INTRODUCTION
L-Ascorbic acid (Vitamin C) has been used in pharmaceutical and cosmetic preparations for a long time on the basis of its many favourable effects on the skin [1]. It is an important antioxidant that protects the skin by scavenging and destroying free radicals and reactive oxygen-derived species [2]. It could improve the morphogenesis of dermal epidermal junction, and is also known for its skin lightening properties [3, 4]. L-Ascorbic acid is also used topically because of its ability to reduce wrinkles by promoting collagen synthesis and its skin-depigmenting activity [5]. However, its low stability is a serious limitation. It is easily oxidized, especially under aerobic conditions and light exposure, being degraded first in a reversible step to dehydroascorbic acid and second to oxalic acid in an irreversible fashion [6]. Chemical modification of ascorbic acid has led to more stable derivatives such as ascorbyl esters with C6 to C18 fatty acids or ascorbyl phosphate salts. Among the lipophilic derivatives, ascorbyl palmitate is often used in topical preparations against oxidative changes of biological components of the skin, and as an anti-oxidant to protect lipophilic ingredients in formulations [7]. Ascorbyl palmitate has been studied in the food, pharmaceutical, and cosmetic industry due to its stability and antioxidant activity [8]. In this research, it was selected as the active material. Ascorbyl palmitate has been proposed as an oxygen scavenger to remove headspace oxygen [9]. This derivative of vitamin C is beneficial for its skin penetration; emulsions of ascorbyl palmitate have been prepared with a view to apply them for topical use [10]. Given the low stability of ascorbic acid dissolved in aqueous vehicles, these new forms may be considered of great potential value. Well the technology of production of these alternative forms of vitamin C can raise the cost of the final product.

Accordingly, in this study the present study was undertaken to investigate the physical, chemical and microbiological stability of O/W emulsions containing ascorbic acid and ascorbyl palmitate in order to make a comparative assessment of these active principles.

MATERIALS AND METHODS
Formulations
The components and concentrations of the formulations used in the study are shown in Table 1. Glycerol monostearate, ceteth-25, isopropyl palmitate, cetearyl alcohol, ethyl paraben, polyethylene glycol, cetyl lactate, laureth 7 and disodium EDTA were added or not of 2% l-ascorbic acid and ascorbyl palmitate at pH 5.5, the active principle optimal pH. The O/W formulations were prepared and mixed in a Heidolph RZR 2021 shaker at 600 rpm.

Physical stability
The obtained emulsion was submitted to a set of organoleptic (colour, thickness, look, feel) and physical (creaming and phase separation) analyses.
Stability Tests
Stability tests were performed at different conditions for emulsions to investigate the effect of these conditions on the storage of emulsions. These tests were performed on samples kept at 8°C ±2°C (in refrigerator), 25°C ±2°C (in incubator) and 40°C ±2°C (in incubator). The physical, i.e. colour, and the organoleptic, i.e. liquefaction and phase separation, characteristics of emulsions were observed at various intervals for 28 days [11, 12].

Centrifugation Tests
Centrifugal tests were performed for emulsions immediately after preparation. Those tests were repeated for emulsions after 24 hours, 7 days, 14 days, 21 days, and 28 days of preparation. They were performed at 5000 rpm and 25°C for 10 minutes by placing 10g of the sample in centrifugal tubes [11, 12].

Skin Measurement (Measurement of Transepidermal Water Loss)
TEWL is determined using skin biophysical technique, Tewameter® CM 210 from Courage Khazaka (Cologne, Germany). TEWL, which is related to skin barrier function, was expressed as g/m². h [13].

pH determination
The pH value of freshly prepared emulsions and emulsions kept at different conditions were determined using a digital pH-meter. The pH tests were repeated for emulsions after 24 hours, 3 days, 7 days, 14 days, 21 days, and 28 days of preparation [14].

Chemical stability
All samples were stored in well-closed 25 ml glass flasks. During storage samples were kept at room temperature (22±1 °C) in the dark, except for those used for studying the influence of light. The amount of non-degraded active ingredient in samples was determined quantitatively at the beginning of storage and subsequently on the 1st, 2nd, 3rd, 7th, 14th and 28th day.

