

## Research Article

**DEVELOPMENT OF STABLE O/W EMULSIONS OF THREE DIFFERENT OILS**Mostafa Shahin<sup>1,5</sup>; Seham Abdel Hady<sup>2</sup>; Mohammed Hammad<sup>3</sup>; Nahed Mortada<sup>4</sup>**Address for Correspondence**<sup>1</sup> Department of Drug Technology, Faculty of Pharmacy and Pharmaceutical Sciences, Ain Shams University, Cairo, Egypt.<sup>2</sup> Department of Pharmaceutics, Faculty of Pharmacy, King Abdul Aziz University, Geddah, Kingdom of Saudi Arabia.<sup>3</sup> Department of Pharmaceutics, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt<sup>4</sup> Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Ain Shams University, Cairo, Egypt.<sup>5</sup> Corresponding author current address: Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8, Canada.

E-mail address: mostafashahin@hotmail.com

**ABSTRACT**

This study describes the formulation of different stable plain o/w emulsions containing several oils (jojoba oil, liquid paraffin and isopropyl myristate) with variable oil contents (20%, 30% and 40% w/w) together with several surfactant blends (Span 60, Span 83, Span 80, Myrj 53, Brij 35 and Tween 80). In the first place, the required hydrophilic lipophilic balance (RHLB) for jojoba oil was determined based on three different methods including the assessment of the degree of creaming after centrifugation and after shelf storage for 28 days at room temperature and the turbidimetric method. While the RHLB of liquid paraffin and isopropyl myristate were taken from the literatures. As such RHLB for jojoba was found to be 12.50.

On the other hand, the proper non ionic surfactant type was selected by the use of two methods, namely: the degree of creaming after 28 days shelf storage at room temperature and the turbidimetric method. Results revealed that a blend of span 60 and brij 35 gave the most stable emulsion for all oils used. Finally, the most suitable emulsifier concentration for each oil type and level was determined using the turbidimetric method. Nine formulae were obtained that could be used for variety of purposes.

**KEY WORDS** O/W emulsion, jojoba oil, isopropyl myristate, heavy liquid paraffin, emulsion physical stability, turbidimetric method.

**INTRODUCTION**

Emulsions are the basis of a wide variety of natural and manufactured materials, including foods, pharmaceuticals, biological fluids, agrochemicals, petrochemicals, cosmetics and explosives [1-3]. Stable emulsions represent an effective approach for the resolution of problems in drug and cosmetic agents' delivery. The emulsion stability have been studied extensively by many research groups and various methods of determining the emulsion stability have been proposed such as droplet size analyses [4], measuring physical properties of emulsion [5], accelerated tests [6], and light-scattering [7]. Among these methods, the most common are assessment of physical properties after centrifugation and shelf storage, which are time-consuming procedures. Turbidity measurements were also used to determine emulsion stability and they provide a faster approach to evaluate emulsion stability. In this work, we aimed at preparing stable o/w emulsions using different oily phases, namely: liquid paraffin, jojoba oil, and isopropyl myristate. At the same time we evaluated the effect of many formulation variables like, the effect of emulsifier type and emulsifier concentration as well as oily phase content on emulsion stability.

**MATERIALS AND METHODS**

Jojoba oil was purchased from Egyptian Natural Oil Company (Cairo, Egypt). Isopropyl myristate, sorbitan sequeioleate (Span 83), polyoxyethylene23 lauryl ether (Brij 35), polyoxyethylene 50 monostearate (Myrj53), polyoxyethylene 20 sorbitan monooleate (Tween80), sorbitan monostearate (Span60) and sorbitan monooleate (Span80) were obtained from Sigma Chemical Company (USA). Heavy liquid paraffin was purchased from (El Nasr Pharmaceutical Chemicals (Cairo, Egypt).

