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# FORTIFICATION OF FACIAL SKIN COLLAGEN EFFICACY BY COMBINED ASCORBYL PALMITATE AND SODIUM ASCORBYL PHOSPHATE

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**Abstract:** Skin care products specifically formulated for the purpose of hydrating skin by increasing collagen efficacy required to contain hydrophobic or hydrophilic antioxidants with the aim of maintaining the hydration status of the horny layer. Ascorbyl palmitate (AP) and sodium ascorbyl phosphate (SAP) are reported as lipophilic and hydrophilic antioxidants, respectively, used for skin care. Present study was aimed to assess the combined AP (in oil phase) and SAP (in aqueous phase) of a multiple emulsion (ME) for fortifying the facial skin collagen efficacy in healthy human females. Active formulation ME was applied to left and a control-placebo on the right side of face of female volunteers over 12-weeks treatment study. Instrumental measurements, Corneometer® CM825, Elastometer® E25 and Visioscan® VC 98 with a sole UVA-light video camera were carried out for skin hydration, skin elasticity and SELS parameters (roughness (SEr), scaliness (SEsc), smoothness (SEsm) and wrinkling (SEw), respectively, at baseline visit and on each subsequent visit at specific time intervals. Combined AP and SAP of ME enhanced *stratum corneum* moisture content (25%), skin elasticity (10%) and SELS parameters i.e. SEr (-5%), SEsc (-12%), SEsm (+9%) and SEw (-9%) after a period of 12-weeks when equated with baseline values. In conclusion, combined treatment with lipophilic and hydrophilic combination of ascorbyl palmitate and sodium ascorbyl phosphate in a single formulation (ME) presented superior results in improving skin moisture, elasticity and SELS parameters, thus provided a way to enhance the facial skin collagen efficacy.

**Keywords**: fortification, collagen efficacy, ascorbyl palmitate, sodium ascorbyl phosphate, combined effects

The skin is fortified with a setup of lipophilic and hydrophilic, enzymatic and non-enzymatic antioxidant systems (1). The skin texture represents the skin smooth surface. Many factors affect skin texture e.g., diet, hydration, amount of collagen and hormones. A gradual decline in skin upon chronic sun exposure ultimately leads to skin aging (2, 3). The skin care products have been designed to improve the functions on the dermis to stimulate the collagen synthesis and firmness of skin. Antioxidants (natural or synthetic) protect human skin against the action of free radicals from UV radiations (4).

Vitamin C deficiency in human has been known for centuries as scurvy. Its activity against free radicals, role in collagen biosynthesis and its photoprotective properties have made it potential candidate for the treatment and prevention of skin aging (5). Vitamin C is potent antioxidant and is known to stimulate collagen formation (6) but has the drawback of instability in formulations. That's why deprivatives of ascorbic acid have been synthesized having an action similar to ascorbic acid but are more stable. Sodium ascorbyl phosphate and ascorbyl esters are the stable vitamin C derivatives (7). Ascorbyl palmitate (AP) and sodium ascorbyl phosphate (SAP) are reported as lipophilic and hydrophilic antioxidants, respectively, used for skin care. Sodium ascorbyl phosphate acts as an *in vivo* antioxidant. It is a stable vitamin C derivative with hydrophilic nature that is converted into simple vitamin C after penetration into skin, thus promotes the development of skin (8). Ascorbyl palmitate is a synthetic vitamin C derivative with improved chem-

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ical stability and lipophilicity, which increased its absorption ability through the skin (9, 10).

Multiple emulsions play an important role when our focus is to prepare skin care products with long-term moisture holding capacity, skin firmness improvement and prolonged action (11). W/O/W multiple emulsions present many interesting possibilities for the controlled delivery of drug, initially entrapped in the internal aqueous compartment and promote the delivery of hydrophilic as well as hydrophobic drugs (12).

Current investigation aimed to assess the combined ascorbyl palmitate and sodium ascorbyl phosphate *via* a multiple emulsion (ME) for fortifying the facial skin collagen efficacy in healthy human females through non-invasive bioengineering techniques.

## MATERIALS AND METHODS

### Test products

Test products (control and ME) were multiple emulsions of W/O/W type. ME was loaded with ascorbyl palmitate and sodium ascorbyl phosphate as functional ingredients while control was without active compounds. Compositions of control and ME are shown in Table 1. Two-step emulsification procedure was implemented for the preparation of W/O/W multiple emulsions (control and ME) (13). Primary W/O emulsion was prepared by heating cetyl dimethicone copolyol plus liquid paraffin (AP also for ME), and water plus magnesium sulfate to 75°C in a digital water bath (Heidolph, Germany) and then mixing by IKA Mixing Overhead Stirrer, Eurostar (IKA, Werke, Germany) at 2000 rpm. In secondary emulsification step, W/O emulsion was dispersed in mixture of water plus polysorbate-80 (SAP also for ME) at 700 rpm for 40 min to attain W/O/W multiple emulsion. Formulated emulsions were confirmed by photomicrographs (Figs. 1a, 1b).

