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RESEARCH ARTICLE

Formulation and Characterization of Multiple Emulsion System Containing Olive Oil and two Antiaging Agents

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ABSTRACT

The intention of the study was to formulate a stable multiple emulsion system containing two skin antiaging agents using a natural oil vitamin C, known to be a very unstable ingredient and is decomposed in the presence of oxygen, was entrapped in the internal aqueous phase of w/o/w multiple emulsions. In this way, vitamin C is expected to exhibit slow release and the effect of vitamin C can be improved as it has been protected from the external environment. The other ingredient, which is a product of wheat protein was also used as an antiaging agent. Both of the ingredients increase the synthesis of collagen fibers in the dermis. Therefore, a synergistic effect can be produced by using the two ingredients in one formulation. In this study, multiple emulsions were prepared by the two-step emulsification method. Basic formulation containing no active material and a formulation containing vitamin C in the internal aqueous phase and wheat protein in the oily phase were prepared. The oil selected was Olive oil since it consists mainly of oleic acid (up to 83%), with smaller amounts of other fatty acids including linoleic acid (up to 21%) and palmitic acid (up to 20%) which is the natural ingredient of the young skin. Basic formulation as well as the formulation containing active ingredients were stored at different accelerated conditions for six months and characterized. Globule size, pH, viscosity and physical changes were tested to characterize the emulsion systems. Basic emulsion was found to be more stable at all the different conditions than the formulation containing the active ingredients.

Keywords: Multiple emulsion, Vitamin C, Wheat protein, Physical properties

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CONTENTS

1. Introduction

Multiple emulsions are defined as emulsions in which both types of emulsions exist simultaneously [1]. They combine the properties of both w/o and o/w emulsions. In addition, they have the potential advantage of prolonged release of

drug, incorporation of incompatible materials and protection of active substances when they are dispersed in the internal phase [2, 3]. The preparation of multiple emulsions with natural oils is a challenging work due to the stability problems. In this study, olive oil which is natural has been used. This oil was preferred because of its cosmetic benefits to the skin. It contains proteins, fats, carbohydrates, vitamins and minerals [4, 5]. Two active agents were incorporated into two different phases of the multiple emulsion.

The role of vitamin C as an antioxidant agent and in the protection of skin against the deleterious effects of UVB light has been recognized by many workers [6, 7, 8]. In addition to this, vitamin C improves the synthesis of collagens increasing the suppleness of the skin [9]. This vitamin has been incorporated into the internal phase of the multiple emulsions prepared, in this study.

Proteins from wheat are being used in cosmetic products as antiaging agents [10]. One such protein condensed with palmitic acid is also reported to be antiaging [11]. This protein is claimed to increase the synthesis of collagen at low concentrations and to act as moisturizer at high concentrations. This material was used in our study in the oily phase of multiple emulsion.

After the preparation of the multiple emulsions, characterization and accelerated stability studies were performed for six months by keeping the samples of the formulations, with and without the active agents at different conditions. During this period, globule size, changes in pH, changes in viscosity and any physical change in the multiple emulsions were periodically investigated.

2. Materials and Methods

Aqueous phase was distilled water prepared in the laboratory. It contained L (+) ascorbic acid (E. Merck, Germany) and magnesium sulphate (E Merck, Germany). Two types of surfactants were used. W/o surfactant was Abil EM 90 (polysiloxane polyalkyl polyether copolymer) (Goldschmidt, Germany) and o/w surfactant was Synperonic PE/F 127 (ethylene oxide/propylene oxide block copolymers) (Uniqema, Belgium). Olive oil (Alban Müller International, France) was used as the oily phase which contained Lipacide PVB (wheat proteins). pH value of multiple emulsions was adjusted by triethanolamine (Carlo Erba, Italy).

2. 1 Emulsion Systems

Multiple emulsions were prepared by the two-step emulsification process [12, 13].

2.1.1Preparation of the Basic Formulation

Primary emulsion (PE) was prepared by heating the oily phase, consisting of Olive oil and lipophilic surfactant (Abil EM 90), to 75°C □ 1°C. Aqueous phase consisting of water and magnesium sulphate was also heated to the same temperature in the water bath (GFL 1052). Aqueous phase was added to the oily phase drop by drop while stirring at 2000 rpm (Heidolph RZR 2101). Agitation was continued until cooling to room temperature of 25°C.