**Determination of the Required hydrophilic lipophilic balance (RHLB) of jojoba oil****Preparation of emulsion**

- The required amount of span 80 was dissolved in the oily phase and that of tween80 in the aqueous phase. The oil phase was added in a stepwise manner to the aqueous phase and shaken vigorously for 10 minutes using overhead mixer (Hiedolph, Germany) at 1400 rpm then the prepared emulsion was homogenized using homogenizer (Erweka, type AR 401, Germany) at 10000 rpm for 5 minutes.
- The emulsifiers span 80 (sorbitan monooleate, HLB=4.3) and tween 80 (polyoxyethylene 20

sorbitan monooleate, HLB=15) at a total blend concentration 2%w/w were used for the preparation of jojoba oil emulsions. The amount of each emulsifier added is calculated according to the following equation [8] :

$$[\text{HLB} = xA + (1-x)B] \quad (\text{Eq.1})$$

Where, x is the proportion of a surfactant having an HLB value of A, and the other surfactant has an HLB value of B.

A set of seven o/w emulsions each of 100 mL and containing 20% w/w jojoba oil were prepared as previously mentioned. The emulsifiers (span 80 and tween 80) were mixed in different ratios to cover an HLB range from 4.3 to 15 in the prepared set of seven emulsions.

- A second set of ten emulsions was then prepared using the same blend of emulsifiers but at closer ratio intervals between the most two stable emulsions obtained from the first set. To determine the most stable emulsion, the prepared emulsions were subjected to evaluation using several methods, regarding their stability, such as measurement of degree of creaming after centrifugation, measurement of degree of creaming after shelf storage and turbidimetric method.

#### **Assessment of emulsion stability**

##### **Degree of creaming after centrifugation**

Samples of each emulsion were subjected to centrifugation at 2000 rpm for 10 minutes and the degree of creaming was expressed as % v/v aqueous phase separated.

##### **Degree of creaming after 28-days shelf storage**

Emulsion samples each of 10ml were poured into stoppered 10ml graduated cylinders immediately after preparation. The degree of creaming expressed as % aqueous phase separated was determined at room temperature as a function of time over 28 days [9].

##### **Turbidimetric method**

Samples each of 5 ml of the emulsion preparations were withdrawn into colorless plastic syringe, the syringes were stored in inverted position with its plunger upward and kept undisturbed at room temperature. On the 7<sup>th</sup> day, 0.5 ml sample was gently taken from the syringe, diluted to 25 ml with distilled water and the percentage transmittance (%T) was measured at 600nm (previously determined for distilled water as a blank control) using Ultraviolet spectrophotometer

(Jenway 6505 UV. vis, UK) [9]. With the blank control set at 100% transmission, the turbidity of diluted emulsion was calculated as: Turbidity = 100-%T.

In each of the previously mentioned methods of stability assessment the results obtained were an average of three determinations.

#### **Effect of non ionic surfactant type on emulsion stability**

##### **Preparation of emulsion**

Blends of emulsifiers, namely: [span 60: myrj 53], [span 60:brij 35], [span 83:brij 35] ,and [span 80:tween 80] were used at a total blend concentration of 2%w/w with the three oils under investigation ( liquid paraffin, jojoba oil, isopropyl myristate). O/W emulsions, 100ml per sample, containing 20%w/w of the oily phase, were prepared using the method mentioned before. The emulsifiers were mixed in ratios giving blends with the required HLB for each oil i.e. 10.5 for heavy liquid paraffin [10], 11.7 for isopropyl myristate [11], 12.5 for jojoba oil (determined experimentally ). The amount of each emulsifier added is calculated according to the previously mentioned Eq. (1).

Assessment of emulsion stability was carried out to select the proper emulsifier type.

#### **Assessment of emulsion stability**

##### **Degree of creaming after 28 days shelf storage**

As described previously

##### **Turbidimetric method**

As described previously.

