FORMULATION AND *IN VITRO* EVALUATION OF A COSMETIC EMULSION FROM ALMOND OIL

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ABSTRACT

Several processes are used for the manufacture of multiple emulsions. The most widely used procedure is two step method. 1st step consists of preparing a primary emulsion. In the 2nd step, a definite amount of this primary emulsion is dispersed in an external phase containing the secondary emulsifier. Although it is a simple method, the 2nd step of the procedure includes many critical factors. In this work water-in-oil-in-water w/o/w multiple emulsion was prepared by using almond oil, Abil-EM 90, magnesium sulfate, Tween 80. The multiple emulsion was prepared by using two-step process. Stability studies of this multiple emulsion were carried out at different storage conditions i.e. 8^{o}C, 25^{o}C, 40^{o}C and 40^{o}C+ 75% relative humidity (RH) for 28 days. It was found that samples of multiple emulsion stored at 8^{o}C, 25^{o}C were stable through out the study period but samples stored at 40^{o}C and 40^{o}C+ 75% RH were found to show some changes in color, liquefaction and phase separation from third week. The changes in pH values and electrical conductivity of multiple emulsions stored at these storage conditions were also measured. Globules sizes of multiple emulsion were also measured at these storage conditions. Results indicate that there were significant changes in pH values and electrical conductivity of multiple emulsion stored at different conditions and non significant changes in globule sizes of multiple emulsion stored at these conditions were observed.

Keywords: Multiple emulsions, stability, almond oil, pH, electrical conductivity, rheology.

INTRODUCTION

Multiple emulsions are defined as emulsions in which both types of emulsions i.e., w/o and o/w exist simultaneously (Becher, 1965). They combine the properties of both w/o and o/w emulsions. These have been described as heterogeneous system of one immiscible liquid dispersed in another liquid in the form of fine droplets which have diameter greater than 1 micron (Becher, 1965). Multiple emulsions have very low thermal stability (Florence and Whitehill, 1982). Water-in-oil-in-water (W/O/W) multiple emulsions are vesicular systems composed of aqueous micro globules contained in oily globules which, in turn, are dispersed in an aqueous phase. The applications of these systems range from pharmaceutical drug delivery, to foods and cosmetic. The main advantages of (W/O/W) multiple emulsions are the protection of entrapped substances, their capacity to incorporate several activities in the different emulsion compartments, and their sustained release effects (Laetitia *et al*., 2003).

W/O/W multiple emulsions are thermodynamically unstable thus leading to various problems such as instability during storage, leaching of contents from inner phase, flocculation of inner aqueous and multiple oil droplets, phase separation all of which should be addressed before the multiple emulsion can practically be accepted (Raynal *et al*., 1993).

The unique property of w/o/w multiple emulsion compared to simple emulsions is the diffusions of water through the oil phase because of unbalanced osmotic pressure between the internal and external aqueous phases. Polar molecules dissolved either in the inner aqueous phase or external aqueous phase can pass through the oil layer by the process of simple diffusion. Water is transported from inner aqueous phase to the external aqueous phase by osmotic pressure. Water diffusion causes swelling, bursting or shrinkage of the internal aqueous droplets affecting the stability as well as the release profile of active ingredients loaded in inner aqueous phase (Silva- Cunha *et al*., 1997).

To prepare a stable w/o/w multiple emulsion, at least two emulsifiers are required. One of them is lipophilic emulsifier to prepare w/o primary emulsion and other is hydrophilic emulsifier to prepare w/o/w multiple emulsion. The lipophilic and hydrophilic emulsifiers are dissolved in oil and aqueous phases respectively (Vaziri and Warburton, 1995). Most commonly used method for the preparation of multiple emulsion is the two step process as used by various researchers for the preparation of w/o/w multiple emulsions (Akhtar and Yazan, 2005;

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Table 1: Organoleptic parameters and centrifugation tests for primary emulsion

Time	Liquefaction				Color				Phase separation				Centrifugation			
(days)	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
0d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	-	-
0.042d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	-	-
1d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	-	-
3d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	-	-
7d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	-	-
14d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	-	-
21d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	+	+
28d	-	-	-	-	CW	CW	CW	CW	-	-	-	-	-	-	+	+