Each volunteer was provided with two vessels with 50 g emulsion. The vessels with emulsions were marked as "right and left" which were specified for right and left cheeks, respectively. The right cheek was specified for control while the left cheek was specified for active formulation (ME). Participants were instructed to use about 1 g of control and active formulation (ME) on respective half of the cheeks at bed time and in the morning over a 12 weeks trial period.

### Measurement site

A measurement area was set on the cheek (the sample area on the cheek; 2 x 2 cm) in such a way that cross of the parallel line of the bottom of the

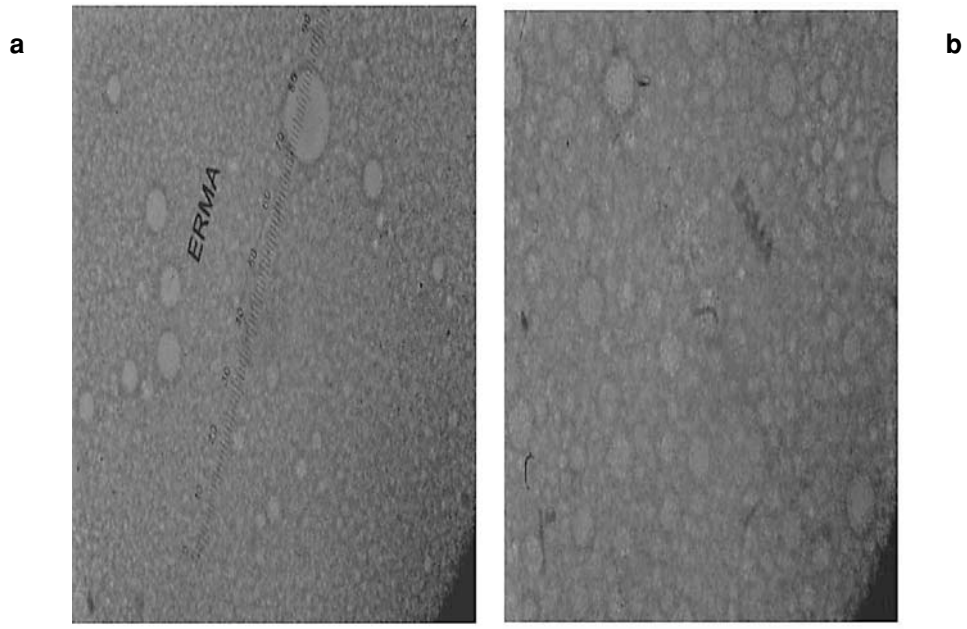


Figure 1. Photomicrograph of (a) control and (b) ME (active multiple emulsion) immediately after preparation

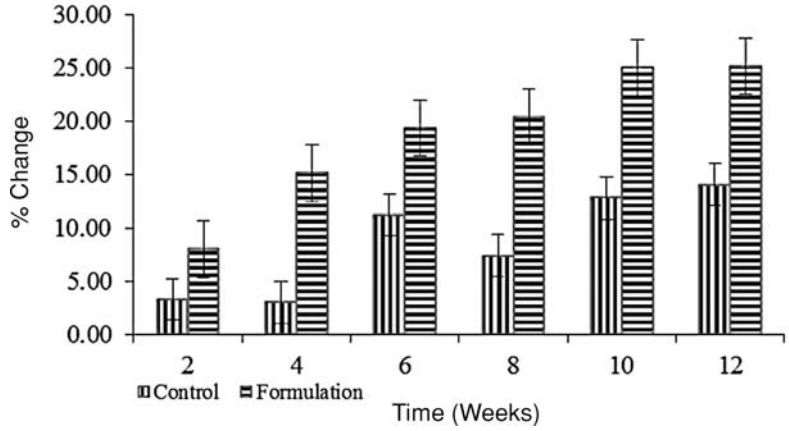


Figure 2. Percentage change in skin moisture after the application of active formulation ME and control

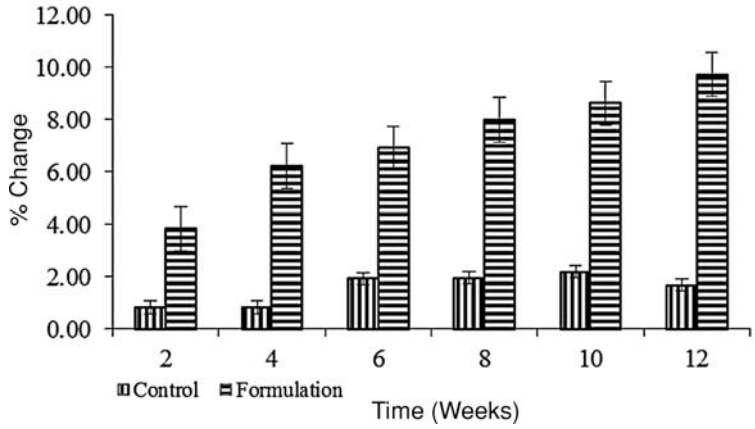


Figure 3. Percentage change in skin elasticity contents for active formulation ME and control