#### **Effect of emulsifier concentration on emulsion stability**

##### **Preparation of emulsion**

O/W emulsions, 100ml per sample, containing 20, 30 or 40%w/w of the oily phase were prepared using the method previously described. Span 60 and brij 35 were mixed in a ratio giving a blend with the indicated RHLB for each oil (liquid paraffin, jojoba oil, isopropyl myristate). The emulsifiers were examined at different total blend concentrations namely; 2, 3, 4, 5 and 6 %w/w for all oils investigated.

#### **Assessment of emulsion stability**

Emulsion stability was measured using the turbidimetric method previously described.

#### **Data analysis.**

Tests of significance were carried out using one way ANOVA followed by Bonferroni test for

multiple comparisons using graph pad instate software (version 2.04a).

## RESULTS AND DISCUSSION

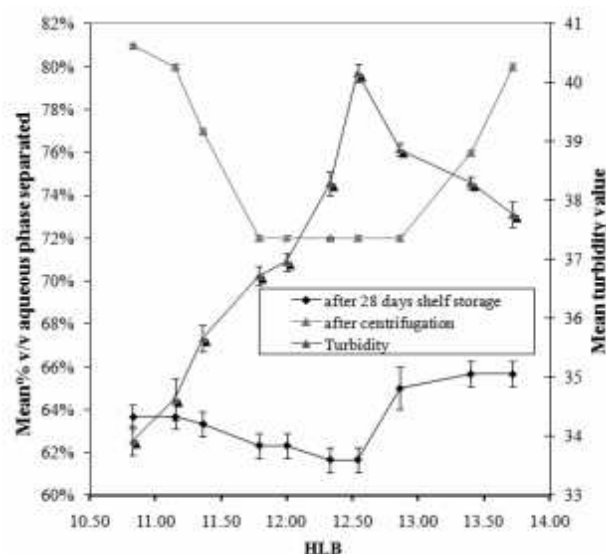
### Required hydrophilic lipophilic balance (RHLB) of jojoba oil

The optimum HLB of the emulsifier system for a given composition of oil and water phases provides a useful starting point in the selection of emulsifiers which will give an emulsion of good stability [8]. To achieve this goal a set of seven emulsions were prepared, the first member of which had span 60 as emulsifier. Brij 35 was added in a gradual increment so that the seventh member in the set had brij 35 as emulsifier. Consequently, the emulsion samples between the first and the seventh member had an emulsifier blend of HLB range from 4.3 (HLB of span 60) to 15 (HLB of brij 35). The most two stable emulsions were determined and the corresponding ratios of span 60 and brij 35 were identified. In a second step, another set of ten emulsion samples was prepared at a closer ratio intervals between the most two stable emulsions of the first set. This procedure gave emulsions having an emulsifier blends with an HLB range from 10.83 to 13.72.

### Degree of creaming after centrifugation

While the degree of creaming after centrifugation was studied for all the emulsions of the first set (HLB range 4.3 to 15), only the values for emulsions prepared within HLB 10.83 to 13.72 are indicated in table I, as the others showed unacceptable products. Data in the table I reveal a considerable resistance of jojoba oil emulsions (20% oil and 2% emulsifier) to centrifugation (No

oily phase separation with little creaming) over the HLB range of 10.83 to 13.72.



**Figure (1): Profiles of degree of creaming and turbidity of jojoba oil emulsions versus HLB.**

The % v/v aqueous phase separated after centrifugation showed a minimum value in the HLB range 11.79 to 12.86 as shown in table I and figure 1, which means that the RHLB value of jojoba oil could probably fall within this range.

### Degree of creaming after 28-days shelf storage

The degree of creaming after 28 days of the emulsions prepared within the HLB range of 10.8 to 13.7 is shown in table I and figure 1. It is obvious that a minimum value of % aqueous phase separated occurs between HLB 12.33-12.54.