-=No Change; + =Slight Change, A= 8°C; B= 25°C; C=40°C; D=40°C+75% Relative Humidity; CW= Creamy White

Table 2: Organoleptic parameters and centrifugation tests for multiple emulsion

Time	Liquefaction				Color				Phase separation				Centrifugation			
(days)	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
0d	-	-	-	-	W	W	W	W	-	-	-	-	-	-	-	-
0.042d	-	-	-	-	W	W	W	W	-	-	-	-	-	-	-	-
1d	-	-	-	-	W	W	W	W	-	-	-	-	-	-	-	-
3d	-	-	-	-	W	W	W	W	-	-	-	-	-	-	-	-
7d	-	-	-	-	W	W	W	W	-	-	-	-	-	-	-	-
14d	-	-	-	-	W	W	W	W	-	-	-	-	-	-	-	-
21d	-	-	+	+	W	W	YW	YW	-	-	-	-	+	+	+	+
28d	-	-	+	+	W	W	YW	YW	-	-	-	-	+	+	++	++

- =No Change; + =Slight Change; W= White; YW= Yellowish White; ++ = More Change; A= 8°C; B= 25°C; C=40°C; D=40°C+75% Relative Humidity

Florence and Whitehill, 1982; Nakhare and Vyas, 1996; Cournarie *et al*., 2004).

In this study Abil EM 90 (a silicone polymeric surfactant) was used as lipophilic emulsifier and Tween 80 (a sorbitan derivative) was used as hydrophilic emulsifier as used by Silva-Cunha *et al*. (1997) in their work.

The purposed of this work was to formulate the stable w/o/w multiple emulsion for cosmetic applications by using almond oil and its *in vitro* evaluations.

For in vitro evaluation, various parameters were studied including organoleptic parameters (color, liquefaction and phase separation), centrifugation, pH and electrical conductivity. Organoleptic parameters and Centrifugation tests were conducted both for primary and multiple emulsions.

MATERIALS AND METHODS

Materials and apparatus

Abil EM 90 was purchased from Franken chemicals (Gebindc), Tween 80 and magnesium sulfate were purchased from Merck (Germany), Double distilled water prepared by using distillation plant (Germany), digital pH meter WTW and digital Conductivity meter of WTW (Germany), Stability Chambers were of Sanyo (Japan). Water bath (China), Electrical balance (Precisa, Switzerland), Digital Humidity Meter (TES Electronic Corp,UK), Centrifuge machine (Hettich, Germany), Mechanical mixer (IKA, Germany), Refrigerator (Dalwance, Pakistan), Microscope (Nikon, Japan), Microscopic Camera (Germany), Microscopic software (MiniSee, Japan) and SPSS version 10.0 were used.

Methods

Preparation of multiple emulsion

Primary emulsion was prepared by heating oil phase consisting of swe*et al*mond oil and lipophilic surfactant (Abil EM 90) (Silva-Cunha *et al*., 1997) to 75°C ± 1°C. Aqueous phase consisting of water and magnesium sulfate was also heated to the same temperature. Aqueous phase was added to the oil phase drop by drop while stirring at 2000 rpm. Agitation was continued until cooling to room temperature of 25°C. For the multiple emulsion, aqueous phase consisted of water and hydrophilic surfactant (Tween 80) (Silva-Cunha *et al*., 1997). Primary emulsion was added little by little to the aqueous phase at 1000 rpm for 10 minutes. Emulsion was then homogenized at 800 rpm for 5 minutes and further at 500 rpm for 5 minutes more.

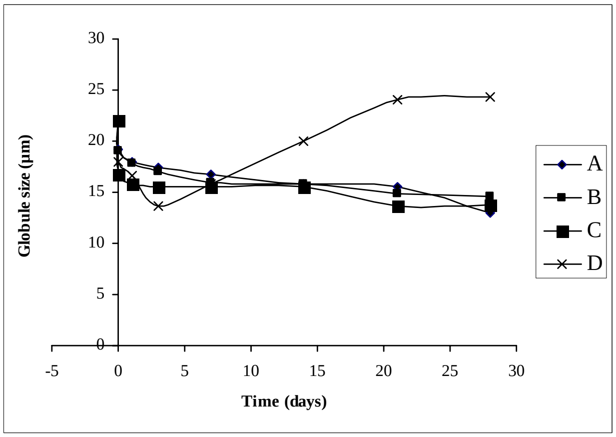

Fig. 1: Globule sizes of samples kept at conditions A=8°C, B=25°C, C=40°C and D=40°C+ 75% RH.