External aqueous phase consisted of water and the hydrophilic surfactant (Synperonic PE/F 127). PE was added slowly to the aqueous phase at 1000 rpm for 10 minutes. Emulsion was then homogenized at 700 rpm for 7 minutes. The stirring rates and times were determined following preformulation studies.

2.1.2Preparation of the Active Formulation

Oily phase which consisted of Olive oil and surfactant (Abil EM 90) was heated up to 75°C□1°C. Lipacide® was added when the temperature was acquired. At the same time, aqueous phase consisting of water and magnesium sulphate was heated to the same temperature. Vitamin C was added finally to the aqueous phase. Aqueous phase was added to the oil phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for about 25 minutes, until the emulsion cooled to room temperature. In the next step, multiple emulsion was prepared. Aqueous phase consisted of Synperonic®, the hydrophilic emulsifier, and water. Triethanolamine was added to adjust the pH of the emulsion. PE was added slowly to the aqueous phase at 700 rpm in 20 minutes. After the complete addition of the PE, the speed of the mixer was reduced to 400 rpm for homogenization, for a period of 35 minutes.

Basic Formulation

Primary Emulsion (PE)			**%**
	Olive oil		26
	Abil EM 90®		4
Magnesium sulphate			0.7
	Distilled water	q.s.	100
Multiple Emulsion			
	PE		80
	Synperonic®		0.8
	Distilled water	q.s.	100

Active Formulation

Primary Emulsion			
	Olive oil		26
	Abil EM 90®		6
	Lipacide PVB®		0.5
Magnesium sulphate			0.7
	Ascorbic acid		1.0
	Distilled water	q.s.	100
Multiple Emulsion			
PE			75
Synperonic PE/F 127			2.0
Triethanolamine			0.7
Distilled water		q.s.	100

2.2 Evaluation of the Emulsions

Primary and multiple emulsions were analyzed to assure the formation and the stability of the emulsion systems.

2.2.1 Physical analysis

Primary and multiple emulsions were analyzed organoleptically (color, thickness, look, feel) and physically (creaming and phase separation).

2.2.2 Types of emulsions

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

2.2.3 Microscopic test

Multiple emulsions were analyzed under microscope (Olympus Bx50), with 100X magnification, using immersion oil [14].

2.2.4 Centrifugation test

Centrifugal tests were performed for the primary emulsions and for the multiple emulsions immediately after preparation. Centrifugal tests were repeated for multiple emulsions after 24 hours of preparation. These tests were performed at 25°C and at 5000 rpm for 5 minutes by placing 10 g of samples in the centrifugal tubes [13].

2.2.5 Globule size

Globule sizes of the multiple emulsions were determined (Malvern, Master sizer 2000) for the freshly prepared emulsions and for the emulsions kept at different conditions, *i.e.,* 4°C±0.1°C, 25°C±0.1°C, 40°C±0.1°C and 40°C±0.1°C with 60 % relative humidity (RH). Analyses were performed every 15 days up to 6 months. Globule sizes were determined by an apparatus, utilizing laser light diffraction phenomenon [15].

2.2.6 pH determination

pH values of the freshly prepared emulsions and the emulsions kept at different conditions were determined by a digital pH-Meter (Lab India).

2.2.7 Rheological tests

Rheological tests were performed for freshly prepared multiple emulsions and multiple emulsions stored at different conditions. For this purpose, programmable cone-plate (Brookfield LV III) rheometer was used. The cone used had an angle of 1.565^0, radius of 1.2 cm and a shear rate of 3.84XN (N=rpm). Approximately 0.2 g samples and a constant temperature of 25°C were used for the tests. Tests were repeated ten times, each containing 9 values of shear rate. Following the determination of the flow type, flow curves were fit to one of the available mathematical models.

2.2.8 Stability Tests

Stability tests were performed on samples kept at 4°C□0.1°C (in refrigerator, Samsung), 25°C□0.1°C (RH apparatus, Shimadzu), 40°C□0.1°C (RH apparatus, RH apparatus, Shimadzu) and at 40°C□0.1°C with 60% RH (in RH apparatus, RH apparatus, Shimadzu). Physical characteristics of multiple emulsions, *i.e.,* the characteristics mentioned above were all tested.

