

Stability comparison of two dermal emulsions containing *Hippophae Rhamnoides* L. oil

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Abstract: Two formulations of multiple emulsion (ME-1 & ME-2) containing *Hippophae rhamnoides* L. oil were prepared. Along with emulsifiers (Abil EM 90, Synperonic F127), Magnesium and Zinc were used as stabilizer in ME-1 and ME-2 respectively. Both formulations were prepared using Two-Step Method and after preparation ME-1 and ME-2 were stored at different storage conditions i.e., 4°C, 25°C, 40°C, 40°C+ 75% RH for four weeks for stability evaluation. At 4°C ME-1 and ME-2 showed phase inversion and were excluded from further stability evaluation. While ME-1 and ME-2 both were stable at 25°C for a period of four weeks as no color change, phase separation and liquefaction occurred. ME-1 and ME-2 at 40°C, 40°C+ 75% RH exhibited change in color, liquefaction and phase separation. The decrease in viscosity and globule size whereas increase in electrical conductivity and pH were observed at 40°C, 40°C+ 75% RH for a period of four weeks. Multiple emulsion from *Hippophae rhamnoides* L. oil are unstable at refrigeration and high temperature conditions. These formulations must be placed at room temperature to increase their shelf life.

Keywords: Multiple emulsion, *Hippophae rhamnoides* L., two step method, evaluation, phase inversion.

INTRODUCTION

Multiple emulsion is a three phase complex system in which oil globules containing internal fine aqueous droplets, dispersed in the outer aqueous phase. This emulsion type is also known as emulsion of emulsion. Multiple emulsion is of two types w/o/w and o/w/o. W/o/w multiple emulsion displays the properties of both w/o and o/w emulsion (Leclercq *et al.*, 2011).

Multiple emulsion is thermodynamically unstable due to several possible mechanisms such as coalescence of internal aqueous droplets within the oil, coalescence of the oil globules of emulsion, movement of the surfactant from the oil within the oil globules to the bulk aqueous outer phase of the multiple emulsion through original w/o interface. Presence of the electrolytes and other additives may also be the other factors causing stability problems (Florence and Whitehill, 1985). Additionally use of a large number of compounds in this emulsion type may also influence the stability. Since the natural oil is also a complex of a large number of compounds so multiple emulsion prepared by it is a challenging job with reference to stability (Akhtar and Yazan, 2005). To obtain a stable emulsion this category two emulsifiers are used while formulating it a lipophilic and a hydrophilic. In case of w/o/w multiple emulsion Lipophilic emulsifier serves to stabilize the primary emulsion (o/w) and the hydrophilic emulsifier the w/o/w multiple emulsion

(Tirnaksiz and Kalsin, 2005; Leal-Calderon *et al.*, 2012).

Multiple emulsion has wide applications in fields of pharmaceuticals, chemistry, food and cosmetics. In pharmaceuticals it is used as controlled release drug delivery system for anti-neoplastic, antibiotic and anti diabetic drugs. These are also used as adjuvant vaccines in pharmaceutical industry. In the food industry it is used to protect functional food ingredient (Kumar *et al.*, 2012).

Hippophae rhamnoides L. oil is obtained from the berries of a deciduous shrub *Hippophae rhamnoides* L. of family Elaeagnaceae (Yang and Kallio, 2002) and is widely distributed throughout the temperate zones of Asia and Europe including northern areas of Pakistan. The berries oil contains a large number of medicinally important compounds as sterols, tocopherols, carotenoids, Vitamin E, saturated and unsaturated fatty acids such as linoleic acid, linoleic acid, oleic acid and palmitoleic acid. This natural oil has long been used orally, systemically and for dermatological conditions. In dermatological conditions psoriasis was one of them due to immunomodulatory properties of the oil (Guliyev *et al.*, 2004).