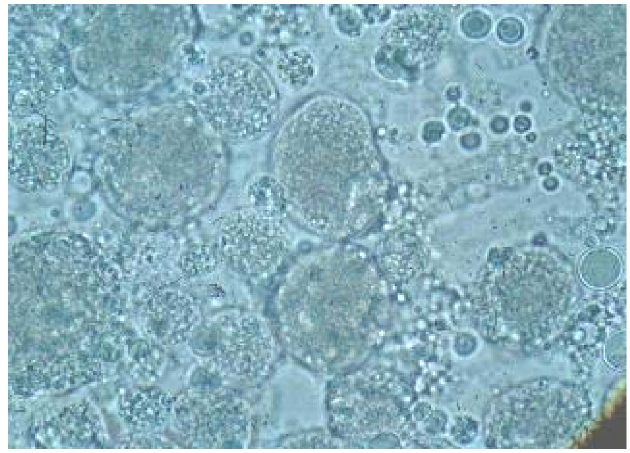

Fig. 2: Freshly prepared multiple emulsion.

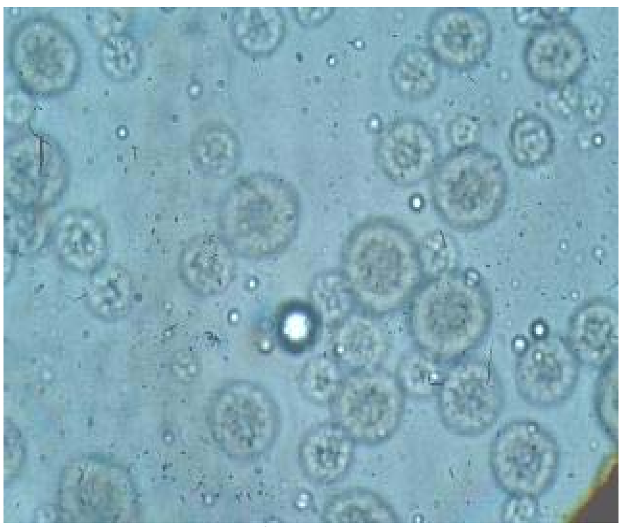

Fig. 3: Sample Kept at 8°C after 28 days.

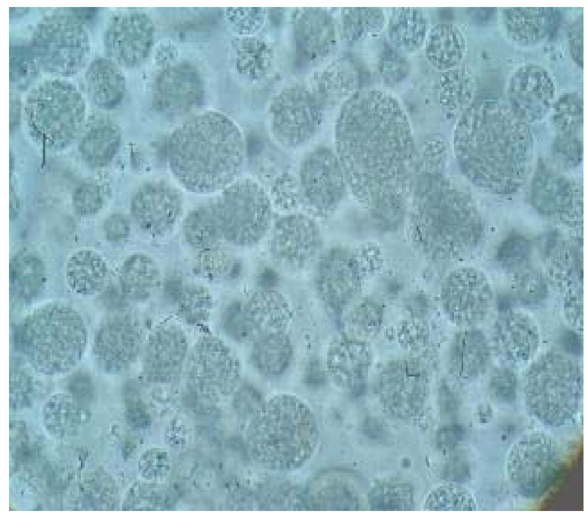

Fig. 4: Sample kept at 25°C after 28 days.

Formula of multiple emulsion

Primary Emulsion	
Sweet almond oil	22%
Abil-EM 90	5%
Magnesium sulfate	0.7%
Distilled water (q.s.)	100%
Multiple Emulsion	
P.E. (Primary Emulsion)	90%
Tween	801.5%
Distilled water (q.s.)	100%

Properties of primary and multiple emulsions

Both primary and multiple emulsions were analyzed organoleptically to assure the formulation of desired emulsions.