**Table I: The effect of HLB variation on degree of creaming and turbidity of jojoba oil emulsion.**

HLB	Mean %v/v aqueous phase separated <sup>a</sup>		Mean Turbidity <sup>a,b</sup>
	After centrifugation	After 28 days shelf storage	
10.83	81%±0.001	63.7%±0.006	33.93 ±0.25
11.15	80%±0.001	63.7%±0.006	34.63 ± 0.35
11.36	77%±0.001	63.3%±0.006	35.67 ± 0.23
11.79	72%±0.001	62.3%±0.006	36.73 ± 0.15
12.00	72%±0.001	62.3%±0.006	36.97 ± 0.15
12.33	72%±0.001	61.7%±0.006	38.30 ± 0.20
12.54	72%±0.001	61.7%±0.006	40.17 ± 0.15
12.86	72%±0.001	65.0%±0.010	38.87 ± 0.12
13.40	76%±0.001	65.7%±0.006	38.30 ± 0.10
13.72	80%±0.001	65.7%±0.006	37.77 ± 0.21

<sup>a</sup> Mean value ± SD, n = 3.

<sup>b</sup> Experiment performed after 7 days shelf storage.z

### Turbidimetric method

The creaming rate of an emulsion as defined by Stoke's law gives only the rate of creaming of a single droplet. However, in a polydisperse system consisting of  $n_i$  droplets of radius  $r_i$ , the mass creaming rate ( $\bar{u}$ ), has been defined as

$$\bar{u} = \sum_i \frac{8\pi}{27V\eta} g n_i r_i^5 (d_i - d_2) \quad (\text{Eq.2})$$

where,  $V$  is the total volume of the disperse phase,  $\eta$  is the viscosity of emulsion,  $g$  is the acceleration due to gravity and  $d_i - d_2$  is the density difference between the dispersed and continuous phases [10]. Equation (2) suggests that the degree of separation of an emulsion is a function of the droplet size and size distribution, all other factors being kept constant. Since larger droplets cream rapidly, an emulsifier blend giving the smallest droplet size should produce the most stable emulsion. The smaller the particle size the higher the turbidity is. The degree of the stability of an emulsion can therefore be determined by turbidimetric method, which is a measure of the reduction of light directly transmitted through the emulsion, particularly through the creamed aqueous layer. Based on turbidity values, data in table I and figure 1 show that, the emulsion sample that exhibit maximum stability is the one having an HLB of 12.54. The turbidity curve was analyzed by two linear regression equations, the intersection of which was used in defining the RHLB value for jojoba oil. By calculation, the RHLB was found to be 12.635. This value is close to the experimentally

determined RHLB values of jojoba oil by the previously discussed methods. An average value is taken which is 12.5. It could be depicted from figure 1 that the turbidity value went through a maximum at HLB of 12.54 which lies in the same HLB range at which the degree of creaming after centrifugation or 28 days shelf storage were minimal, thus confirming the determined RHLB of jojoba oil. The methods used in determination of the RHLB values of jojoba oil in this study are based on the assumption that the droplet size of the emulsions would be smallest at the optimum HLB of the emulsifier blend [12], and would give the highest turbidity over a period of storage due to low creaming rate. Similar results were obtained by Orafiya and Olandimeji [9] who determined the RHLB values of some essential oils.

### Effect of non ionic surfactant type

#### Degree of creaming after 28 days shelf storage

From data of table II and figure 2, it obvious that although all emulsifier blends, used to select the proper non ionic surfactant type, exhibited the most proper HLB for each oil, the most proper emulsifier blend meshing and interacting at the oil water interface was found to be span 60 (sorbitan monostearate, HLB = 4.7) and brij 35 (polyoxyethylene23 lauryl ether, HLB = 16.9). It showed the greatest emulsion stability based on the lowest % v/v aqueous phase separation for all oils. The blend ratios of span 60: brij 35 were 36:64, 52:48, and 43:57 for jojoba oil, liquid paraffin and isopropyl myristate respectively.