Chromatographic conditions
The HPLC apparatus consisted of JASCO HPLC, a sample injector with a 20 μl sample loop and a wavelength detector. For ascorbyl palmitate, the stationary phase was 125 mm x 4 mm column packed with 5 μm LiChrospher RP-

Sample preparation for stability testing
A standard stock solution of ascorbyl palmitate (0.4 mg/mL) was prepared by dissolving appropriate amount in methanol. The standard solution was obtained by diluting the standard stock solution with methanol to yield a solution containing 0.04 mg/mL. Approximately 0.4 g of cream was exactly weighed, placed into a 50 mL volumetric flask, taken to volume with methanol and shaken for about 5 min for ascorbyl palmitate analysis. The solutions were passed through a 0.45-μm membrane filter before injection. The stability of ascorbyl phosphate was determined by HPLC in samples kept at room temperature (22±1 °C) in the dark for 4 weeks.

Microbiological stability
In order to assess the degree of contamination, 1g of material was dispersed in a 4-ml sterile Ringer solution containing 0.25% tween 80. Appropriate dilutions were made in the same dispersing vehicle, and 0.1 ml was plated out on the appropriate solid medium using the surface viable method. Emergent colonies were counted after the necessary incubation. All operations were carried out in duplicates [15].

Aerobic plate count
Aerobic plate counts were determined by inoculating 0.1 ml of the homogenate sample onto triplicate sterile plates of prepared and dried Standard Methods Agar using the surface spread technique. The plates were then incubated for 48 h at 35°C [16]. The Standard Methods Agar is a standardized medium for the enumeration of microorganisms from materials of sanitary importance. Duplicates of each dilution (1 mL) of neutralized and non-neutralized samples were pour-plated using Standard Methods Agar (Oxoid, Basingstoke, Hampshire, England) and incubated at 30 ± 1°C for 48 ± 3 h. Plates containing 25–250 colonies were selected and counted, and the average number of CFU/mL was calculated.

Pseudomonas aeruginosa count
Pseudomonas aeruginosa were enumerated on Pseudomonas Agar Base (CM 559, Oxoid) supplemented with cetrimide, fucidin, and cephaloridine, providing a selective isolation medium for Pseudomonas aeruginosa. Colonies were counted after 2 days of incubation at 25°C [17].

Staphylococcus aureus
Surviving population of Staphylococcus aureus was determined by standard plating methods [18]. At each sampling time, colonies of Staphylococcus were selected, Gram-stained, and observed for catalase and oxidase reactions to confirm the presence of Staphylococcus aureus.

Yeast and mould counts
The method involved enumeration of colonies on Sabouraud dextrose chloramphenicol agar medium. Enumeration was carried out as a pour plate, surface spread, or membrane filtration method [15]. Microbiological tests were repeated for fresh and formulations at 25°C after 7, 14, 21 and 28 days of preparation.

![Table 1. Components and concentrations of the formulations](image)
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RESULTS AND DISCUSSION

Physical stability of formulated emulsions
In this study, formulations were placed in different storage conditions i.e. 8±2°C, 25±2°C and 40±2°C for a period of four weeks in stability chambers. The samples were observed for change in colour, liquefaction, and phase separation, as presented in Table 2.

Color
The findings revealed that the freshly prepared emulsions were white and yellowish white in colour for F1, F2 and F3. Little changes in color were observed for emulsions well the end of storage is marked. (Table2). For example, for F1, the change in colour appeared from the 21st day and persisted up to the 28th day of the analyses period. The change in colour at the end of the observation period was presumably due to the oily phase separation which was promoted at higher temperature. Interestingly, no change in colour was observed for F4 at the different storage conditions, i.e., 8 ±2°C, 25 ±2°C and 40 ±2°C, up to 28 days of observation.

Liquefaction
The viscosity of emulsion is often reported to play a vital role in its flow properties (20). Starting from the emulsion preparation, the time and temperature processes begin to affect its flow properties (20). Starting from the emulsion preparation, it is well known that the stability of the emulsion is highly dependent on the time and temperature. The viscosity of emulsion is often reported to play a vital role in its flow properties (20). Starting from the emulsion preparation, it is well known that the stability of the emulsion is highly dependent on the time and temperature. The viscosity of emulsion is often reported to play a vital role in its flow properties (20). Starting from the emulsion preparation, it is well known that the stability of the emulsion is highly dependent on the time and temperature. The viscosity of emulsion is often reported to play a vital role in its flow properties (20). Starting from the emulsion preparation, it is well known that the stability of the emulsion is highly dependent on the time and temperature. The viscosity of emulsion is often reported to play a vital role in its flow properties (20). Starting from the emulsion preparation, it is well known that the stability of the emulsion is highly dependent on the time and temperature. The viscosity of emulsion is often reported to play a vital role in its flow properties (20). Starting from the emulsion preparation, it is well known that the stability of the emulsion is highly dependent on the time and temperature. The viscosity of emulsion is often reported to play a vital role in its flow properties (20). Starting from the emulsion preparation, it is well known that the stability of the emulsion is highly dependent on the time and temperature.