Types of emulsions

Types of emulsions were analyzed by diluting the emulsion with oil and water separately.

Stability tests

Stability tests were performed at different storage conditions for both primary and multiple emulsions to see the effect of these conditions on the storage of both primary and multiple emulsions. These tests were performed on samples kept at 8°C ± 0.1°C (in refrigerator), 25°C ± 0.1°C (in oven), 40°C ± 0.1°C (in oven) and 40°C ± 0.1°C (in oven) with 75% relative humidity (RH). Organoleptic characteristic of both primary and multiple emulsions, i.e. color, liquefaction and phase separation were noted at various intervals for 28 days.

Centrifuge tests

Centrifugal tests were performed for primary emulsions and for multiple emulsions immediately after preparation. The centrifugal tests were repeated for multiple emulsions after 24 hours, 3 days, 7 days, 14 days and 28 days of preparation. The centrifugal tests were performed at 25°C

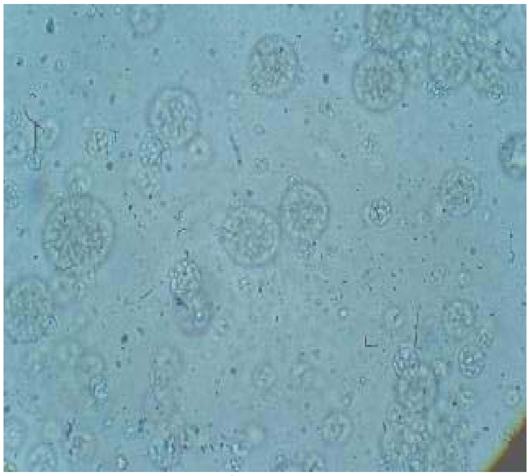

Fig. 5: Sample kept at 40°C after 28 days.

Fig. 6: Sample kept at 40°C+75% (RH) after 28 days.

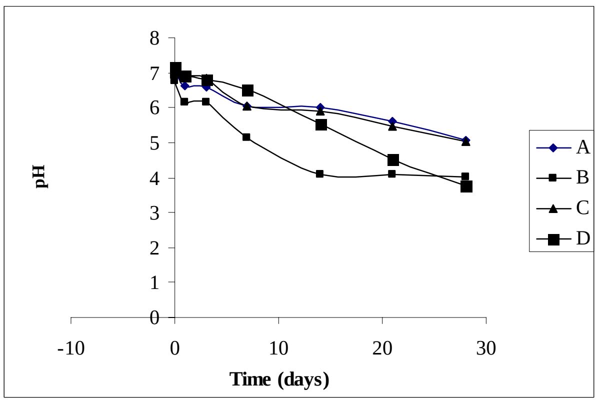

Fig. 7: pH values of samples kept at A= 8°C, B= 25°C, C= 40°C and D=40°C+ 75% RH.

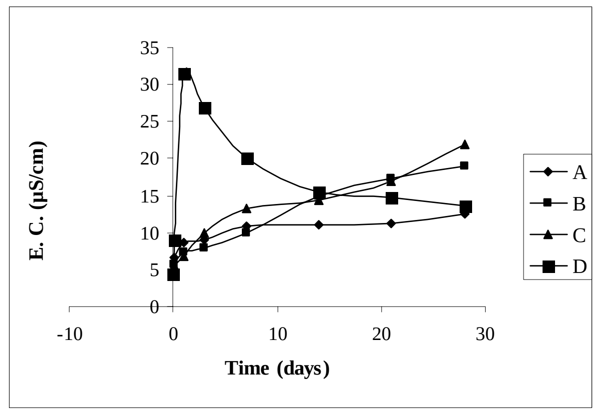

Fig. 8: Electrical conductivity (EC) values of samples kept at A=8°C, B=25°C, C=40°C and D=40°C+ 75% RH.

and at 5000 rpm by placing the 10g 0f sample in centrifugal tubes.

Microscopic Tests

Multiple emulsions were analyzed under microscope to confirm the multiple characters. A drop of multiple emulsion was placed on the glass slide and diluted with water and covered by glass cover. A drop of immersion oil was placed on the cover slide and observed under the microscope (Groves, 1984).

