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ASSESSMENT OF LOADING OF ASCORBYL-2-PHOSPHATE AND 6-O-PALMITOYLASCORBIC ACID IN CETYL DIMETHICONE COPOLYOL BASED W/O/W EMULSIONS

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Abstract

W/O/W emulsions with multifaceted nature ensure the ability to incorporate lipophilic as well as hydrophilic compounds based on their solubility. Loading of these compounds in different phases of W/O/W emulsion may affect their constancy. This study was aimed to evaluate the constancy of Ascorbyl-2-phosphate and 6-O-Palmitoylascorbic acid in W/O/W emulsions (F_1 and F_2). F_1 and F_2 samples containing Ascorbyl-2-phosphate and 6-O-Palmitoylascorbic acid in different phases of cetyl dimethicone copolyol based W/OW emulsions were analyzed for globule size, rheological behavior and HPLC, immediately after preparation and then at different storage conditions (8°C, 25°C, 40°C and 40°C+75%RH) for 90 days. Rheological analysis showed that at accelerated storage conditions F_1 and F_2 had shear thinning behaviour of varying shear stress with no influence of location of functional ingredients in the carrier system. Microscopic analysis showed increase in globule size with time, especially at higher temperatures while

decreased at low temperatures. HPLC analysis at the end of 90 days showed that 6-O-Palmitoylascorbic acid and Ascorbyl-2-phosphate were almost stable in F_1 and F_2 W/OW emulsions with no influence of their location in a carrier system. F_1 and F_2 W/OW emulsions were found stable carriers for Ascorbyl-2-phosphate and 6-O-Palmitoylascorbic acid. This study may contribute to the improvement of formulations with Ascorbyl-2-phosphate and 6-O-Palmitoylascorbic acid to enhance their cosmetic benefits and also provides the direction to evaluate the stability with different concentrations of these compounds.

Keywords

W/OW Emulsions, Cetyl Dimethicone Copolyol, Ascorbyl-2-Phosphate, 6-O-Palmitoylascorbic Acid

1. Introduction

Topical use of ascorbic acid has grown extensive reputation due to its free radical scavenging activity. Owing to many useful effects on the skin, many cosmetic and dermatological products contain ascorbic acid. Due to its antioxidant nature, it is used as an antiaging component in cosmetic products. Various personnel have formulated different ascorbic acid topical preparations but still the stability of ascorbic acid is a major problem (Combs, & McClung, 2016). Thus, derivatives of ascorbic acid like sodium ascorbate, 6-O-Palmitoylascorbic acid, etc. used as antioxidants because they are stable than ascorbic acid (Shibuya et al., 2017). Two derivatives of ascorbic acid are extensively used in cosmetic preparations: Ascorbyl-2-phosphate and 6-O-Palmitoylascorbic acid. These two derivatives are differing in their potential to pervade through the skin because of their different hydro-lipophilic properties. As 6-O-Palmitoylascorbic acid is lipophilic in nature, its penetration is more easy (Gonzalez-Sabin, Moran-Ramallal, & Rebolledo, 2011), while ascorbyl phosphate salts are in prodrugs form which require conversion into ascorbic acid by the process of enzymatic hydrolytic before their penetration in the skin (Gašperlin, & Gosenca, 2011).

W/O/W type has broader areas of application among two basic multiple emulsions (Weitz, Thiele, & Abate, 2018) A unique property of W/O/W multiple emulsions is the diffusion of water through the oil phase (Khan et al., 2015). Polar molecules dissolved in either the internal or external aqueous phases are allowed through the middle oil layer (act as a membrane) by the process of diffusion. In the instance of water, this is compelled by osmotic pressure (Wang et al., 2016). Therefore; active compounds which are loaded in inner aqueous phase may

have different release profile as compared to that which is loaded in outer aqueous phase of W/O/W emulsion. There is also change in volume fraction of the primary emulsion due to water diffusion which changes the rheological properties of multiple emulsion and globule size.

