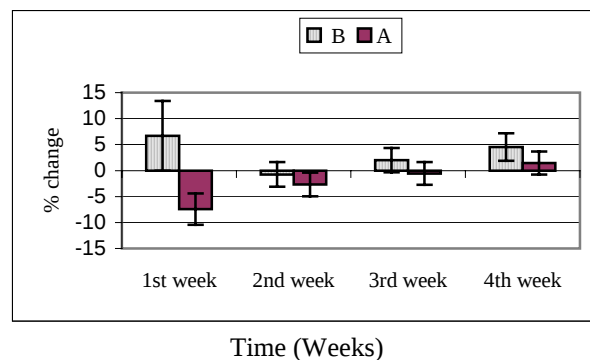
Sebum production is measured using a special opalescent plastic film, which becomes transparent when it is in contact with sebum lipids (Grays, 2000).

The effects of formulations on the sebum contents of human cheeks were investigated in this study. It was found that the active formulation produced no significant effect on the secretion of sebum with respect to the basic formulation in a period of 4 weeks. Sebum was measured every week in all the individuals and with the help of ANOVA test it was found that there was no significant effect of the active formulation on the skin sebum values ($p > 0.05$). It was also found that there was no significant variation between the active and basic formulation regarding the skin sebum content. This shows that neither

vitamin C nor wheat proteins had any effect on the production of sebum using the multiple emulsion prepared within an application period of 28 days.

pH of Skin

The percentages of change in the skin pH values before and after applications of formulations were measured by a Skin pH meter (Courage & Khazaka) and are shown in fig. 6.



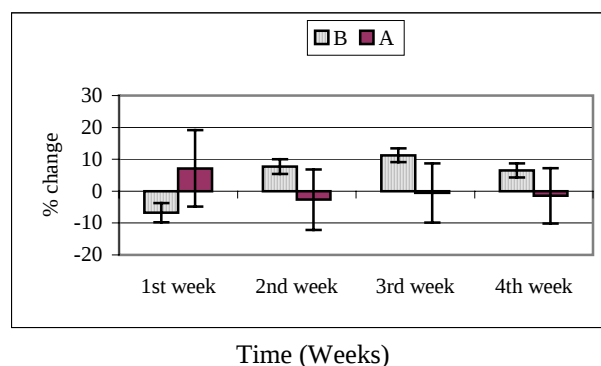
■ B = Basic Formulation ■ A = Active Formulation
Fig. 6: Percentages of change in skin pH values.

Sebum which is produced by sebaceous glands and is a mixture of waxes and esters is slightly acidic. It has a pH of 4.2-5.6 (Gray, 2000).

Skin pH values of volunteers were measured before and after applications of formulation. It was also found that active formulation as well as the basic formulation did not produce any alterations in the pH values of human skin ($p > 0.05$). Therefore, both of the emulsions may be considered to be safe with respect to the effect on skin pH values.

Skin Elasticity

The changes in the skin elasticity values before and after applications of formulations were measured by a Cutometer (Courage & Khazaka) and percentages of change are demonstrated in fig. 7.



■ B = Basic Formulation ■ A = Active Formulation

Fig. 7: Percentages of change in values of net elasticity of skin.

Proteins named collagens are present in the dermis. Most of the collagens are present in bundles running horizontally through the dermis, which are buried in a jelly like material called ground substance. Collagens account for up to 75% of the weight of the dermis, and are responsible for the resilience and elasticity of the skin. The collagen bundles are held together by elastic fibers. These are made of a protein called elastin. Despite their name, they are not involved in the natural elasticity of the skin.

Vitamin C is known to increase the collagen fibers of human skin (Hata and Senoo, 1989, Geesin *et al*, 1988, Pinnel *et al.*, 1987 and Kumano *et al*, 1998). In addition to vitamin C, Lipacide® has also been claimed to increase quantity and quality of collagen fibers (Handjani-vila *et al* 1976). In the active formulation, both of the ingredients were incorporated but there was no significant effect of the active formulation on the net elasticity of human skin in an application period of 4 weeks, since no significant variation was determined by the ANOVA test and there was no significant difference between the two creams as determined by paired sample t-test ($p > 0.05$). 4 week application period may be insufficient for the skin elasticity to be improved. Application period may be increased to further investigate any improvement.

Panel Test

Sensory evaluation of the two creams by the volunteers are presented in table 1.

A questionnaire containing seven questions was prepared and the two copies of this form were given to each volunteer for sensory evaluation of the two creams. Average values were calculated from the values assigned by each volunteer for each question for both of the creams.

Average values for the first question, *ie* ease of application of creams were found to be 4.46 and 4.36 for the active and the basic formulations, respectively. This indicates that both of formulations can be easily applied

on the skin. Average values regarding spreadability were 4.36 for the active formulation and 4.27 for the basic formulation which means that the active formulation spreads on skin better than the basic formulation. Average values for feel on application were 3.91 for the active formulation and 3.64 for the basic formulation. This indicates that the active formulation is felt better on the skin than basic formulation. Average values for the sense in long-term application of creams were 4.18 and 4.27 for the active and the basic formulation, respectively. This shows that the basic formulation produces more pleasant feeling on application to skin than the active formulation. There was no irritation on the skin as both of the creams were assigned 0 values for irritation by all the volunteers. This is in accordance with the patch test results. Shine on skin was 3.73 for the active formulation and 3.82 for the basic formulation. This is expected since the basic formulation contains higher quantity of oil than the active formulation. Similarly, the basic formulation leads to more softness of the skin than the active formulation. The average values for the basic formulation was 4.82 while it was 4.56 for the active formulation. Actually the two preparations seem to be similar upon sensorial evaluation.

CONCLUSION

Conclusively, there was no variation between the active and the basic formulation regarding the different parameters of skin. Both of the creams behaved similarly from the dermatological point of view except for skin hydration as both the creams increased the hydration in the skin.

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Table 1: Average values $\pm$ SE for panel test

	Average values for Basic Formulation	Average values for Active Formulation
Ease of Application	4.36 $\pm$ 0.20	4.46 $\pm$ 0.21
Spreadability	4.27 $\pm$ 0.20	4.36 $\pm$ 0.20
Sense just after application	3.64 $\pm$ 0.20	3.91 $\pm$ 0.25
Sense in long term	4.27 $\pm$ 0.20	4.18 $\pm$ 0.23
Irritation	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Shine on skin	3.82 $\pm$ 0.23	3.73 $\pm$ 0.31
Sense of softness	4.82 $\pm$ 0.12	4.56 $\pm$ 0.28

SE : Standard Error

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