Globules size

Globule size of the multiple emulsions were determined fir the freshly prepared emulsions and for the emulsions kept at different conditions, i.e. 8°C ± 0.1⁰C, 25°C ± 0.1°C, 40°C ± 0.1°C and, 40°C ± 0.1°C with 75% relative humidity (RH) conditions. Analysis was performed after 1 hour to 28 days. Globule sizes were determined by an instrument utilizing light microscopy (Groves, 1984).

pH Determination

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

Electrical conductivity tests

Conductivity tests were performed for multiple emulsion immediately after preparation and for samples kept at different conditions by using digital conductivity meter. Conductivity tests were repeated for multiple emulsions after 24 hours, 3 days, 7 days, 14 days, 21 days and 28 days of preparation.

RESULTS AND DISCUSSION

Results

Stability of primary emulsion

Stability of primary emulsion kept at different storage conditions were studied and organoleptic parameters regarding stability (color, liquefaction, phase separation) and centrifugation tests are presented in the table 1.

Stability of multiple emulsion

Stability of multiple emulsion kept at different storage conditions were studied and organoleptic parameters regarding stability (color, liquefaction, phase separation) and centrifugation tests are presented in the table 2.

Globule sizes of multiple emulsion

Globule sizes of multiple emulsion kept at different storage conditions up to 28 days have been examined and presented graphically in the fig. 1 and photographs are given in the figs. 2-6.

pH tests for multiple emulsion

pH values of multiple emulsion kept at different storage conditions up to 4 weeks have been determined and presented graphically in the fig. 7.

Electrical conductivity tests for multiple emulsion

Electrical conductivity values of multiple emulsion kept at different storage conditions for 28 days have been determined and presented graphically in the fig. 8.

DISCUSSION

Stability

Multiple emulsions of w/o/w may deteriorate by several possible mechanisms (Florence and Whitehill, 1982) which include swelling of internal drops due to osmotic pressure leading to passage of water from external phase to internal phase. Other mechanisms include rupture of the oil layer or coalescence of the oil globules or the coalescence of the internal water droplets. Presence of electrolytes plays an important role in the stability of multiple emulsions by balancing the osmotic pressure between the internal and external aqueous phases or by forming the rigid interfacial layer between the oil and aqueous phase.

In this work, both primary and w/o/w multiple emulsions were divided in to four samples separately and these samples were kept at different storage conditions i.e. at 8^{o}C in refrigerator, at 25^{o}C, 40^{o}C and at 40^{o}C + 75% relative humidity (RH) in stability chambers. These samples at different storage conditions were observed for a period of 28 days at different intervals. Samples were observed with respect to change in color, liquefaction and phase separation.

The freshly prepared primary emulsion was creamy white in color. There was no change in color of primary emulsion at different storage condition i.e. 8^{o}C, 25^{o}C, 40^{o}C and at 40^{o}C + 75% relative humidity up to the observation period of 28 days. This shows that primary emulsion was stable at different storage conditions up to 28 days.

The freshly prepared multiple emulsion was white in color. There was little change in color of samples kept at 40^{o}C and at 40^{o}C + 75% relative humidity. The color of multiple emulsion in these samples became yellowish white. The change in color appeared from 21st day and was persisted up to 28th days of analyses period. The change in color at the end of observation periods is might be due to the oily phase separation which is promoted at higher temperature.

No liquefaction was observed in the primary emulsion at any storage condition i.e. at 8^{o}C, 25^{o}C, 40^{o}C and at 40^{o}C+ 75% RH up to 21st day. While in the case of multiple emulsion no liquefaction was observed in the samples kept at 8^{o}C and 25^{o}C during whole the observation period of 28 days but slight liquefaction was observed in samples kept at 40^{o}C and 40^{o}C + 75% RH from 21st of observation but there was no further increase in liquefaction till the end of the study period. Liquefaction is the sign of instability, might be due to the passage of water from the internal phase to external phase as described by many researchers (Kawashima *et al*., 1992), (Kita *et al*., 1978).

In the case of primary emulsion, no phase separation was observed at any of samples kept at 8^{o}C, 25^{o}C, 40^{o}C and 40^{o}C + 75% relative humidity up to observation period of 28 days. This indicates that primary emulsion is stable at all the storage conditions for 28 days.