To guarantee the selection of most stable and effective formulation, chemical and physical stability studies are essential during development process (Baertschi, Alsante, & Reed, 2016). As regards the physical stability; it is famous for multiple emulsion that its degradation in heating is a result of alternation in the solubility of the constituents or facilitation of the coalescence phenomenon in emulsion. Therefore, study of the rheological behavior, globule size and HPLC analysis during the formulations development is essential for evaluating their stability (Khan et al., 2016). Therefore, in current investigation, an attempt has been made to assess the constancy of cetyl dimethicone copolyol based W/O/W emulsions (F_1 and F_2) holding lipophilic 6-O-Palmitoylascorbic acid and hydrophilic Ascorbyl-2-phosphate in different phases.

2. Materials and methods

2.1 Materials

Paraffin oil (η: 110–230mPa·s, Merck, Germany), Cetyl dimethicone copolyol (Evonik, Germany), Polysorbate 80 (Merck-schuchardt Hohenbrunn, Germany). 6-O-Palmitoylascorbic acid and Ascorbyl-2-phosphate were purchased from sigma Aldrich. Magnesium sulfate heptahydrate (Merck) and purified water.

2.2 Method

2.2.1 Preparation of W/O/W Emulsions

Two step emulsification was adopted for the preparation of W/O/W emulsions (Smaoui & Hilima, 2013). W/O/W emulsions (F_1 and F_2) were prepared individually by heating the components of phase A that is, the oil phase and phase B (water phase) to 75°C (Table 1 and 2). Both phases were then mixed at 75°C by IKA Mixing Overhead Stirrer, Eurostar (Germany). Mixing speed was 2000rpm for first 5min and 1000rpm and 500rpm for next 10 and 5min, respectively. This was the primary emulsion. After that phase B was prepared by mixing its components at room temperature. Phase A was added to phase B at 700 rpm for 40min at room temperature (Matsumoto, Kita, & Yonezawa, 1976). The difference between the preparations of F_1 and F_2 was that in W/O/W emulsion F_1, Ascorbyl-2-phosphate (0.5%) was loaded in inner aqueous phase while in F_2, Ascorbyl-2-phosphate (0.5%) was loaded in outer aqueous phase during preparation.

Table 1: *Composition of F_1*

Phase A	**%Wt**	**Phase B**	**%Wt**
Oil phase			
Cetyl dimethicone copolyol	2.4	Polysorbate 80	0.8
Liquid paraffin	13.6	Purified water	19.2
6-O-Palmitoylascorbic acid	0.5		
Aqueous phase			
Magesium sulfate, heptahydrate	0.56		
Ascorbyl-2-phosphate	0.5		
Purified water	62.44		

Where, % Wt = Percent weight

Table 2: *Composition of F_2*

Phase A	%Wt	Phase B	%Wt
Oil phase			
Cetyl dimethicone copolyol	2.4	Polysorbate 80	0.8
Liquid paraffin	13.6	Ascorbyl-2-phosphate	0.5
6-O-Palmitoylascorbic acid	0.5	Purified water	18.7
Aqueous phase			
Magesium sulfate, heptahydrate	0.56		
Purified water	62.94		

Where, % Wt = Percent weight

2.2.2 Storage of W/OW Emulsions In Incubators

After preparation, F_1 and F_2 samples were stored in different incubators (8°C, 25°C, 40°C and 40°C+75%RH) in tightly closed glass containers for a period of 90 days (3 months). The samples were analyzed periodically for rheology, globule size and HPLC.

2.2.3 Globule Size Analysis

Confirmation foe preparation of multiple emulsion is usually done by globule size analysis (Van der Tuuk, Sørland, Sjöblom, & AS, 2009). Globule size analysis was carried out by means of microscope (Nikon E200, Nikon, Japan). F_1 and F_2 samples were diluted with continuous phase and then cautiously kept individually on a microslide and then covered with a cover slip. Globule size measurements were done for freshly prepared F_1 and F_2 samples, and

then followed at 8◦C, 25◦C, 40◦C and 40◦C with 75% relative humidity to study any alternation in globule size during the testing period of 30, 60 and 90 day.