3. Results and Discussion

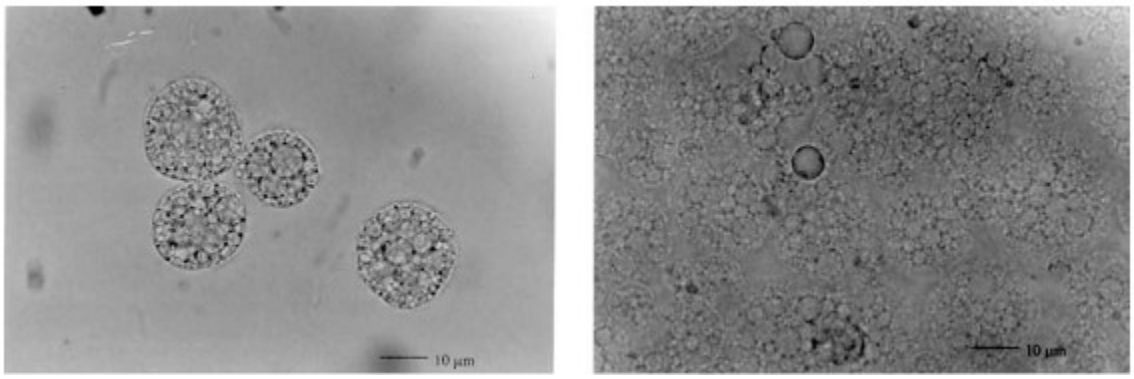
The results of the physical characterization of the emulsion systems prepared are summarized in Table 1.

Table 1. Physical characteristics of freshly prepared basic (B) and active (F) formulations and those kept at different conditions

		Fresh		15 Days		1 Month		45 Days		2 Months		3 Months		6 Months	
		B	F	B	F	B	F	B	F	B	F	B	F	B	F
Color	A	W	W	W	W	W	W	W	W	W	YW	W	YW	W	YW
	B	W	W	W	W	W	W	W	YW	W	YW	W	YW	W	YW
	C	W	W	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW
	D	W	W	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW
Liquifaction	A	No	No	No	No	No	No	No	+	No	+	No	++	No	++
	B	No	No	No	No	No	No	No	+	No	+	No	+	No	+
	C	No	No	No	+	No	+	No	+	No	++	No	++	No	++
	D	No	No	No	+	No	+	No	+	No	++	No	++	No	++
Phase Separatio	A	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	B	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	C	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	D	No	No	No	No	No	No	No	No	No	No	No	No	No	No

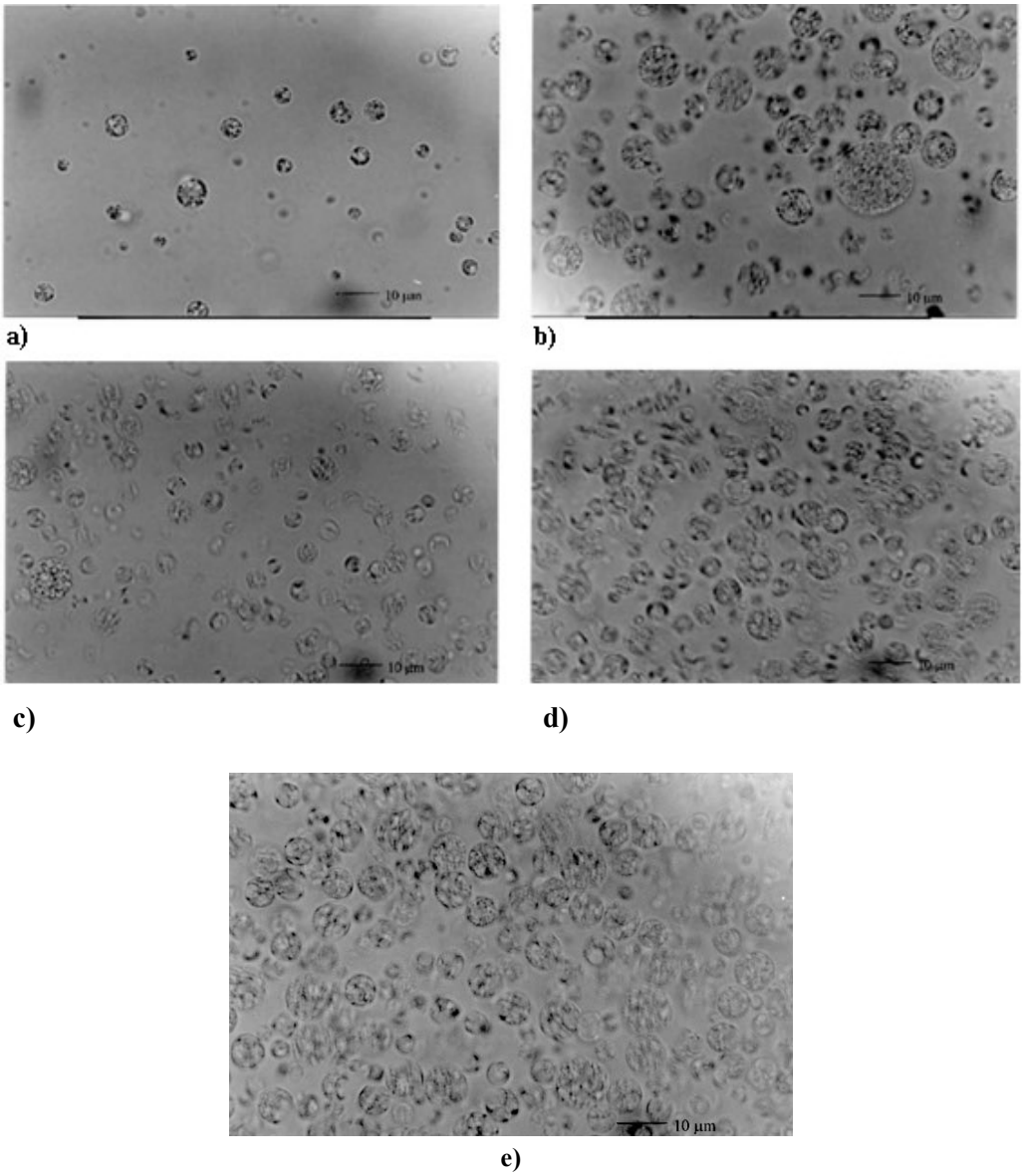
W= White; YW= Yellowish White; + = Little; ++ = More A = 4°C; B= 25°C;
C= 40°C; D= 40°C and 60% RH