In the case of multiple emulsion, no phase separation was seen in any of the samples kept at different storage conditions up to the period of 28 days. This indicates that multiple emulsion is stable at all the storage conditions i.e. 8^{o}C, 25^{o}C, 40^{o}C and 40^{o}C +75% relative humidity during whole the observation period of 28 days.

Centrifugation test

In this study centrifugation test was performed on different time intervals for both primary and multiple emulsions kept at different storage conditions up to a period of 28 days.

In the case of primary emulsion, no phase separation after centrifugation was seen in any of the samples kept at different storage conditions up to 14 days but slight phase separation on centrifugation was seen from 21st day up to 28th day of observation in the samples kept at 40^{o}C and 40^{o}C+ 75% relative humidity and no more phase separation was observed till the end of study period.

In the case of multiple emulsion, no phase separation after centrifugation was seen in any of the sample kept at different storage condition up to 14 days. Slight phase separation on centrifugation was seen in the samples kept at different storage condition after 21st day of observation and there was no further increase in phase separation in the samples kept at 8^{o}C and at 25^{o}C till the end of 28th day but in case of samples kept at 40°C and 40°C + 75% RH phase more phase separation on centrifugation was observed at 28th day.

Globule size

The increase or decrease in the globule sizes indicates the process of instability (Florence and Whitehill, 1982). The multiple droplets may coalesce with the other oil drops, or the internal aqueous droplets may be expelled out individually; or more than one drop may be expelled; or the internal globules may coalesce before being expelled out resulting in the shrinkage of internal droplets; or water may pass from the external phase to the internal aqueous phase resulting in the swelling of internal droplets and then complete rupture of droplets. The globule sizes can be determined by light microscope (Groves, 1984), (Omotosho *et al*., 1986) or by electron microscope (Davis *et al*., 1978), (Adeyeye and Price, 1991). Light scattering and diffraction methods are also used for the determination of globule sizes (Omotosho *et al*., 1986). Coulter counter is also a practical method for the determination of particle sizes (Bunville, 1984).

In this study, light microscope fitted with digital camera was used for the determination of globule sizes. The globule sizes of multiple emulsion samples stored at different storage conditions changed with time. The globule sizes of samples stored at 8°C, 25°C and 40°C were found to decrease with the passage of time. This is might be due to the shrinkage of globules which is might be due to the expulsion of internal aqueous droplets to external water phase as the values of electrical conductivity of samples at most of the conditions increased with the passage of time.

The globule size of sample stored at 40°C+75% relative humidity (RH) was initially decreased up to 72 hours and after 72 hours, there was again increase in globule size. This indicates that the mechanism of instability in the first 72 hours is the shrinkage of globules, might be due to the expulsion of internal aqueous droplets to external water phase. However after 72 hours, the mechanism of instability is might be coalescence of oil droplets as the globule size was increased.

The average globule size of the freshly prepared multiple emulsion was 22µm. The average globule size of sample kept at 8°C was decreased gradually from first 1 hour to last day. On 28th day the globule size was 13.0µm. Globule sizes of samples kept at 25°C and 40°C also decreased gradually and continuously from 1hour up to 28th day of observation. Globule sizes of samples kept at 25°C and 40°C were 14.60µm 13.80µm respectively on 28th day.

Average globule size of sample kept at 40°C+75% RH was initially decreased up to 72 hours after preparation which was 13.65µm then globule size was found to increase up to last day of study and on 28th day, globule size was 24.30µm.

By using two way analysis of variance (ANOVA) technique at 5% level of significance, it was found that results of change of globule sizes are insignificant at different levels of time and temperature. As no significant change in globule sizes of samples kept at different storage conditions was observed for a period of 28 days so there is no need to perform individual comparison test i.e. LSD.

pH

pH of skin ranges from 5 and 6, and 5.5 is considered to be average pH of the skin. Therefore, the formulations intended for application to skin should have pH closer to this range.