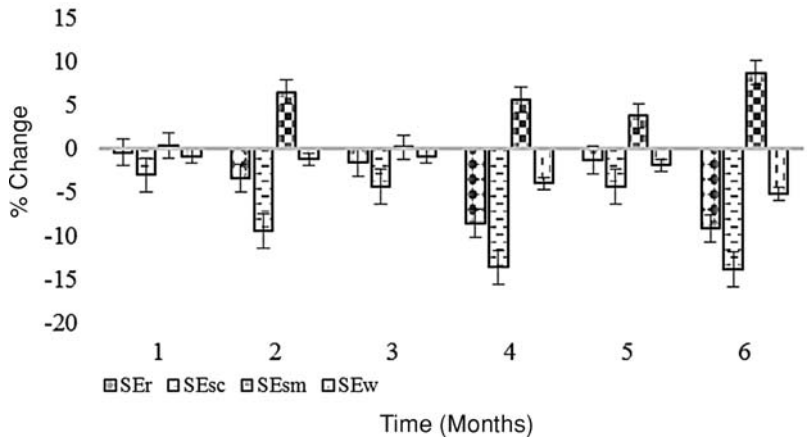


Figure 4. Percentage change in SELS parameters; roughness (SEr), scaliness (SEsc), smoothness (SEsm) and wrinkling (SEw) after application of active formulation ME and control. 1, 3 and 5 represents 3 month results for control while 2, 4 and 6 represents 3 month results for ME

nose and a vertical line to the inner corner of the eye was taken a reference point.

**Subjects**

A sample of 11 Asian female volunteers (students) with an age between 22-25 years (mean age: 23.5 years), non smokers, no history of dermatological diseases or allergy to substances in test product were recruited in this study. Principle of Declaration of Helsinki was charted in this study. The inclusion criteria was: no history of hypersensitivity, every individual should sign the volunteer protocol and have to come to the laboratory for measurements on specific time intervals. The criteria for exclusion were: presence of any dermatitis, smokers and previous treatment with sunscreens, moisturizers or anti-ageing cosmetics. Participants were also instructed not to use any skin care products on the test sites 2 weeks before study and throughout the study period of 12 weeks. Additionally, exposure to solar radiations and use of occlusive clothes on the test area were prohibited. Participants were restricted with use of oral natural or synthetic vitamin C supplements which may affect the results. All the study participants were well informed about the use of study products and essential details of study. All volunteers completed the study successfully.

**Ethical considerations**

A written informed consent was taken from all the participants of the study. Participants were informed about possible adverse reactions, protocols and objectives of this study and they have the rights to quit study without informing about such reasons. The approval of this study was taken from the Advanced Studies and Research Board (No. 975/AS & RB), the Islamia University of Bahawalpur and registered by Institutional Ethical Committee and Board of Faculty, Faculty of Pharmacy and Alternative Medicine (No. 2336/Dean), the Islamia University of Bahawalpur, Bahawalpur- Pakistan.

**Study protocol**

The study was split face placebo controlled. Prior to the study, a cosmetic expert examined each volunteer for any type of skin reaction/sensitivity and no adverse effects were ensured. An expert investigator ensured the proper conductance of Corneometer® CM825, Elastometer® E25 and Visioscan measurements (Courage and Khazaka Electronic GmbH), considering the experimentation protocols for these skin micro-topographic techniques.

**Skin moisture content determination with Corneometer® CM825**

The measurement of the skin moisture is based on the capacitance method which is internationally recognized method of Corneometer® (14). The device represents the values in arbitrary units by measuring the water contents of the superficial epidermal layers down to the depth of about 0.1 mm. Moisture contents of human skin (*stratum corneum* water contents) were measured with Corneometer® before the application of control and active formulation (ME) and then on 2nd, 4th, 6th, 8th, 10th and 12th week of investigation period.

**Skin elasticity determination with Elastometer® E25**

Skin Elastometer® allows easy and quick measurement of the elastic properties of the skin (biolog-

Table 1. Composition of control and active formulation (ME).

| Control | (% wt.) | ME | (% wt.) |
|---|---|---|---|
| Primary emulsion (W/O) | | Primary emulsion (W/O) | |
| Cetyl dimethicone copolyol | 2.40 | Cetyl dimethicone copolyol | 2.40 |
| Liquid paraffin | 13.60 | Liquid paraffin | 13.60 |
| Magnesium sulfate | 0.56 | Magnesium sulfate | 0.56 |
| - | - | Ascorbyl palmitate | 0.5 |
| Deionized water | 63.44 | Deionized water | 62.69 |
| Multiple emulsion (W/O/W) | | Multiple emulsion (W/O/W) | |
| Polysorbate-80 | 0.8 | Polysorbate-80 | 0.8 |
| - | - | Sodium ascorbyl phosphate | 0.5 |
| Water (Q.S) | 100 | Water (Q.S) | 100 |

ME stands for active multiple emulsion formulation. W/O/W = Water-in-oil-in-water, Q.S = Quantity sufficient.