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<th>Liquefaction</th>
<th>Colour</th>
<th>Phase separation</th>
<th>Centrifugation</th>
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<tbody>
<tr>
<td>8°C</td>
<td>F1 -</td>
<td>W W W</td>
<td>-</td>
<td>-</td>
<td>6.93</td>
</tr>
<tr>
<td>25°C</td>
<td>F2 -</td>
<td>W W W</td>
<td>-</td>
<td>-</td>
<td>6.93</td>
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<tr>
<td>40°C</td>
<td>F3 -</td>
<td>YW YW YW</td>
<td>-</td>
<td>-</td>
<td>6.93</td>
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<tr>
<td>24 h</td>
<td>F4 -</td>
<td>W W W</td>
<td>-</td>
<td>-</td>
<td>6.93</td>
</tr>
<tr>
<td>3 day</td>
<td>F1 -</td>
<td>W W W</td>
<td>-</td>
<td>-</td>
<td>6.72</td>
</tr>
<tr>
<td>7 day</td>
<td>F2 -</td>
<td>W W W</td>
<td>-</td>
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<tr>
<td>14 day</td>
<td>F3 -</td>
<td>YW YW YW</td>
<td>-</td>
<td>-</td>
<td>6.46</td>
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<tr>
<td>21 day</td>
<td>F4 -</td>
<td>W W W</td>
<td>-</td>
<td>-</td>
<td>6.34</td>
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<tr>
<td>28 day</td>
<td>F1 -</td>
<td>W W W</td>
<td>-</td>
<td>-</td>
<td>5.98</td>
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Table 2: Physical characteristics of F1, F2 and F3, formulations kept at 8 ±2°C, 25 ±2°C and 40 ±2°C.

*No Change; + = Slight Change; YW= Yellowish White; W= White.
is in excess. These also occur in larger extent in more dilute systems, indicating that the initial concentration of the active ingredient is an important factor concerning the extent of its degradation (27). When ascorbyl palmitate used as an antioxidant to stabilize formulations if used as an active ingredient the concentrations are higher, usually 1–2%. In our study, ascorbyl palmitate was incorporated in emulsion at 2.00%. Fraction of nondegraded ascorbyl palmitate determined at different time intervals of storage in the dark is shown in Table 3.

After 28 days, 37% of ascorbyl palmitate remained fraction against only 8% of ascorbic acid. Compared to L-ascorbic acid, ascorbyl palmitate was significantly more stable. These results support the use of ascorbyl palmitate as an active ingredient in cosmetic and pharmaceutical preparations.

Microbiological stability of formulated emulsions

Aerobic plate count

The increase in storage time resulted in significant proliferations in Aerobic plate counts regardless of the type of treatment being applied (Table 4). The log mean count recorded for the Aerobic plate count of samples on day 0 was about 2 log10 CFU/g. On day 28 of storage, the log mean count of Aerobic plate count reached 3.13, 2.93 and 2.35 for F1, F2, and F3, respectively, which did not approximate the maximum limit of 6.9 log10 CFU/g for Aerobic plate count recommended by ISO NF-21149 (2006) in processed cosmetics (16).

Chemical stability

Chromatographic analysis of ascorbyl palmitate (F3)

Several chromatographic analytical methods for quantifying the amount of ascorbyl palmitate are described in the literature. Ascorbyl palmitate has been determined using amino and cyanopropyl columns (6, 25, 26). The instability of ascorbyl palmitate is a result of its oxidative degradation. Generally, the kinetics of oxidative reactions are second order, but can usually be simplified to pseudo first order if oxygen

Table 3. Percentages of non-degraded L-ascorbic acid (F2) and ascorbyl palmitate (F3) \( n=3 \) in o/w emulsions at 2.00%.

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for the formulated sample F3 were noted to show delayed growth when compared to F1 and F2 (Table 4).

CONCLUSIONS

L-ascorbic acid and its derivative: ascorbyl palmitate seems to be very interesting since it preserved physicochemical properties of the product and was efficient against the proliferation of various spoilage microorganisms. In fact, their stability (physical, chemical and microbiological) was studied in o/w emulsions. In fact, ascorbyl palmitate is more stable than L-ascorbic acid at initial concentration used 2.00%. Therefore, ascorbyl palmitate can be used as an active ingredient in cosmetic and pharmaceutical preparations on the basis of its stability. The newly formulated cream proved to exhibit a number of promising properties and attributes that might open new opportunities for the construction of more efficient, safe, and cost-effective skin-care, cosmetic, and pharmaceutical products.

REFERENCES AND NOTES