Two formulations ME-1 and ME-2 of multiple emulsion were prepared using *Hippophae rhamnoides* L. oil, one formulation (ME-1) of multiple emulsion containing Magnesium Sulphate and the other formulations (ME-2) zinc sulphate in inner aqueous phase. To determine the state of stability parameters of both the formulations were kept at different storage temperatures as 4°C, 25°C, 40°C,

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40°C+ 75% RH for a total of 28 days. The stability of these formulations during the said period was assessed through different in vitro parameters like organoleptic, change in pH, change in electrical conductivity changes in globule size and change in viscosity.

MATERIALS AND METHOD

Materials

Abil EM 90 (Goldschmidt, France), Synperonic PE/F127 (CRODA, Germany), Zinc Sulphate (Merk, Germany), Magnesium Sulphate (Merk, Germany), *Hippophae rhamnoides* L oil (Cheema Laboratories, Pakistan), Carboxymethylcellulose (BDH, England).

Preparation of W/O/W emulsions

ME-1 and ME-2 were prepared by Two-Step Method using different proportion of components (Table 1). In first step oil phase containing lipophilic emulsifier (Abil EM90) and aqueous phase containing magnesium sulphate were heated in the water bath (PCSIR, Pakistan) at 80°C separately. The aqueous or internal phase was added drop by drop into the oily phase with constant stirring at 2000 rpm till all the aqueous phase was incorporated and temperature dropped down to 25°C. In the second step external aqueous phase was prepared by mixing hydrophilic emulsifier (synperonic PE/F 127) in water (Dogru *et al.*, 2002) and then primary emulsion incorporated in the external aqueous phase in small portions at a speed of 1000 rpm for 10 minutes. The emulsion was then homogenized with the same stirrer (PCSIR, Pakistan) at 800 rpm for 5 minutes and then further for 5 minutes at 800 rpm (Erdal and Araman, 2006 ; Akhtar and Yazan, 2008; Akhtar *et al.*, 2008).

Stability evaluation of ME-1 and ME-2

First of all emulsion type was analyzed by diluting the multiple emulsion in water and oil and then the developed formulations were kept at different storage temperatures at 4°C in refrigerator, 25°C in cold incubator, 40°C in cold incubator and at 40°C+ 75% RH in oven and different stability tests as pH, conductivity, globule size, and viscosity were performed at 0 hour, 24 hours, 48 hours, 72 hours, 1st week, 2nd week, 3rd week and 4th week.

Organoleptic parameters

Organoleptic parameters as color liquefaction and phase separation of both the formulations ME-1 and ME-2 kept at different storage conditions (in fresh, 24 hours, 48 hours, 72 hours, 1st week, 2nd week, 3rd week and 4th week were observed by visual inspection (Akhtar *et al.*, 2010).

Globule size

Globule sizes of different samples of both ME-1 and ME-2 kept at different storage temperatures i.e., 4°C, 25°C, 40°C, and at 40°C+ 75% RH (PEL, Pakistan; SANYO,

Japan) were determined at the time intervals of fresh, 24 hours, 48 hours, 72 hours, 1st week, 2nd week, 3rd week and 4th week (Akhtar *et al.*, 2010). A glass slide with a water drop and a minute amount of sample was mixed and covered with a cover slip. Then immersion oil was put on the cover slip and placed under 100x power lens of microscope by Labomed, USA (Leclercq *et al.*, 2011).

pH determination

At different time and temperatures both ME-1 and ME-2 were subjected to pH changes by a digital pH meter by HANNA Instruments, Romania (Leclercq *et al.*, 2011; Akhtar *et al.*, 2008).

Electrical conductivity

Electrical conductivity of different samples of ME-1 and ME-2 kept at different storage conditions was determined at the time intervals by Conductivity Meter by HANNA Instruments, Romania (Akhtar *et al.*, 2008).

Viscosity studies

The viscosity change in different samples of both ME-1 and ME-2 kept at different storage conditions 4°C, 25°C, 40°C, and at 40°C+ 75% RH were determined at the time intervals of fresh, 24 hours, 48 hours, 72 hours, 1st week, 2nd week, 3rd week and 4th week by Viscometer (Brookfield, USA) at 100 rpm using spindle S-06 Tirmaksiz and Kalsin, 2005; Erdal and Araman, 2006).