Table 2. Values of moisture after application of control.

| Volunteer | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 | Week 12 |
|---|---|---|---|---|---|---|---|
| 1 | 81 | 66 | 69 | 59.6 | 83 | 95 | 103 |
| 2 | 93 | 83 | 79 | 66 | 58 | 78 | 78 |
| 3 | 47.5 | 47.1 | 47.3 | 49 | 55 | 56.9 | 61 |
| 4 | 36 | 36.6 | 41 | 59.1 | 42 | 38 | 38 |
| 5 | 53.2 | 54.5 | 51 | 49.2 | 50.8 | 49.7 | 51.3 |
| 6 | 48 | 51.2 | 53 | 53 | 57 | 56 | 53.6 |
| 7 | 43 | 55 | 58.2 | 58 | 46 | 46 | 48 |
| 8 | 48 | 39.4 | 40 | 50.4 | 50 | 59.7 | 58.3 |
| 9 | 22 | 36 | 29 | 31 | 31 | 34 | 32 |
| 10 | 84 | 62 | 54.2 | 62 | 55 | 57 | 55 |
| 11 | 45.2 | 48.9 | 58 | 69.5 | 68.8 | 68 | 71 |
| Sum | 600.9 | 579.7 | 579.7 | 606.8 | 596.6 | 638.3 | 649.2 |
| Mean | 54.62727 | 52.7 | 52.7 | 55.16364 | 54.23636 | 58.02727 | 59.01818 |
| STDev | 21.92488 | 13.97512 | 13.8202 | 10.47724 | 13.60024 | 17.5128 | 19.59831 |
| SEM | 6.62383 | 4.222091 | 4.175288 | 3.165329 | 4.108834 | 5.290876 | 5.920939 |

Table 3. Values of moisture content after application of active formulation (ME).

| Volunteer | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 | Week 12 |
|---|---|---|---|---|---|---|---|
| 1 | 50 | 69 | 72.4 | 77 | 71 | 74 | 74 |
| 2 | 93 | 83 | 79 | 66 | 58 | 78 | 78 |
| 3 | 38.7 | 41 | 45 | 42 | 52.2 | 57.1 | 58 |
| 4 | 50.2 | 51.3 | 54 | 57 | 55.2 | 58.3 | 58.9 |
| 5 | 36.7 | 38.5 | 49 | 52.1 | 41.2 | 52 | 54 |
| 6 | 59 | 57.3 | 56 | 49 | 53.5 | 51.9 | 50.9 |
| 7 | 47.3 | 52.7 | 55.3 | 61.3 | 60.5 | 61.9 | 62 |
| 8 | 41.5 | 49 | 45 | 42.3 | 51.8 | 54.7 | 55 |
| 9 | 38 | 37 | 39 | 47 | 51 | 49 | 44 |
| 10 | 57.2 | 56 | 61 | 58 | 60 | 61.5 | 63 |
| 11 | 46.7 | 52 | 55.6 | 55 | 53 | 49 | 51 |
| Sum | 516.3 | 556.8 | 591.1 | 610.6 | 615.2 | 635.4 | 636.8 |
| Mean | 46.93636 | 50.61818 | 53.73636 | 55.50909 | 55.92727 | 57.76364 | 57.89091 |
| STDev | 7.547486 | 9.226681 | 9.110903 | 10.92954 | 8.057554 | 7.706137 | 8.258869 |
| SEM | 6.62383 | 4.222091 | 4.175288 | 3.165329 | 4.108834 | 5.290876 | 5.920939 |

ical skin age) measured by suction. The elasticity is expressed in % in the display. Elastometer was used to measure the cutaneous elasticity before application of control (C) and active formulation (ME) and then on 2$^{nd}$, 4$^{th}$, 6$^{th}$, 8$^{th}$, 10$^{th}$ and 12$^{th}$ week of investigation period.

**Surface evaluation of living skin (SELS)**

Visioscan® VC 98 with a special UV light video camera was used to measure four (SELS) parameters i.e., roughness (SEr), scaliness (SEsc), smoothness (SEsm) and wrinkling (SEw). SEr is the roughness parameter which calculates the proportion of dark pixels. SEsm is the index of smoothness and is calculated from the mean width and depth of wrinkles. SEsc is the index of scaliness of skin which shows the level of dryness of the skin. SEw identifies aging including wrinkles and is calculated from the proportion of horizontal and vertical wrinkles (15). In our study, evaluation was done by cal-

culating the percentage changes in different SELS parameters (SEr, SEsc, SEsm and SEw) for a study period of 3 months. Measurements were taken on right and left cheeks of female adults at baseline visit and on 1st, 2nd and 3rd month. Before any measurements, all volunteers had to rest in Cosmetic Lab, under constant environmental conditions of 22 ± 2°C and 50 ± 5% relative humidity, for at least 30 minutes Basic principle of SELS is based on evaluation of an image of living skin by obtaining the picture which is finally electronically processed for quantitative results. The measuring probe comprises of two counter rotating halogen lamps which uniformly illuminate specific measuring field on the skin. A CCD camera is fitted in the measuring head which records the picture of skin and camera is connected to the computer directly *via* EPP-port. Skin smoothness, skin roughness, scaliness and wrinkles are evaluated by the grey level distribution of the image as an index.