2.2.4 Rheological Analysis

Rheological analysis of F_1 and F_2 samples were done using CP41 spindle in a Brookfield programmable rheometer (Model DV.III, USA) via Rheocalc V 2.6 software. Readings were taken for fresh F_1 and F_2 samples and then followed for the testing period of 30, 60 and 90 day. Power law was applied using Rheocalc V 2.6 software for consistency index and flow index.

2.2.5 Hplc Analysis

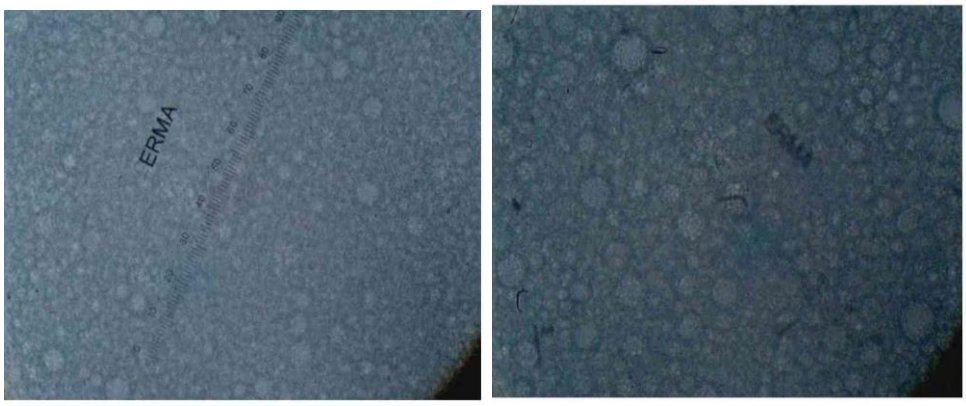
High performance liquid chromatography method was adopted for quantitative analysis of 6-O-Palmitoylascorbic acid and Ascorbyl-2-phosphate in F_1 and F_2 W/O/W emulsions. Before the sample injection into the HPLC system extraction of active ingredients from excipients was carried out (van der Tuuk Oedal, Sørland, Sjöblom & AS, 2009)

Percentage of active ingredients in F_1 and F_2 was expected by constructing a standard curve. By using standard curve parameters (Intercept and Slop), percentage of Ascorbyl-2-phosphate and 6-O-Palmitoylascorbic acid were calculated.

3. Results

3.1 Globule Size Analysis

The information about the confirmation of W/O/W emulsion formation and size of the multiple globules was obtained by taking photomicrographs. Photomicrographs of F_1 and F_2 after preparation are shown in Figure 1. Average globule sizes of $F_1$and F_2 with time are given in Table 3. Average globule size of F_1 was initially 9.3± 2.13µm. During the testing period of 30, 60 and 90 day, globule size decreased at 8°C and 25ºC while, at 40°C and 40ºC with 75%RH globule size increased. Average globule size of F_2 was initially 8.9± 2.01µm which was observed smaller than F_1. This globule size was found to decrease gradually at 8°C and 25ºC during the testing period of 30, 60 and 90 day. However, at 40°C and 40ºC with 75%RH, globule size of F_1 first decreased at day-60 and then increased at day-90.



A **B**

Figure 1: *Photomicrographs of multiple emulsions immediately after preparation, (A) F_1 and (B) F_2.*

Table 3: *Mean globule size (µm) as a function of storage time and storage conditions*

	F_1				F_2			
Fresh	9.3±2.13				8.9± 2.01			
Temperature	8ºC	25ºC	40ºC	40ºC+RH	8ºC	25ºC	40ºC	40ºC+RH
After 30days	8.2±1.48	7.9±2.62	9.6±2.37	10.1±3.60	7.5±3.03	7.1±4.24	8.2±2.22	8.4±2.57
After 60days	6.2±7.50	7.1±2.85	11.1±5.04	13.1±2.24	6.2±3.01	6.8±4.27	8.7±2.21	13.2±6.18
After 90days	5.5±7.50	6.1±2.85	12.7±2.85	13.4±5.99	4.4± 7.50	4.4±2.85	7.8± 5.04	12.3±5.91

Where, RH=relative humidity, ±= S.D(Standard deviation)

3.2 Flow Analysis

The viscosities of F_1 and F_2 samples were measured at 100 to 200 rpm speed using shear rates from 200 to 400 (with 20 increments) on each sample. Shear rates were gradually increased on each sample and changes in viscosities were noted (Figure 2, 3 and 4). The viscosities of F_1 and F_2 were decreased with increasing shear rates which showed their shear thinning behavior.