STATISTICAL ANALYSIS

Two- way ANOVA at 5% significance was used to observe the change of each parameter at different time intervals and temperature levels. Change in each parameter in both the preparations was then compared by using independent T-test using SPSS 12.0.

RESULTS

Organoleptic parameters

Multiple emulsion ME-1 kept at 4°C in the refrigerator exhibited phase inversion on the second day of storage and excluded from further stability evaluation but at 25°C showed no liquefaction, phase separation or color variation was observed throughout the period of four weeks. At 40°C, ME-1 showed no phase separation till the end of the four weeks and slightly liquefied after 72 hours, whereas the color was dark yellow at the time of preparation which turned yellow at 48 hours and light yellow from 72 hours till one month. The dark yellow color changed to yellow after 72 hour with no liquefaction and phase separation at 40°C + 75%.

Multiple emulsion ME-2 kept at 4°C in the refrigerator also exhibited phase inversion on the second day hence also not included in further stability studies. At 25°C no

liquefaction, color change and phase separation was observed but at 40°C color changed dark yellow to yellow after 48 hours and light yellow after 2nd week along with high liquefaction and slight phase separation. The dark yellow color turned yellow on 2nd week and light yellow on 3rd week at 40°C + 75%. A slight liquefaction and phase separation also took place at these conditions.

Globule size determination

Globule sizes of samples of ME-1 and ME-2 at different conditions of storage as 25°C, 40°C, and at 40°C+ 75% RH at different intervals during storage were determined (fig. 1). The globules size of freshly prepared samples of ME-1 and ME-2 was 17 and 9.6 µm and a decreasing trend the values after one month at all storage temperature were observed with both (fig. 2).

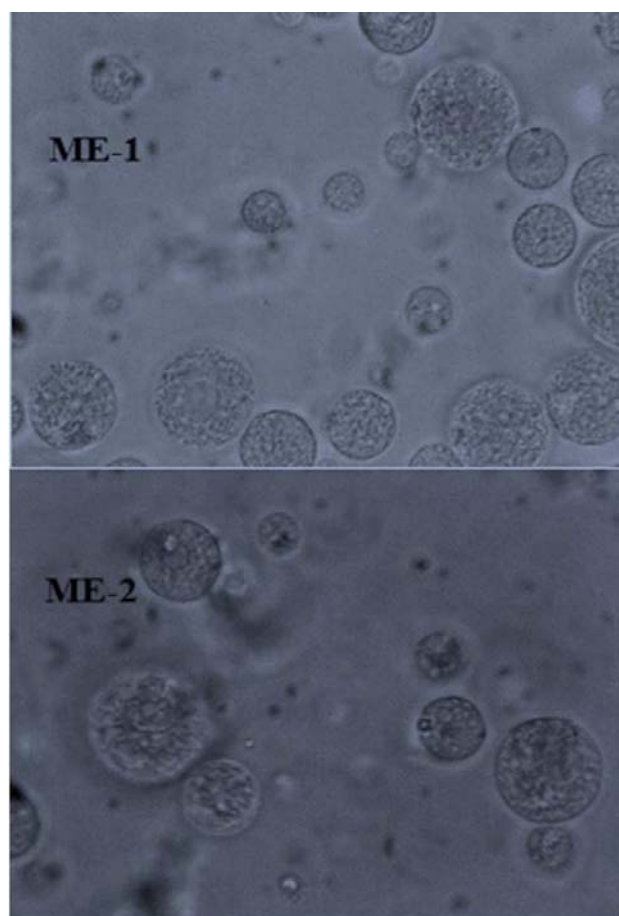


Fig. 1: Microscopic Examination of ME-1 and ME-2 after 1 week at 40°C/ 75%RH 128x189mm (300 x 300 DPI)

pH values of different samples of formulations ME-1 and ME-2 kept at different storage conditions of 25°C, 40°C, and at 40°C+ 75% RH at different intervals (fresh, 24 hours, 48 hours, 72 hours, 1st week, 2nd week, 3rd week and 4th week) during storage were observed. A slight increase in pH of both formulations with the passage of time was found (fig. 3).