Table 4. Values of elasticity (%) after application of control.

| Volunteer | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 | Week 12 |
|---|---|---|---|---|---|---|---|
| 1 | 73 | 83 | 75 | 75 | 81 | 81 | 72 |
| 2 | 62 | 61 | 59 | 61 | 64 | 63 | 65 |
| 3 | 51 | 52 | 60 | 57 | 54 | 54 | 52 |
| 4 | 64 | 62 | 62 | 65 | 64 | 62 | 65 |
| 5 | 85 | 84 | 86 | 86 | 85 | 84 | 85 |
| 6 | 81 | 77 | 79 | 81 | 85 | 84 | 85 |
| 7 | 63 | 61 | 61 | 64 | 66 | 66 | 64 |
| 8 | 65 | 63 | 67 | 61 | 53 | 58 | 59 |
| 9 | 49 | 55 | 55 | 54 | 54 | 55 | 56 |
| 10 | 58 | 58 | 58 | 59 | 58 | 58 | 59 |
| 11 | 56 | 55 | 48 | 55 | 56 | 56 | 55 |
| Sum | 707 | 711 | 710 | 718 | 720 | 721 | 717 |
| Mean | 64.27273 | 64.63636 | 64.54545 | 65.27273 | 65.45455 | 65.54545 | 65.18182 |
| STDev | 11.44632 | 11.36022 | 11.23711 | 10.72465 | 12.54084 | 11.78443 | 11.27669 |
| SEM | 3.458102 | 3.43209 | 3.394898 | 3.240076 | 3.788774 | 3.56025 | 3.406854 |

Table 5. Values of elasticity (%) after application of active formulation (ME).

| Volunteer | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 | Week 12 |
|---|---|---|---|---|---|---|---|
| 1 | 72 | 68 | 65 | 68 | 70 | 88 | 95 |
| 2 | 64 | 67 | 75 | 75 | 88 | 74 | 75 |
| 3 | 45 | 47 | 42 | 48 | 48 | 52 | 51 |
| 4 | 63 | 65 | 85 | 92 | 92 | 96 | 95 |
| 5 | 64 | 65 | 72 | 72 | 60 | 60 | 60 |
| 6 | 83 | 90 | 90 | 92 | 97 | 95 | 95 |
| 7 | 72 | 68 | 58 | 72 | 82 | 88 | 88 |
| 8 | 63 | 65 | 71 | 74 | 65 | 52 | 52 |
| 9 | 63 | 66 | 61 | 60 | 61 | 62 | 63 |
| 10 | 58 | 67 | 59 | 59 | 60 | 61 | 62 |
| 11 | 80 | 86 | 96 | 59 | 58 | 58 | 59 |
| Sum | 727 | 754 | 774 | 771 | 781 | 786 | 795 |
| Mean | 66.09091 | 68.54545 | 70.36364 | 70.09091 | 71 | 71.45455 | 72.27273 |
| STDev | 10.47334 | 11.30808 | 15.76244 | 13.5901 | 16.13691 | 17.25899 | 17.84988 |
| SEM | 3.164152 | 3.416339 | 4.762067 | 4.105771 | 4.875201 | 5.214196 | 5.392713 |

Table 6. Skin roughness (SEr) after the application of control.

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 0.64 | 0.52 | 0.47 | 0.48 |
| 2 | 0.2 | 0.13 | 0.11 | 0.16 |
| 3 | 0.33 | 0.02 | 0.06 | 0.04 |
| 4 | 0.1 | 0.04 | 0.11 | 0.05 |
| 5 | 0.35 | 0.08 | 0.04 | 0.04 |
| 6 | 0.11 | 0.33 | 0.36 | 0.39 |
| 7 | 0.15 | 0.29 | 0.23 | 0.25 |
| 8 | 0.71 | 0.8 | 0.7 | 0.5 |
| 9 | 0.86 | 0.2 | 0.08 | 0.08 |
| 10 | 0.06 | 0.09 | 0.08 | 0.09 |
| 11 | 0.35 | 0.35 | 0.32 | 0.37 |
| Sum | 3.86 | 2.85 | 2.56 | 795 |
| Mean | 0.350909 | 0.259091 | 0.232727 | 0.222727 |
| STDev | 0.272193 | 0.236831 | 0.21029 | 0.182214 |
| SEM | 0.082234 | 0.07155 | 0.063532 | 0.055049 |

Table 7. Skin roughness (SEr) after the application of active formulation (ME).

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 0.21 | 0.13 | 0.09 | 0.04 |
| 2 | 0.32 | 0.32 | 0.35 | 0.35 |
| 3 | 0.17 | 0.14 | 0.13 | 0.18 |
| 4 | 0.1 | 0.17 | 0.18 | 0.17 |
| 5 | 0.46 | 0.31 | 0.37 | 0.35 |
| 6 | 0.8 | 0.8 | 0.87 | 0.89 |
| 7 | 0.45 | 0.45 | 0.45 | 0.46 |
| 8 | 0.27 | 0.26 | 0.24 | 0.25 |
| 9 | 0.56 | 0.54 | 0.54 | 0.47 |
| 10 | 0.02 | 0.02 | 0.01 | 0.01 |
| 11 | 0.32 | 0.28 | 0.23 | 0.25 |
| Sum | 3.68 | 3.42 | 3.46 | 3.42 |
| Mean | 0.334545 | 0.310909 | 0.314545 | 0.310909 |
| STDev | 0.223086 | 0.219338 | 0.242914 | 0.243781 |
| SEM | 0.067398 | 0.066265 | 0.073388 | 0.07365 |

### Statistical analysis

All measured data for skin moisture content, elasticity and SELS parameters were analyzed using SPSS (version 20) software. ANOVA (analysis of variance) and paired t-test was used for the comparison of effects at baseline and after subsequent intervals of treatment.