Power law analysis of F_1 and F_2 showed pseudo plastic behavior, with a flow index below 1(Table 4).

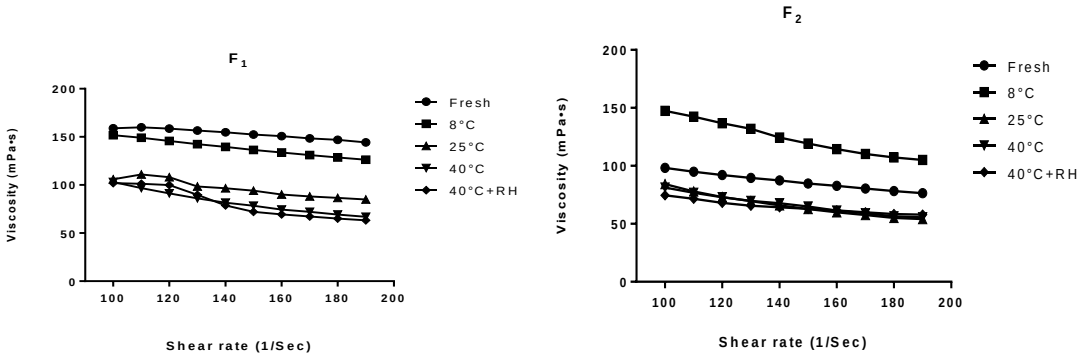


Figure 2: *Flow curves of F_1 and F_2 samples as a function of shear rate when kept in different storage conditions, fresh and at 30 day of preparation (n = 3)*

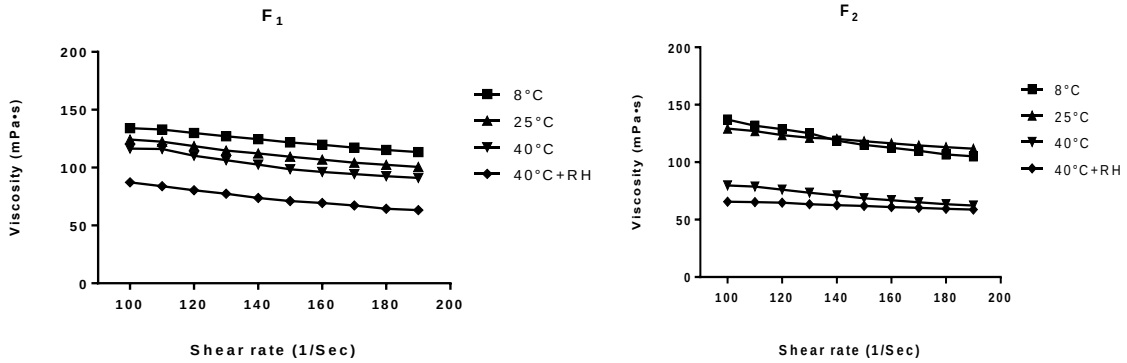


Figure 3: *Flow curves of F_1 and F_2 samples as a function of shear rate when kept in different storage conditions at 60 day of preparation (n = 3)*