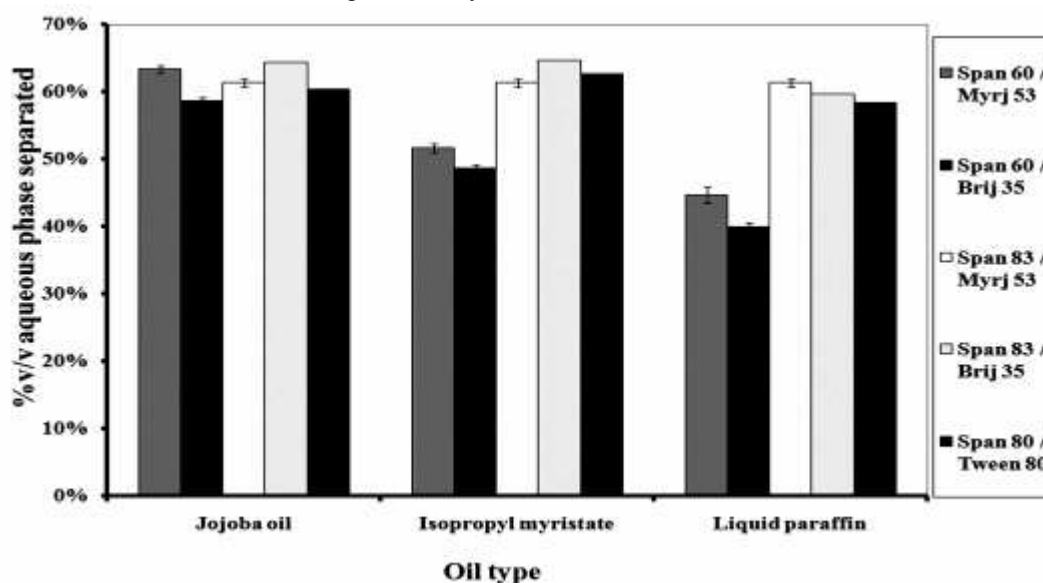


Figure (2): Degree of creaming after 28 days shelf storage for emulsions prepared with 20% w/w oily phase and different emulsifier blends.

**Table II: Degree of creaming and turbidity values for emulsions prepared with different oils and emulsifier blends.**

Emulsifier blend *	Jojoba oil **		isopropyl myristate **		liquid paraffin **	
	a	b	a	b	a	b
Span 60 / Myrj 53	63% ± 0.006	31.10 ± 2.62	52% ± 0.006	29.30 ± 0.36	45% ± 0.006	33.73 ± 1.44
Span 60 / Brij 35	59% ± 0.006	40.53 ± 1.68	49% ± 0.006	29.53 ± 1.53	40% ± 0.006	35.23 ± 1.40
Span 83 / Myrj 53	61% ± 0.011	34.73 ± 1.25	61% ± 0.006	19.90 ± 1.66	61% ± 0.006	18.50 ± 1.32
Span 83 / Brij 35	64% ± 0.006	27.17 ± 2.72	65% ± 0.006	13.40 ± 1.15	60% ± 0.006	20.70 ± 2.19
Span 80 / Tween 80	60% ± 0.006	38.80 ± 1.10	63% ± 0.006	19.00 ± 0.70	58% ± 0.006	28.10 ± 0.10

\*Each emulsifier blend (2% w/w) is adjusted to the optimum HLB for each oil.

\*\*Used at a concentration of 20 % w/w

a Mean % v/v aqueous phase separated after 28 days shelf storage ± SD, n = 3 .