## RESULTS

### Skin moisture content

Active formulation (ME) studied enhanced *stratum corneum* moisture content after 12-week period of daily applications when compared with the baseline values (Tables 2, 3). The percentage of

changes in skin moisture contents after the application of control and ME have been shown in Figure 2. All values were measured in triplicates (n = 3). ME produced about 25% increase in skin moisture contents while its respective control produced 14% increase in skin moisture contents at the end of study period of 12 weeks. After applying the statistical analysis (two-way ANOVA test) it was found that ME as well as control showed significant increase in moisture content with respect to time. With the help of paired sample t-test analysis, significant differences in the skin moisture values were observed by ME and control at the end of study.

**Skin elasticity**

Average percentage changes in skin elasticity values after the application of control and ME have been shown in Fig. 3. Comparison with baseline val-

Table 8. Skin scaliness (SEsc) after the application of control.

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 0.09 | 0.04 | 0.04 | 0.01 |
| 2 | 0.04 | 0.05 | 0.08 | 0.08 |
| 3 | 0.12 | 0.09 | 0.07 | 0.16 |
| 4 | 0.24 | 0.09 | 0.05 | 0.03 |
| 5 | 0.19 | 0.17 | 0.11 | 0.14 |
| 6 | 0.18 | 0.17 | 0.19 | 0.21 |
| 7 | 0.24 | 0.24 | 0.23 | 0.23 |
| 8 | 0.08 | 0.08 | 0.01 | 0.02 |
| 9 | 0.03 | 0.03 | 0.02 | 0.03 |
| 10 | 0.02 | 0.04 | 0.06 | 0.03 |
| 11 | 0.06 | 0.06 | 0.05 | 0.08 |
| Sum | 1.29 | 1.06 | 0.91 | 1.02 |
| Mean | 0.117273 | 0.096364 | 0.082727 | 0.092727 |
| STDev | 0.082352 | 0.067863 | 0.069151 | 0.079761 |
| SEM | 0.02488 | 0.020503 | 0.020891 | 0.024097 |

Table 9. Skin scalliness (SEsc) after the application of active formulation (ME).

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 0.09 | 0.08 | 0.09 | 0 |
| 2 | 0.04 | 0.04 | 0.03 | 0.1 |
| 3 | 0.15 | 0.11 | 0.1 | 0.12 |
| 4 | 0.06 | 0.07 | 0.04 | 0.04 |
| 5 | 0.07 | 0.08 | 0.08 | 0.09 |
| 6 | 0.19 | 0.12 | 0.17 | 0.18 |
| 7 | 0.93 | 0.8 | 0.7 | 0.2 |
| 8 | 0.17 | 0.01 | 0.02 | 0.01 |
| 9 | 0.43 | 0.36 | 0.36 | 0.17 |
| 10 | 0.1 | 0.17 | 0.18 | 0.18 |
| 11 | 0.15 | 0.14 | 0.13 | 0.12 |
| Sum | 2.38 | 1.98 | 1.9 | 1.21 |
| Mean | 0.216364 | 0.18 | 0.172727 | 0.11 |
| STDev | 0.259433 | 0.2253 | 0.199253 | 0.070143 |
| SEM | 0.078379 | 0.068066 | 0.060197 | 0.021191 |

Table 10. Skin smoothness (SEsm) after the application of control.

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 0.09 | 0.08 | 0.09 | 0 |
| 2 | 0.04 | 0.04 | 0.03 | 0.1 |
| 3 | 0.15 | 0.11 | 0.1 | 0.12 |
| 4 | 0.06 | 0.07 | 0.04 | 0.04 |
| 5 | 0.07 | 0.08 | 0.08 | 0.09 |
| 6 | 0.19 | 0.12 | 0.17 | 0.18 |
| 7 | 0.93 | 0.8 | 0.7 | 0.2 |
| 8 | 0.17 | 0.01 | 0.02 | 0.01 |
| 9 | 0.43 | 0.36 | 0.36 | 0.17 |
| 10 | 0.1 | 0.17 | 0.18 | 0.18 |
| 11 | 0.15 | 0.14 | 0.13 | 0.12 |
| Sum | 2.38 | 1.98 | 1.9 | 1.21 |
| Mean | 18.49455 | 18.33182 | 18.18091 | 18.94091 |
| STDev | 2.528214 | 2.792847 | 2.224754 | 4.083338 |
| SEM | 0.763811 | 0.843761 | 0.672131 | 1.233637 |

Table 11. Skin smoothness (SEsm) after the application of active formulation (ME).