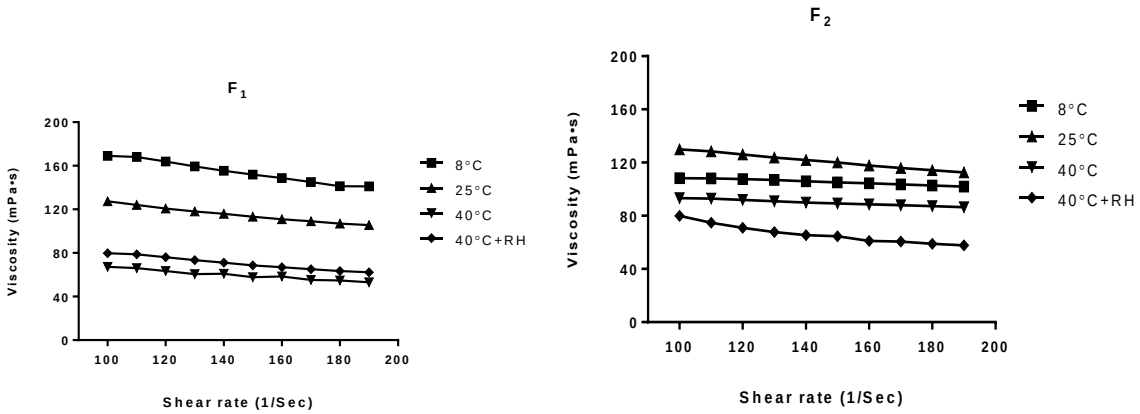


Figure 4: *Flow curves of F_1 and F_2 samples as a function of shear rate when kept in different storage conditions at 90 day of preparation (n = 3)*

Table 4: *Power law analysis of F_1 and F_2 fresh sample and samples kept at different storage conditions after, 30, 60 and 90 days*

	F_1				F_2			
	Fresh				Fresh			
Flow index	0.70				0.56			
Consistency index	426.7				596.8			
	After 30 Days				After 30 Days			
Temperature	8ºC	25ºC	40ºC	40ºC+RH	8ºC	25ºC	40ºC	40ºC+RH
Flow index	0.70	0.52	0.54	0.48	0.50	0.52	0.54	0.55
Consistency index	641.2	911.4	182.2	942.6	573.1	734.1	872.9	761.8
	After 60 Days				After 60 Days			
Temperature	8ºC	25ºC	40ºC	40ºC+RH	8ºC	25ºC	40ºC	40ºC+RH
Flow index	0.62	0.66	0.73	0.58	0.51	0.54	0.77	0.82
Consistency index	918.4	611.0	479.7	826.9	734.1	993.4	367.2	151.2
	After 90 Days				After 90 Days			
Temperature	8ºC	25ºC	40ºC	40ºC+RH	8ºC	25ºC	40ºC	40ºC+RH
Flow index	0.54	0.63	0.70	0.67	0.61	0.77	0.88	0.90
Consistency index	549.5	651.8	741.5	665.3	590.5	377.1	165.2	172.0

3.3 HPLC Analysis

Quantitative analysis of F_1 and F_2 formulations via HPLC method is given in Table 5. Slight degradation of active compounds after 90 days showed their stability in W/O/W emulsions.

Table 5: *HPLC analysis of F_1 and F_2*

	6-O-Palmitoylascorbic acid			Ascorbyl-2-phosphate		
F.Code	Theoretical concentration (mg)	Obtained concentration (mg)	%age	Theoretical concentration (mg)	Obtained concentration (mg)	%age
F_1	500	434.3	86.9	500	456.2	91.3
F_2	500	455.8	91.2	500	467.5	93.5

mg= milligram

4. Discussion

The planning of any stable formulation requires the consideration of an amount of factors. In current investigation, stability of F_1 and F_2 multiple emulsion has been investigated in terms of globule size, flow changes and HPLC during the storage period**.**

Confirmation of W/O/W emulsion formation and stability are determined by the size of globules which is an important parameter (Akhtar et al., 2010) Globule sizes of F_1 and F_2 are given in Table No. 3. F_1 and F_2 showed the decrease in globule size at 8ºC and 25ºC while increase in globule size at 40ºC and 40ºC +75% RH with time. Decrease in globule size is owing to contraction of globules results from the exclusion of internal water droplets towards external water phase by diffusion (Gaspar & Campos, 2003). and increase in globule size under accelerated conditions is due to the joining of globules (Vasiljević, Parojčić, Primorac & Vuleta, 2009)