Upon microscopic analyses, multiple emulsion systems were photographed (100X) randomly and some of those photographs are given in Figure 1 and Figure 2.



a) **b)**

Figure 1. Basic formulation, (a) freshly prepared; (b) formulation kept at 25°C for 3 Months



a) **b)**

c) **d)**

e)

Figure 2. Active formulation, (a) freshly prepared; (b) formulation kept at 4°C for 3 months; (c) formulation kept at 25°C for 3 months; (d) formulation kept at 40°C for 3 months; (e) formulation kept at 40°C and 60 % RH for 3 months

The average globule size of the freshly prepared basic formulation was 12.29 µm which is in the range of average globule size of multiple emulsions (Table 2) [16]. This size increased with time upon storage at different conditions; the most increase was seen in the samples kept at 4°C (18.92 µm). For the active formulation, the average globule size of 5.43 µm has decreased to around 4.40µm in two-months period. After two months, there was an increase in the average globule size being 7.37 µm at 4°C, at the end of 6 months (Table 2).

Table 2. Globule sizes (µm) ± standard errors of basic (B) and active (F) formulations kept at different conditions (n=3)

Globule Sizes (µm) ± Standard Errors														
Conditions	**Fresh**		**15 Days**		**1 Month**		**45 Days**		**2 Months**		**3 Months**		**6 Months**	
	B	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**
	12.29 ± 0.46	5.43 ± 0.04	-	-	-	-	-	-	-	-	-	-	-	-
A	-	-	-	-	-	-	-	-	15.70 ± 0.41	4.65 ± 0.05	14.98 ± 0.23	6.69 ± 0.25	18.92 ± 0.73	7.37 ± 0.16
B	-	-	-	-	-	-	-	-	14.38 ± 0.28	4.51 ± 0.06	14.18 ± 0.55	5.03 ± 0.02	14.76 ± 0.48	5.10 ± 0.05
C	-	-	-	-	-	-	-	-	13.51 ± 0.42	4.16 ± 0.13	16.16 ± 0.22	6.22 ± 0.03	13.70 ± 0.34	6.34 ± 0.24
D	-	-	12.59 ± 0.19	4.42 ± 0.10	11.74 ± 0.39	4.41 ± 0.14	15.16 ± 0.29	4.19 ± 0.10	13.80 ± 0.33	4.21 ± 0.03	14.52 ± 0.56	6.53 ± 0.08	14.59 ± 0.56	6.53 ± 0.18

A = 4°C; B = 25°C; C = 40°C; D = 40°C and 60 % RH

pH values of the freshly prepared basic formulation was 6.64 which is close to the pH range of the skin. pH values of the basic formulations kept at different conditions have decreased with time (Table 3). pH of the freshly prepared active formulation was found to be 2.66. Therefore, it was adjusted to 6.27 using triethanolamine. pH values of the active formulations stored at different conditions also decreased with time. The most prominent decrease in pH was seen in the sample kept at 4°C.