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 18.34 | 19.71 | 14.68 | 14.42 |
| 2 | 17.28 | 16.86 | 15.71 | 20.96 |
| 3 | 21.62 | 18.06 | 17.81 | 16.8 |
| 4 | 17.65 | 22.59 | 24.55 | 24.83 |
| 5 | 19.14 | 21.44 | 16.93 | 17.4 |
| 6 | 18.98 | 19.78 | 19.55 | 19.94 |
| 7 | 16.54 | 16.75 | 20.9 | 18.67 |
| 8 | 23.64 | 25.1 | 27.9 | 25.87 |
| 9 | 25.1 | 32.87 | 34.56 | 32.91 |
| 10 | 19.01 | 18.51 | 20.77 | 24.65 |
| 11 | 17.65 | 18.09 | 15.23 | 17.32 |
| Sum | 214.95 | 229.76 | 228.59 | 233.77 |
| Mean | 19.54091 | 20.88727 | 20.78091 | 21.25182 |
| STDev | 2.747659 | 4.711212 | 6.112969 | 5.355854 |
| SEM | 0.830108 | 1.423327 | 1.846818 | 1.618083 |

ues (Tables 4, 5) indicates that elasticity contents were found an increase to greater extent after the application of ME. ME caused about 10% increase in elasticity of skin while respective control produced slight effects (2% increase) on cutaneous elasticity. When two-way ANOVA test was applied, control showed insignificant changes while ME produced significant changes in elasticity values with respect to time after 12 weeks of study. With the help of paired sample t-test, statistically significant differences in elasticity results of ME and control were observed.

**Surface evaluation of living skin**

The percentage of changes (average) in skin roughness (SEr), scaliness (SEsc), smoothness (SEsm) and wrinkles (SEw) were measured for surface evaluation of living skin (SELS) after applica-

tion of control and ME for 12 weeks at specific time intervals i.e. at zero hour, 1st month, 2nd month and 3rd month of study period. Results have been shown in Figure 4. All values were measured in triplicates (n = 3). Obtained results indicated that smoothness values (Tables 10, 11) were increased while roughness, scaliness (Tables 6-9) and wrinkling values (Tables 12, 13) were decreased after three months as compared to baseline values. After calculating percentage changes in skin roughness at specific time intervals in female volunteers; it was observed that the control produced too little effects on skin roughness index (SEr) which was seen after completion of 2nd and 3rd month; whereas ME showed a pronounced decrease in the skin roughness values. A pronounced decrease in skin scaliness was also observed after topical application of ME. Although control formulation also decreased the skin scali-

Table 12. Skin wrinkling (SEw) after the application of control.

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 45.5 | 45 | 52.19 | 47.66 |
| 2 | 38.14 | 39.34 | 38.54 | 38.42 |
| 3 | 33.97 | 35.97 | 31.23 | 32.44 |
| 4 | 39.93 | 35.78 | 52.41 | 52.41 |
| 5 | 33.86 | 36.15 | 33.34 | 34.76 |
| 6 | 40.75 | 47.21 | 37.09 | 37.69 |
| 7 | 45 | 42.12 | 42.01 | 42.13 |
| 8 | 52.32 | 47.21 | 52.28 | 48.7 |
| 9 | 43.27 | 42.61 | 37.82 | 38.76 |
| 10 | 41.47 | 42.31 | 39.43 | 39.76 |
| 11 | 40.23 | 34.29 | 34.47 | 31.58 |
| Sum | 454.44 | 447.99 | 450.81 | 444.31 |
| Mean | 41.31273 | 40.72636 | 40.98273 | 40.39182 |
| STDev | 5.282715 | 4.708701 | 7.838022 | 6.753941 |
| SEM | 1.595987 | 1.422568 | 2.367982 | 2.040465 |

Table 13. Skin wrinkling (SEw) after the application of active formulation (ME).

| Volunteer | Zero | 30 Days | 60 Days | 90 Days |
|---|---|---|---|---|
| 1 | 33.97 | 37.55 | 40.64 | 42.65 |
| 2 | 40.29 | 38.14 | 37.78 | 39.87 |
| 3 | 36.33 | 37.94 | 38.5 | 36.78 |
| 4 | 39.93 | 32.14 | 33.41 | 34.43 |
| 5 | 54.54 | 39.41 | 33.07 | 49.34 |
| 6 | 36.15 | 47.21 | 36.65 | 37.21 |
| 7 | 47.21 | 46.69 | 45.95 | 44.54 |
| 8 | 40.91 | 40.89 | 39.79 | 32.41 |
| 9 | 50.6 | 50.6 | 53.78 | 35.67 |
| 10 | 39.93 | 41.23 | 38.17 | 38.87 |
| 11 | 37.82 | 34.29 | 35.36 | 35.98 |
| Sum | 457.68 | 446.09 | 433.1 | 427.75 |
| Mean | 41.60727 | 40.55364 | 39.37273 | 38.88636 |
| STDev | 6.462707 | 5.625576 | 5.967821 | 4.941824 |
| SEM | 1.952479 | 1.69957 | 1.802967 | 1.492998 |

ness, but this effect was very low. After applying two-way analysis of variance test on skin roughness, scaling, smoothness and wrinkling values, it was observed that control produced statistically insignificant ($p > 0.05$) effects while ME showed significant effects at various time intervals of three-month study period. Paired sample t-test revealed that ME showed significant differences during the whole evaluation period of three months when compared with control.