While comparing globule size of F_1 and F_2 at 8ºC and 25ºC, there was no/little change observed with respect to time. However, at 40ºC globule size of F_1 at 60 and 90-day was markedly larger than F_2 but at 40ºC+75% RH globule sizes of both were almost similar. These changes showed instability of F_2 at accelerated condition as Ascorbyl-2-phosphate was present in outer aqueous phase and no Ascorbyl-2-phosphate was present in inner aqueous phase due to which osmotic pressure difference was generated in the system which ultimately led to destabilize the globules (Rojas, Staton, John & Papadopoulos, 2008). Osmotic pressure difference causes the penetration of water from the outer to the inner water phase. The multiple globules swell and may then split due to breakdown of the oily membrane (Nikovska, 2010), thus destabilization occur.

The knowledge of rheological performance of formulations throughout the process of development is crucial as such studies provide performance of the product during use (Tirnaksiz & Kalsin, 2005) Rheological analysis helps in the characterization of emulsion formulations, by describing the changes occur in the emulsion formulations provoked through aging, shear stress and temperature (Pokorski & Marczak, 2005).

The decrease in viscosities of F_1 and F_2 were observed on applying shear rates, indicating their shear thinning behavior. The aggregates may be distorted at low shear. While at high shear rate, the aggregates may be split to separate globules that lead to decrease in viscosity. The decrease in viscosity was more at 40ºC and 40ºC with 75% relative humidity. At higher temperatures, there is diffusion of water molecules (Matsumoto, Kita & Yonezawa, 1976) F_1 and F_2 have shown thinning behaviors on increasing shear rates at all storage conditions till the end of study period.

While comparing viscosity of F_1 and F_2, viscosity of fresh F_1 was 158.90 cP while, viscosity of fresh F_2 sample was 98.24 cp. After 30 days, viscosity of F_1 slightly decreased (152.78 cP) at 8ºC while viscosity of F_2 increased (147.36 cP) at 8ºC on applying shear rates. At 25ºC, 40ºC and 40ºC + 75% RH, viscosity of F_1 further decreased and similar behavior was observed for F_2 on applying shear rates. After 60 days, viscosity of F_1 got further decreased and this effect was seen at all storage conditions on increasing shear rates. However, maximum decrease in viscosity was observed at high temperatures. For F_2 sample, somewhat random behavior was observed. Its viscosity increased at 8ºC and 25ºC while decreased at 40ºC and 40ºC + 75% RH. At 90 day, viscosity of F_1 increased at 8ºC while decreased further at 25ºC, 40ºC and 40ºC + 75% RH and viscosity of F_2 increased at 25ºC while increased at all other storage conditions. The decrease in viscosity of F_1 at all storage conditions is due to the migration of inner aqueous phase towards the outer aqueous phase while in case of F_2 decrease in viscosity at 25ºC, 40ºC and 40ºC+ 75% RH owing to burst out of multiple globules resulting from osmotic pressure (Akhtar, Ahmad, Masood & Aleem, 2008; Gianeti, Gaspar, Bueno & Berardo, 2012)

Power law results of F_1 and F_2 samples showed pseudo plasticity (flow index below 1) at all storage conditions. Pseudo plasticity is an enviable rheological property in cosmetic formulations as it provides a pleasant sensory feeling and improves application and spreading.[22] It is reported that, there is production of an intelligible film covering the skin surface by the use of formulations with a pseudo plastic flow. There may be progressive disintegration of the internal structure of the formulations that cause pseudo plastic flow (Ali & Akhtar, 2014).