Table 3. pH values ± standard errors of basic (B) and active (F) formulations kept at different conditions (n=3)

pH Values ± Standard Errors														
	Fresh		**15 Days**		**1 Month**		**45 Days**		**2 Months**		**3 Months**		**6 Months**	
	B	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**
	6.64 ± 0.00	6.27 ± 0.00	-	-	-	-	-	-	-	-	-	-	-	-
4°C	-	-	-	-	-	-	-	-	5.69 ± 0.01	4.81 ± 0.00	5.54 ± 0.03	4.28 ± 0.00	4.64 ± 0.02	4.36 ± 0.01
25°C	-	-	-	-	-	-	-	-	5.67 ±	5.21 ±	5.45 ±	4.78 ±	5.49 ±	4.77 ±

									0.05	0.10	0.02	0.00	0.01	0.01
40°C	-	-	-	-	-	-	-	-	4.02 ± 0.18	5.13 ± 0.00	3.44 ± 0.04	4.60 ± 0.0	3.31 ± 0.04	4.59 ± 0.01
40°C and 60 % RH	-	-	6.46 ± 0.02	5.46 ± 0.03	6.37 ± 0.05	5.30 ± 0.01	6.14 ± 0.00	5.05 ± 0.00	6.17 ± 0.00	4.89 ± 0.01	4.76 ± 0.00	4.53 ± 0.00	3.79 ± 0.02	4.53 ± 0.01

In this study, all the rheological measurements were taken at 25°C. Increasing shear stresses were applied to the samples and the changes in viscosities were noted (Table 4). Rheograms of shear stress versus shear rate were obtained.

Table 4. Viscosities (mPas.s) ± standard errors of basic (B) and active (F) formulations kept at different conditions (n= 10)

Viscosities (mPas.s) ± Standard Errors			
		B	**F**
Fresh		16 065.47 ± 1 758.64	7 476.62 ± 486.62
4°C	3 months	11 341.22 ± 1 626.47	2 900.90 ± 37.66
	6 months	12 917.07 ± 1 750.44	2 769.35 ± 449.30
25° C	3 months	9 589.66 ± 915.17	7 080.40 ± 1 044.80
	6 months	15 808.24 ± 1 462.59	12 725.18 ± 1 874.29
40° C	3 months	13 461.99 ± 1 192.09	6 099.23 ± 776.58
	6 months	14 206.88 ± 1 970.48	30 249.63 ± 3 712.16
40°C and 60 % RH	3 months	12 225.83 ± 1 144.51	6 451.03 ± 972.07
	6 months	18 649.33 ± 2 531.94	18 542.75 ± 2 334.69

Shear thinning, thixotropy and flow indices were calculated for each rheogram (Table 5). Then, different mathematical models were applied to the rheograms. 'Power Law' was found to fit all the rheograms of the basic formulation and the confidences of fit were found to be in the range of 94.9 % and 99.2 % (Table 5). The same tests were performed on the active formulation. 'Power Law' was also found to fit all the rheograms. Different parameters of viscosity, *i.e* shear thinning, thixotropy and flow indices were calculated and are shown in Table 5. Confidences of fit were found to be 96.2 % - 99.0 %.

Table 5. Different rheological parameters of basic (B) and active (F) formulations