## DISCUSSION

### Skin moisture content

The improvement of skin hydration promotes collagen formation (16). A dramatic increase in skin moisture contents after application of ME containing ascorbic acid derivatives (ascorbyl palmitate and sodium ascorbyl phosphate) was due to the reason that ascorbyl palmitate has skin moisturizing effects (10). In the molecule of ascorbyl palmitate with favorable effects as an excellent skin antioxidant, the fatty acid ester moiety is sited in 6-position and the inorganic ester group is sited in 2-position promotes its penetration into skin and promise various advantages for skin applications including moisturizing potential which was found to be higher as compared to the other hydrophilic compounds (17). Moreover, sodium ascorbyl phosphate acts as an *in vivo* antioxidant and improves hydration of skin by promoting collagen formation. It is a stable vitamin C derivate with hydrophilic nature that protects the skin, promotes its development and improves its appearance (18). Both compounds synergistically improved the skin moisture contents.

### Skin elasticity

Collagen is present in fibroblast of human dermis and essential for healthy firm skin (19). External factors such as exposure to sunlight, especially UV radiations decrease the quality and quantity of collagen leading to aging skin. In the past era, topical applications of soluble animal collagen was used to stimulate the formation of collagen in the skin. However, these tests were unsuccessful because collagen cannot penetrate the epidermis (20, 21). We observed that after topical application of ME (containing ascorbic acid derivatives in 1% concentration), improvement of cutaneous elasticity and hydration properties was due to high penetration ability and synergistic antioxidant effects of ascorbyl palmitate and sodium ascorbyl phosphate. Ascorbyl palmitate has greater chemical stability, lipophilicity and skin absorption ability (9). Sodium ascorbyl phosphate is hydrolyzed by the skin cells, which cause the *in situ* liberation of the ascorbic acid (22). Ascorbic acid and its derivatives such as ascorbyl palmitate work as potent antioxidants to protect the skin from free radical damage and also promote collagen production (23). Sodium ascorbyl phosphate also has photo-protective effects and has ability to increase collagen production in human fibroblasts (24). As both compounds synergistically increased the collagen production thus both can be used safely in combination in new anti-aging products.

### Surface evaluation of living skin

Structural changes in the skin surface around the prominent pores, are induced not only by changes in epidermal cells, but also by strong anisotropy of the dermal fiber structure due to collagen (25). Structural changes are evaluated by (SELS) parameters i.e., roughness (SEr), scaliness (SEsc), smoothness (SEsm) and wrinkling (SEw).

SEr values describe the skin's roughness. Higher the SEr content, less will be the smoothness of skin and vice versa. SEsc (surface evaluation of skin's scaliness) predicts skin scaliness index. Scaliness index is inversely related to *stratum corneum* hydration level. The lower is the value of SEsc, the higher will be skin hydration level and vice versa. SEsm represents the surface evaluation of skin smoothness. SEw designate the width and number of wrinkles and relates to skin fitness. An increase in SEw index will be the more skin wrinkles (15).

Thousands of natural and synthetic compounds have been assessed for their efficiency against free radicals. Scientists are trying to develop novel synthetic antioxidants directed at retarding the effects of free-radical-induced damage (22, 26). We have explored the combined effects of ascorbyl palmitate and sodium ascorbyl phosphate on SELS parameters of skin (smoothness, roughness, scaliness and wrinkling) and found some interesting results. In combination, these compounds showed synergistic/combined antioxidant effects as one compound is hydrophobic (ascorbyl palmitate) and the other (sodium ascorbyl phosphate) is hydrophilic which might enhance their penetration into the skin. All four parameters of SELS were improved after the application of ME in comparison to control. ME showed decrease in mean values of skin roughness (SEr), scaling (SEsc), and wrinkling (SEw) which indicated that the active formulation has anti-aging properties. Moreover, pronounced effects were on skin smoothness. A decline in the values of the skin

wrinkles (SEw) indicated the reduction in the fine wrinkles and improvement in the texture of living skin. Improvement of SELS parameters after topical application of ME was due to antioxidant properties of ascorbyl palmitate and sodium ascorbyl phosphate. With its antioxidant properties, ascorbyl palmitate helped to maintain normal connective tissue, promoted the synthesis of collagen and maintained the healthy skin and blood vessels (27).

## CONCLUSION

From the study, it was observed that combined use of ascorbyl palmitate and sodium ascorbyl phosphate *via* a multiple emulsion (ME) enhanced the skin facial collagen by improving skin moisture, skin elasticity and SELS parameters when compared with baseline values. Thus, combined application of two different lipophilic and hydrophilic nature of antioxidants, ascorbyl palmitate and sodium ascorbyl phosphate loaded in multiple emulsion (ME) have potential influence on facial skin parameters, thus provide a way to fortify collagen efficacy of facial skin.

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### Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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