Although flow indexes of F_1 and F_2 were changed by stress and consistency indexes were also changed. In our results, at 8ºC and 25ºC the consistency index of F_1 and F_2 got increased. At 40ºC and 40ºC with 75%RH, consistency index of F_1 got increased while consistency index of F_2 got decreased after 60 and 90 days showing instability. It is reported by many researchers that consistency indexes generally decline during storage stating the instability of product. It seems that this was due interactions of ascorbic acid derivatives with other components of W/O/W emulsion. These interactions have been reported when these compounds were analyzed by mass spectroscopy (Jurkovič, 2003)

HPLC showed little degradation of 6-O-Palmitoylascorbic acid and hydrophilic Ascorbyl-2-phosphate at the end of 90 days. After 90 days, above 90% of non-degraded Ascorbyl-2-phosphate and above 85% of non-degraded 6-O-Palmitoylascorbic acid remained in F_1 and F_2 W/O/W emulsions. Literature data indicate that Ascorbyl-2-phosphate is the stable ascorbic acid derivative. In the structure of Ascorbyl-2-phosphate, phosphate group is located in the second position on the cyclic ring which defends the enediol system of the molecule against oxidation (Van der, Sørland, Sjöblom & AS, 2009; Yaqoob et al., 2006). The fractions of non-degraded 6-O-Palmitoyl ascorbic acid and ascorbyl-2-phosphate lasting after 90 days of storage (Table 5).

5. Conclusions

6-O-Palmitoylascorbic acid and Ascorbyl-2-phosphate were combined in W/O/W type multiple emulsions (F_1 and F_2), irrespective of their quantitative composition and location of active compounds in the carrier system. W/O/W emulsions were then characterized for globule size, flow changes and HPLC. Globule size and flow changes showed acceptable stability of F_1 and F_2 except under accelerated conditions. HPLC also revealed minor degradation of active compounds after 90 days. As compared to simple vitamin C, addition of 6-O-Palmitoyl ascorbic acid and ascorbyl-2-phosphate in combination have no considerable effect on the stability of F_1 and F_2 multiple emulsions except under accelerated conditions. Multiple emulsion was found a stable carrier for vitamin C derivatives. Current study may contribute the improvement of formulations with Ascorbyl-2-phosphate and 6-O-Palmitoylascorbic acid with different combinations to GET their combined antioxidant effects.

6. Conflict of interest

Authors have no conflict of interest.

References

Akhtar, N., Ahmad, M., Khan, H. M. S., Akram, J., Gulfishan, G., Mahmood, A., & Uzair, M. (2010). Formulation and characterization of a multiple emulsion containing 1% L-ascorbic acid. Bulletin of the Chemical Society of Ethiopia, 24 (1), 1-10. https://doi.org/10.4314/bcse.v24i1.52955

Akhtar, N., Ahmad, M., Masood, M. I., & Aleem, M. (2008). Formulation and in vitro evaluation of a cosmetic emulsion from almond oil. Pakistan journal of pharmaceutical sciences, 21 (4), 430-437.

Ali, A., & Akhtar, N. (2014). Changes in the Characteristics of Water-in-Oil-based High Internal Phase Emulsion Containing Moringa Leaves Extract at Various Storage Conditions. Tropical Journal of Pharmaceutical Research, 13 (5), 677-682. https://doi.org/10.4314/tjpr.v13i5.4

Baertschi, S. W., Alsante, K. M., & Reed, R. A. (Eds.). (2016). Pharmaceutical stress testing: predicting drug degradation. CRC Press.

Combs Jr, G. F., & McClung, J. P. (2016). The vitamins: fundamental aspects in nutrition and health. Academic press.

Gašperlin, M., & Gosenca, M. (2011). Main approaches for delivering antioxidant vitamins through the skin to prevent skin ageing. Expert opinion on drug delivery, 8 (7), 905-919. https://doi.org/10.1517/17425247.2011.581657

Gianeti, M. D., Gaspar, L. R., Bueno de Camargo Júnior, F., & Berardo Gonçalves Maia Campos, P. M. (2012). Benefits of combinations of vitamin A, C and E derivatives in the stability of cosmetic formulations. Molecules, 17 (2), 2219-2230. https://doi.org/10.3390/molecules17022219

González-Sabín, J., Morán-Ramallal, R., & Rebolledo, F. (2011). Regioselective enzymatic acylation of complex natural products: expanding molecular diversity. Chemical Society Reviews, 40 (11), 5321-5335. https://doi.org/10.1039/c1cs15081b