Electrical Conductivity Tests

The electrical conductivity values of the samples of ME-1 & ME-2 at different storage temperature is listed in fig 4. A great change in the electrical conductivity was observed in both ME-1 (80-460 µS/cm) and in ME-2 (90-1510 µS/cm) as well.

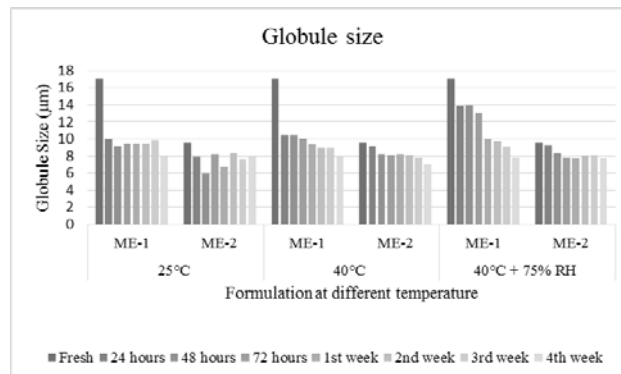


Fig. 2: Globule size of ME-1 and ME-2 at Different Temperatures

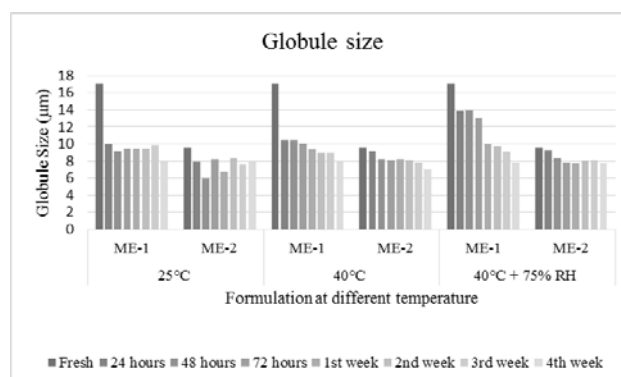


Fig. 3: pH of ME-1 and ME-2 at Different temperatures

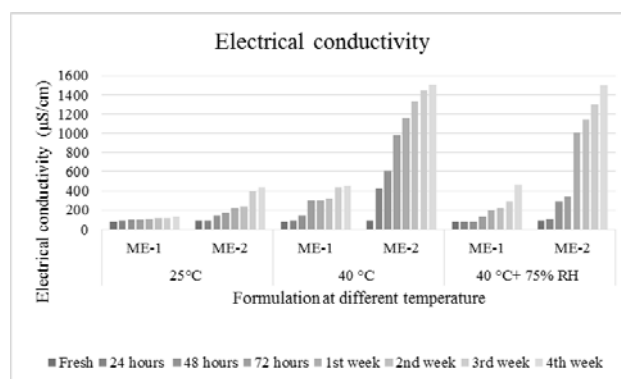


Fig. 4: Comparison of electrical Conductivity between ME-1 and ME-2

Viscosity studies

Viscosities of ME-1 and ME-2 at start were high but at the end of specified stability period their viscosities decreased 5790 to 490 mPa.s for ME-1 and 6430 to 130 mPa.s for ME-2 (fig. 5).

DISCUSSION

ME-1 and ME-2 are thermodynamically unstable and this instability may be due to four possible mechanisms, including, coalescence of internal droplets, coalescence of oil globules, rupture of the oil globules and thus release of inner phase water to the external water phase, transport of inner aqueous phase and its soluble components to the external aqueous phase. These can occur either by diffusion or reverse micellar transport that arises as a result of osmotic difference between the two aqueous phases. The presence of two thermodynamically unstable interfaces is the reason for the instability of multiple emulsion. Two emulsifiers lipophilic and hydrophilic are needed to stabilize oil-in-water and water-in-oil interfaces. Several approaches are there to overcome the instability in multiple emulsion as to stabilize the inner phase by using the better emulsifier, preparing L2-microemulsion and micro spheres, increasing the viscosity of either inner or outer phase by adding carriers (Kumar *et al* 2012; Leal-Calderon *et al.*, 2012; and Fernando *et al.*, 2007).