			ST	**TI**	**FI**
Fresh		**B**	1.69 ± 0.03	2.57 ± 0.07	0.58 ± 0.01
		F	1.35 ± 0.02	1.81± 0.05	0.74 ± 0.01
4°C	**B**	**3 m**	2.07 ± 0.06	3.28 ± 0.11	0.48 ± 0.01
		6 m	1.99 ± 0.06	3.13 ± 0.11	0.51 ± 0.01
	F	**3 m**	1.95 ± 0.09	3.07 ± 0.18	0.50 ± 0.03
		6 m	2.54 ± 0.03	4.04 ± 0.04	0.39 ± 0.0
	B	**3**	1.69 ±	2.57 ±	0.58 ± 0.01

		m	0.03	0.07	
		6 m	1.56 ± 0.04	2.29 ± 0.09	0.63 ± 0.02
	F	**3 m**	2.03 ± 0.11	3.56 ± 0.18	0.45 ± 0.03
		6 m	2.17 ± 0.04	3.46 ± 0.08	0.44 ± 0.02
40°C	**B**	**3 m**	1.51 ± 0.03	2.18 ± 0.06	0.64 ± 0.01
		6 m	2.13 ± 0.08	3.39 ± 0.14	0.47 ± 0.01
	F	**3 m**	2.02 ± 0.19	3.16 ± 0.33	0.51 ± 0.05
		6 m	1.14 ± 0.01	1.32 ± 0.01	0.57 ± 0.02
40°C and 60 % RH	**B**	**3 m**	1.57 ± 0.03	2.30 ± 0.07	0.63 ± 0.01
		6 m	2.06 ± 0.07	3.28 ± 0.14	0.47 ± 0.01
	F	**3 m**	2.35 ± 0.25	3.70 ± 0.38	0.44 ± 0.04
		6 m	1.82 ± 0.59	2.69 ± 1.16	0.48 ± 0.06

3 m: 3 months; 6 m: 6 months; **ST:** Shear Thinning Index; **TI:** Thixotropy Index; **FI:** Flow Index n= 10

When the globules are aggregated, the reaction of the aggregates to shear can result in a shear thinning flow. The 'shear thinning index' is a convenient way to characterize non-Newtonian flow. 'Shear thinning index' means that the viscosity decreases with increasing shear rates (17). At low shear rates, the aggregates may be deformed but remain intact. As the shear rate is increased, the aggregates may be broken down to individual globules, decreasing friction and therefore viscosity. 'Shear thinning index' increased to some extent at all conditions at the end of 6 months. A change in the fluid's viscosity over time indicates time-dependent behavior; a decrease signifies thixotropy. If a material's flocculated structure is destroyed with time as it is sheared, a time-dependent type of flow behavior will be observed. If the shear rate is decreased after destruction of some or all of the flocculated structure, the material's viscosity may be lower than it previously was at the same shear rate. Since flocs begin to link together after destruction, the rate at which this occurs affects the time required for viscosity to attain previous levels. If the relinking rate is low, viscosity will be lower. This procedure is commonly known as the 'thixotropy index'. The name is misleading since this ratio does not quantify thixotropy. For pseudoplastic (shear thinning) materials, the ratio will exceed 1.0 as the degree of pseudoplastic behavior increases (18). 'Thixotropy index' of the two emulsion systems were all above 1.0. 'Thixotropy index' for the basic and active formulations increased at the end of 6 months indicating the increase in pseudoplastic behavior.

3.1 Discussion

Two-step procedure has been used in this study with critical stirring rates and temperatures. The lipophilic surfactant has been used in the preparation of the PE with an amount five times as much as the secondary hydrophilic surfactant. Both of the surfactants were selected to be of nonionic character because of cutaneous concerns. The multiple emulsions prepared had a homogeneous appearance with good consistency. This is an advantage of multiple emulsion system where there is no need of incorporation into another system for application. Characterization of the multiple emulsions prepared in this study included the determination of physical appearance, type of emulsion, globule size and pH and centrifugation and rheological tests. The same measurements and tests were applied to the samples kept at different conditions. The external phases of the emulsion systems prepared were determined to be aqueous. Microscopic analysis has shown the presence of multiple characters for both of the emulsion systems prepared. This multiple character lasted for 6 months even under accelerated conditions. Stability of the systems is of great concern for the emulsions. The products intended for cosmetic use have to be designed to be with good consistency, tolerable cutaneously and stable until consumption by the consumers. Stabilities of the emulsions systems depend on the preparation method, apparatus used oils and surfactants selected and their concentration, the sizes of the globules obtained and the temperature use, besides other conditions. By centrifugation of the emulsions prepared, the effect of gravity has been accelerated. When the freshly prepared

and the 24-hour old emulsions were centrifuged, no phase separation could be detected. While centrifugation gives a definite idea about the stability of the simple emulsion, it can only be used in the prediction of the stability of the multiple systems. Regarding the basic formulation, there was no change in the color of the emulsion up to six months in any of the samples. The color was white throughout the period of analysis, *i.e.,* six months. This shows that the basic formulation is stable at all conditions investigated (Table 1).