Jurkovič, P., Šentjurc, M., Gašperlin, M., Kristl, J., & Pečar, S. (2003). Skin protection against ultraviolet induced free radicals with ascorbyl palmitate in microemulsions. European journal of pharmaceutics and biopharmaceutics, 56 (1), 59-66. https://doi.org/10.1016/S0939-6411(03)00062-6

Khan, H., Akhtar, N., Ali, A., Khan, H. M. S., Sohail, M., Naeem, M., & Nawaz, Z. (2016). Physical and chemical stability analysis of cosmetic multi-ple emulsions loaded with

ascorbyl palmitate and sodium ascorbyl phosphate salts. Acta poloniae pharmaceutica, 73 (5), 1339-1349.

Khan, H., Akhtar, N., Mahmood, T., Jameel, A., & Mohsin, S. (2015). Preliminary 1 month stability screening of cosmetic multiple emulsions (W/O/W) prepared using cetyl dimethicone copolyol and P olysorbate 80. International journal of cosmetic science, 37 (1), 76-81. https://doi.org/10.1111/ics.12172

Matsumoto, S., Kita, Y., & Yonezawa, D. (1976). An attempt at preparing water-in-oil-in-water multiple-phase emulsions. Journal of Colloid and Interface Science, 57 (2), 353-361. https://doi.org/10.1016/0021-9797(76)90210-1

Nikovska, K. (2010). Oxidative stability and rheological properties of oil-in-water emulsions with walnut oil. Advance Journal of Food Science and Technology, 2 (3), 172-177.

Pokorski, M., & Marczak, M. (2005). Stability of ascorbyl palmitate molecule in the rat brain. Journal of physiology and pharmacology, 56, 197.

Rojas, E. C., Staton, J. A., John, V. T., & Papadopoulos, K. D. (2008). Temperature-induced protein release from water-in-oil-in-water double emulsions. Langmuir, 24 (14), 7154-7160. https://doi.org/10.1021/la703974n

Shibuya, S., Sakaguchi, I., Ito, S., Kato, E., Watanabe, K., Izuo, N., & Shimizu, T. (2017). Topical Application of Trisodium Ascorbyl 6-Palmitate 2-Phosphate Actively Supplies Ascorbate to Skin Cells in an Ascorbate Transporter-Independent Manner. Nutrients, 9 (7), 645. https://doi.org/10.3390/nu9070645

Smaoui, S., & Hilima, H. B. (2013). Application of l-ascorbic acid and its derivatives (sodium ascorbyl phosphate and magnesium ascorbyl phosphate) in topical cosmetic formulations: Stability studies. Journal of the Chemical Society of Pakistan, 35 (4), 1096-1102.

Tirnaksiz, F., & Kalsin, O. (2005). A topical w/o/w multiple emulsions prepared with Tetronic 908 as a hydrophilic surfactant: formulation, characterization and release study. Journal of pharmacy & pharmaceutical sciences, 8 (2), 299-315.

Van der Tuuk O. N., Sørland, G., Sjöblom, J., & AS, A. T. (2009). Methods for droplet size distribution determination of water-in-oil emulsions using low-field NMR. Diffusion fundamentals, 7, 1-29.

Vasiljević, D. D., Parojčić, J. V., Primorac, M. M., & Vuleta, G. M. (2009). Rheological and droplet size analysis of W/O/W multiple emulsions containing low concentrations of

polymeric emulsifiers. Journal of the Serbian Chemical Society, 74 (7), 801-816. https://doi.org/10.2298/JSC0907801V

Wang, W. C., Shi, K., Pan, Y. X., Peng, C., Zhao, Z. L., Liu, W., ... & Ji, X. L. (2016). Fabrication of polymersomes with controllable morphologies through dewetting W/O/W double emulsion droplets. Chinese Journal of Polymer Science, 34 (4), 475-482. https://doi.org/10.1007/s10118-016-1769-5

Weitz, D. A., Thiele, J. W., & Abate, A. R. (2018). U.S. Patent Application No. 15/656,415.