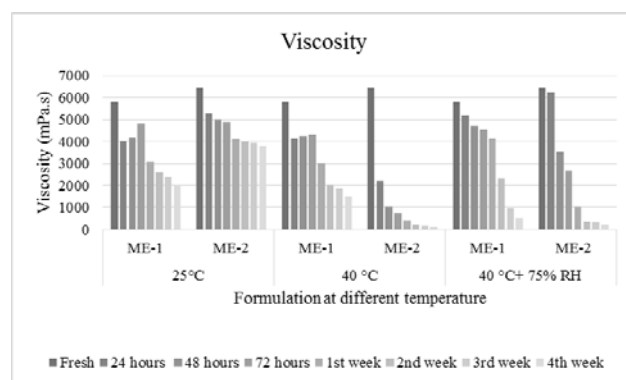


Fig. 5: Viscosity of ME-1 and ME-2 at Different Storage Conditions

The electrolytes are added to the either as additive or active components and diffusion is the possible mechanism for the release of electrolytes from oil layer membrane and it also effect the release behavior of the electrolytes. This release is also affected by the concentration and solubility of the electrolyte in the aqueous phase but it does not depend on the internal phase viscosity. So the stability of multiple emulsion containing electrolytes is achieved by controlling and limiting the diffusion mechanism.

As color of the *Hippophae rhamnoides L.* oil was dark orange and preparation formed were dark yellow. Oil phase contains carotenoides (Arimboor *et al.*, 2006) therefore the changes in the color at 40°C and 40°C + 75% RH might be due to the oxidation of carotenoides at high temperature and presence of humidity (Partanen *et al.*, 2005) Liquefaction occurred in ME-2 at 40°C and 40°C+75% RH and in ME-1 in the sample at 40°C. The

liquefaction and phase separation might be due to the transport of inner phase water to external water phase and was a sign of instability (Jiao and Burgess, 2003) that was promoted by high temperature (Lutz *et al* 2009; Jiao and Burgess 2003).

Table 1: Proportion of Chemicals in ME-1 and ME-2

Chemicals (%)		ME-1	ME-2
Primary Emulsion	Hippophae rhamnoides L. oil	20	20
	Abil EM90	7	7
	Magnesium Sulphate	2.5	-
	Zinc Sulphate	-	2.5
	Distilled water (q.s.)	100	100
Multiple Emulsion	Primary emulsion	75	75
	Synperonic F127	3	3
	Carboxyethyl cellulose	0.2	0.2
	Distilled water (q.s.)	100	100

The increase or decrease in the globule size defines instability of a multiple emulsion. The internal droplets shrinkage occur due to the coalescence of the multiple droplets with the other oil droplets, similarly more than one droplets may coalesce or expelled out or water may pass from the external phase to internal water phase that results in the complete rupture of the internal droplets due to the swelling of internal water droplets. In formulations ME-1 and ME-2 the globule sizes decreased with passage of time to the average of 7.5µm. Using two-way ANOVA test at 5% significance level change in globule sizes was highly significant as $p < 0.05$ at different time and temperature levels. The globule size change in our formulations of ME-1 and ME-2 at 25°C was taken as standard and change in globule size at other temperature levels was compared insignificant change was observed at temperature of 40°C and 40°C + 75% RH. By applying independent T-test to compare the results of globule sizes in both preparations ME-1 and ME-2 highly insignificant difference was observed 25°C and 40°C and at 40°C at 75% RH temperature highly significant difference was observed. The decrease in globule size was because of globules shrinkage due to the diffusion of inner aqueous phase to the external aqueous phase at high temperature (Jiao and Burgess 2003; Olivieri *et al.*, 2003). The globule size decreased due to the: i) transfer of internal aqueous phase to the external water phase resulting in reducing the globules volume ii) bursting of multiple globules because of osmotic pressure (Tirnaksiz and Kalsin, 2005).