In this work pH of freshly prepared multiple emulsion was 7.15 which is near to the neutral pH. pH of samples of multiple emulsion kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C + 75% RH were found to decrease continuously up to the 28th day of observation. The pH of multiple emulsion sample kept at 8°C decreased continuously from the first day to the last day. On 28th day pH was 5.05. The pH of sample kept at 25°C also decreased continuously and on 28th day pH was found to be 4.02. pH of samples kept at 40°C and 40°C+ 75% RH also showed continuous decrease and on 28th day pH were 5.03 and 3.77 respectively. The decrease in pH is might be due to the production of highly acidic by-product from any of the acidic ingredients of the almond oil such as palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, linoleic acid, arachidic acid, eicosenic acid, behenic acid and erucic acid.

By using two way analysis of variance (ANOVA) technique at 5% level of significance, it was found that the change in pH of different samples is significant both at different levels of time and temperature.

By using LSD technique and taking the average pH of multiple emulsions at 0 hour as a standard and comparing it with average pH at other time levels it was found that most significant change in pH was observed from 7th day up to the total observation period of 28 days.

By taking the average pH of sample kept at 8°C as a standard and comparing it with pH of other temperature levels, it was found that most significant change in pH was observed in the sample kept at 25°C. So it can be concluded that most prominent change in pH was seen in sample kept at 25°C after 7th day.

Electrical conductivity

The increase in electrical conductivity values of multiple emulsions is due to the transfer of electrolytes entrapped in the inner aqueous phase of multiple emulsions from inner aqueous phase to external aqueous phase and decrease in electrical conductivity is due to the transfer of electrolytes which are lost in to external aqueous phase during the process of manufacturing towards internal aqueous phase.

In this work, electrical conductivity of freshly prepared multiple emulsion was 4.5µS/cm. Electrical conductivity of samples kept at 8°C, 25°C and 40°C was found to increase with the passage of time but the electrical conductivity of sample kept at 40°C+ 75% RH was found to increase initially up to 24 hours and then found to decrease gradually for remaining observation period of 28 days. The initial increase in electrical conductivity is might be due to transfer of $MgSO_4$ from inner aqueous phase to external aqueous phase and after 24 hours the decrease in electrical conductivity is might be due to the transfer of $MgSO_4$ back into inner aqueous phase from external aqueous phase.

The electrical conductivity of multiple emulsion sample kept at 8°C increased continuously from first hour to the last day. On 28th day electrical conductivity was 12.5µS/cm. Electrical conductivity of samples kept at 25°C and 40°C also increased continuously and on 28th day were 19µS/cm and 22µS/cm respectively. Electrical conductivity of sample kept at 40°C+ 75% RH initially increased suddenly up to 24 hours and this value was 31.5µS/cm then this value decreased continuously up to 28th day and it was 13.6µS/cm.

By using two way analysis of variance (ANOVA) technique at 5% of level of significance, it was found that change in electrical conductivity is significant at both different levels of time and temperature.

By using LSD technique and taking the average value of electrical conductivity of 0 hour as standard and comparing it with the electrical conductivity of samples at other time levels it was found that, significant change in electrical conductivity was observed from 24th hour observation to 7th day of observation and highly significant change in electrical conductivity was observed from 14th day of observation to 28th day of observation.

By taking the average electrical conductivity of sample kept at 8°C and comparing the electrical conductivity of samples kept at other storage conditions, it was found that most significant change in electrical conductivity was observed in sample kept at 40°C+ 75% RH. So it can be concluded that the most prominent change in conductivity was started on 14th day in the sample kept at 40°C + 75% RH after preparation of multiple emulsion.

CONCLUSIONS

It can be concluded that:

- There was no phase separation in primary emulsion at different storage conditions for a period of 28 days.
- There was no phase separation in multiple emulsion at different storage conditions for a period of 28 days.
- Liquefaction of multiple emulsion in samples started at increased temperatures after 21st day of storage.
- Phase separation on centrifugation was seen in both primary and multiple emulsions started at higher temperature conditions after 21st day of storage.
- Globule sizes of multiple emulsion at most of the storage conditions decreased with the passage of time.
- pH values of multiple emulsion at all the storage conditions decreased with the passage of time.
- Electrical conductivity values of multiple emulsion at all the storage conditions increased with the passage of time.
- Stability of multiple emulsion formulated in this study can be improved by using better emulsifying agents.

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