The white color of the formulation containing the active ingredients, which were kept at 40°C and 40°C and 60% RH, turned to yellowish white in 15 days. The same color change was observed in the samples kept in room temperature in 45 days. There was little change in the color of the sample kept at 4°C which appeared after two months (Table 1). This change in color may be due to the presence of vitamin C in the external phase due to the immigration of vitamin C from the internal phase to the external phase [19]. Liquifactions noted in Table 1 are relative to the freshly prepared formulations and are not significant when compared to rheological measurements. Therefore, these observed results may give only a hint to an instability in the emulsion systems. Examining visually the samples of the basic formulation stored at different conditions, it may be concluded that there was no liquifaction seen in any of the samples (Table 1). The samples' consistencies remained the same up to six months. The liquifaction properties of the formulation containing the active ingredients which were kept at different conditions showed results parallel to the color change (Table 1). The traces of liquifaction which is a sign of instability may be due to the passage of water from the internal phase to the external aqueous phase as has been described by many workers [20, 21].

No complete phase separation was seen in the samples of the basic and the active formulations kept at different conditions up to a period of six months. However, there was a tendency of phase separation in the samples of active formulation at 4°C after a period of three months. This tendency of phase separation was determined by the visible coalescence of globules. The average globule size of basic formulation stored at different conditions increased with time which indicated coalescence of globules as defined in the literature; this ultimately shows the instability of multiple emulsions [22, 16]. For the active formulation, decrease in globule size with time up to two months followed by an increase may indicate that the mechanism of instability in the first two months is the shrinkage of globules, possibly due to the expulsion of internal aqueous droplets to the external water phase. However, later on after two months, the mechanism of instability may be the coalescence of oil droplets. pH values of the facial skin range between 5 and 6, and 5.5 is considered to be the average pH of the skin. Therefore, formulations applied to the face are planned to have a pH value in the range of 5-6. pH values of multiple emulsions were adjusted by triethanolamine to bring them to higher values and citric acid was added to bring the pH value towards the acidic values [19]. pH value of the formulation plays an important role for many enzymes and proteins to work efficiently. If the pH value of the formulation is not in the optimum range, proteins in the formulations cannot produce the required activity [23]. The decrease in pH may be due to the diffusion of water from internal phase to the external phase since the pH of distilled water ranges from 5-7. pH of the distilled water used in the preparation of emulsions was 4.65. The other theory which can be suggested for the decrease in the pH values of the emulsions is the production of highly acidic by-product from any of the ingredients in the oily phase since the pH of basic formulation which contained no active material also decreased with time. Rheological analysis is a leading test for defining the aging phenomena of cosmetic emulsions. Shape of the particles making up the dispersed phase is of significance in determining a system's rheology. Particles suspended in a flowing medium are constantly being rotated. If the particles are essentially spherical, rotation can occur freely. Microscopic evaluation of the two systems has shown that the globules are mostly spherical which means the globules in the emulsions flow freely. Viscosities of the active formulation were found to be lower than the basic formulation, except for the samples kept at 40°C for 6 months. All viscosity values decreased with the increase in shear stress. Viscosities were also found to decrease at the end of 3 months. However, viscosity values at the end of 6 months increased at all conditions except for the samples at 4°C. This may be due to the rotation of the globules with time.

The flow of the two emulsion systems prepared was found to fit 'Power Law':

$\tau = kD^n$

Where, τ = shear stress

D = shear rate

k = consistency index

n = flow index

"Power law" means that preparations display one type of non-Newtonian flow rather than shifting from one type to another as shear rate is varied. In a 'Power Law model', there are two parameters: 1) *k*, the viscosity at a reference shear rate (often 1/sec) and, 2) *n*, the power law index, flow index, that is the slope of the viscosity curve with respect to shear rate [18]. Flow index is tan θ (θ= angle formed by plot line with Y-axis of graph). If θ is less than 45 degrees, the fluid is pseudoplastic which is valid for the two emulsion systems prepared. Flow index of the active formulation was most affected for the sample at 4°C after 6 months.

4. Conclusion

It can be concluded that the multiple emulsion prepared using olive oil containing no active ingredient is a very stable multiple emulsion system. The active ingredients in the multiple emulsion formulation have led to some instability characteristics which need to be further modified.

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