Using two-way ANOVA test at 5% significance level the values for pH change of different samples was very significant at different time intervals while insignificant at different storage temperatures ($p < 0.05$) in ME-1. Whereas significant change was observed at both time and temperature levels in ME-2. When the change in pH of freshly prepared sample of both ME-1 and ME-2 was taken as the standard to compare the pH change in other

samples at other time intervals significant change was observed especially from 1st week to the forth week. While to observe the pH change at temperature level taking the change in pH of sample of multiple emulsion at 25°C was compared to pH changes at other temperatures insignificant change was observed at 40°C ($p > 0.05$) and significant results obtained at 40°C at 75% RH ($p < 0.05$) in ME-2. By applying independent T-test to compare the change of pH of both ME-1 and ME-2 highly insignificant ($p > 0.05$) difference was observed at 25°C and 40°C and at 40°C at 75% RH temperature significant ($p < 0.05$) difference was observed. The increasing in the pH of the all the samples with the time might be due to production of alkaline products within the formulation due to the oxidation of any of the compounds of *Hippophae rhamnoides* L. oil (Partanen *et al.*, 2005).

Electrical conductivity of preparations ME-1 and ME-2 was increased more at 40°C and 40°C + 75% RH than at 25°C. Using two-way ANOVA test at 5% significance level of electrical conductivity the change was significant ($p < 0.05$) at different levels of time and temperature in both ME-1 and ME-2. The change in electrical conductivity of sample placed at 25°C was taken as standard and change in electrical conductivities at other temperature levels was compared. The most significant changes ($p < 0.05$) in values were observed at 40°C and 40°C + 75% RH in both ME-1 and ME-2. Hence, the results showed that ME-1 and ME-2 were stable at temperature at 25°C. T-test of electrical conductivity of both formulations ME-1 and ME-2 showed highly significant difference of electrical conductivity change ($p < 0.05$) at 25°C, 40°C and 40°C at 75% RH. This increase in the electrical conductivity was due to the transport of electrolytes ($MgSO_4$ and $ZnSO_4$) from internal water droplets to the external water phase (Bonnet *et al.*, 2010).

Viscosity of ME-1 and ME-2 was decreased at 40°C and 40°C+75% RH and less decrease in viscosity was observed at 25°C. Using two-way ANOVA test at 5% significance level that the decrease in viscosity of different samples was very significant in both ME-1 and ME-2 at different levels of time while insignificant at different levels of temperature ($p < 0.05$) in ME-1 and highly significant at different time intervals and temperature levels in ME-2. The change in viscosity of freshly prepared sample of both formulations ME-1 and ME-2 was taken as the standard and comparing it with change in viscosity at different time and temperature levels, significant change ($p < 0.05$ in time was observed especially from 1st week to forth week in ME-1 and at 72 hours 4th week in ME-2. The independent T-test results of change in viscosity of both ME-1 and ME-2 were having insignificant difference ($p > 0.05$) at 25°C, 40°C and 40°C + 75% RH. Viscosity of the multiple emulsion decreased with aging and was attributed to the coalescence of internal water droplets with the external aqueous phase

(Lutz *et al.*, 2003; Vasiljevic *et al.*, 2006) and it also depends on the globule size. Smaller the globular size of the w/o/w multiple emulsion lower will be the viscosity.

CONCLUSION

The study concluded that $ZnSO_4$ is better stabilizer than $MgSO_4$. In future it can be the promising compound for not only as a stabilizer but also as source of Zinc for skin formulations and also for the products prepared from natural oils. Secondly refrigeration and high temperature is fatal for the stability of multiple emulsion so the shelf life can be improved by storing the multiple emulsion at room temperature.

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