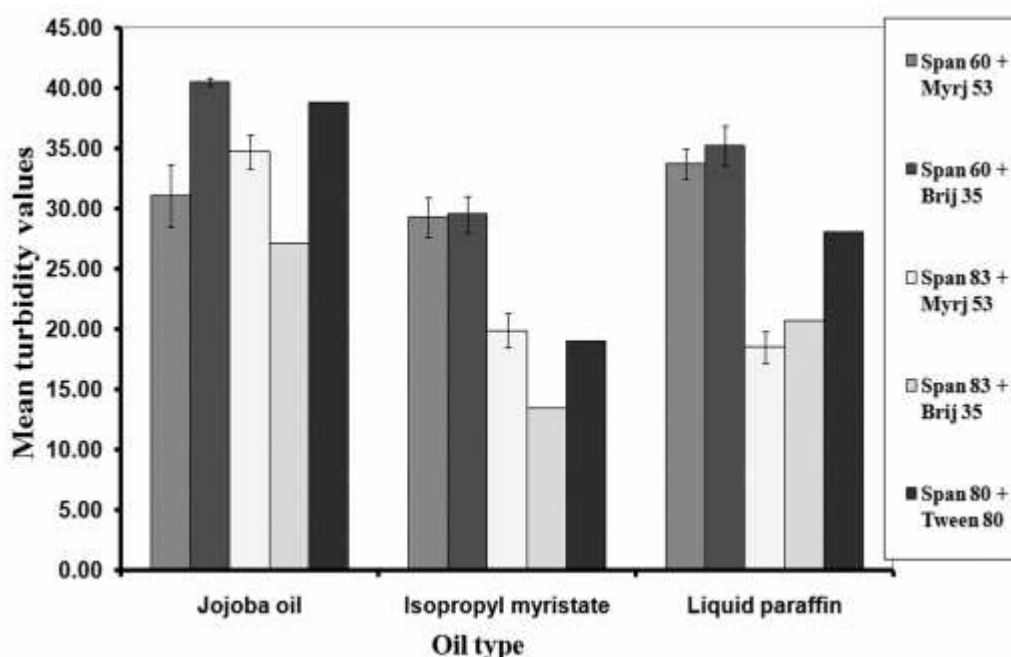
b Mean turbidity values after 7 days shelf storage ± SD, n = 3 .

HLB of each surfactant: span 60 (4.7), myrj 53 (17.9), brij 35 (16.9), span 83 (3.7), span80 (4.3) and tween80 (15).

### Turbidimetric method

From data in table II and figure 3 revealed the superiority of the emulsifier system span 60/ brij 35 over all the other surfactant blends, when used at the previously mentioned ratio for each oil obtained from the results of degree of creaming study (2.1). Emulsifiers are thought to form a film around the suspended dispersed phase droplets and strengthening of this film could attain a much greater degree of the droplet stability. This could be accomplished by using a combination of hydrophilic and lipophilic emulsifiers. In such a system the hydrophilic and lipophilic emulsifiers are thought to align alongside each other imparting more rigidity and strength to the film through hydrogen bonding [13, 14]. Therefore, the stability of macroemulsions is considerably enhanced by mixing of surfactants compared with a single

surfactant as reported by many authors like Falbe [15] and Kunieda et al [16] who studied the mixing effect of polyethylene type non ionic surfactants on the liquid crystalline structures and they reported that the lower surface tension and the better stability of macroemulsions and the larger solubilizing capacity of micro-emulsions result from the mixing effect of these surfactants. Selection of emulsifiers based on the difference in their structural features and having the same HLB was also reported [17-22], disregarding the resultant HLB value of the emulsifier blend and the required HLB of the oil, considered the improved stability of emulsion containing sorbitan monooleate ( span 80) and ethoxylated sorbitan monopalmitate ( Tween 40) to be a result of a convenient meshing of the molecules at the oil droplet water interface due to steric considerations.



**Figure (3): Turbidity value for emulsions after 7 days shelf storage prepared with 20% w/w oily phase and different emulsifier blends.**

**EFFECT OF EMULSIFIER CONCENTRATION****Jojoba oil**

For emulsions containing either 20, 30 or 40% w/w oil loading and after 7 days shelf storage, the turbidity values approached a plateau as the emulsifier concentration was  $\geq 3\%$  w/w (table III, figures 4 and 5), and by carrying out one way ANOVA a non significant increase in the mean turbidity value was observed after 3%w/w ( $P>0.05$ ) in case of 20 and 30% w/w oily phase. Emulsions containing 40%w/w jojoba oil showed the maximum permissible turbidity reading of the equipment i.e. 100%, so these emulsions samples were subjected to shelf storage for 14 days in order to discriminate between these high values. These emulsions had turbidity values approaching a plateau only when the emulsifier was  $\geq 4\%$  w/w, and the increase in the mean turbidity value was non significant ( $P>0.05$ ) as checked by one way ANOVA. It is well noted that stability of emulsion increases with increase of the emulsifier concentration till a maximum after which no

further increase in emulsion stability. This finding is in agreement with those obtained by [23]. Therefore, we can deduce from the previous findings that an emulsifier system (span 60:brij 35, 36:64) at a concentration of 3% w/w is the most appropriate for emulsification of 20 and 30% w/w oil loading, while a 4%w/w is preferred for 40%w/w oil loading.

**Liquid paraffin**

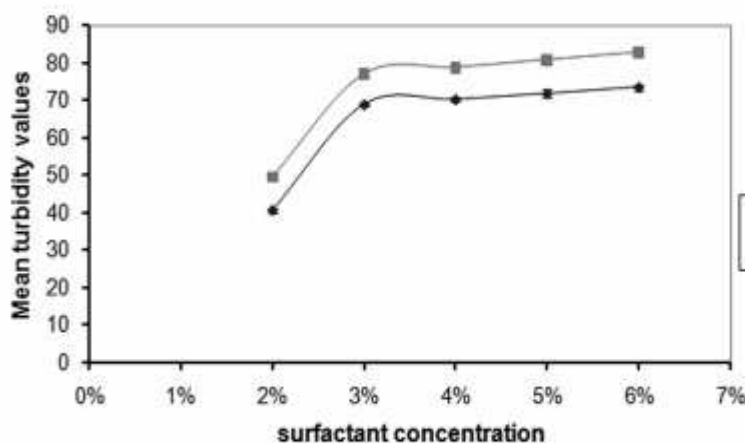
For emulsions containing either 20 or 30% w/w oil loading the surfactant concentration turbidity profile (after 7 days shelf storage) exhibited a plateau as the emulsifier concentration was  $\geq 4\%$  w/w (table IV, figures 6 and 7). ANOVA proved that differences in the mean turbidity value were non significant ( $P>0.05$ ) after 4%w/w. Values in table IV revealed that emulsions containing 40% w/w and were subjected to 7 days shelf storage, showed maximum turbidity starting from 3% w/w emulsifier.

**Table III: Effect of emulsifier concentration on the turbidity of jojoba oil emulsions after 7 and 14 days shelf storage.**

surfactant concentration	*Mean turbidity values			
	20% oil	30% oil	40% oil	40% oil **
2%	40.57 $\pm$ 0.493	49.53 $\pm$ 0.96	57.73 $\pm$ 0.80	37.63 $\pm$ 0.05
3%	68.83 $\pm$ 0.35	76.93 $\pm$ 0.73	100.00 $\pm$ 0.00	57.63 $\pm$ 0.05
4%	70.20 $\pm$ 0.60 <sup>a</sup>	78.70 $\pm$ 0.52 <sup>a</sup>	100.00 $\pm$ 0.00 <sup>a</sup>	86.57 $\pm$ 0.50
5%	71.73 $\pm$ 1.02 <sup>b</sup>	80.73 $\pm$ 0.37 <sup>b</sup>	100.00 $\pm$ 0.00 <sup>a,b</sup>	87.00 $\pm$ 0.43 <sup>b</sup>
6%	73.47 $\pm$ 0.83 <sup>c</sup>	82.70 $\pm$ 1.11 <sup>c</sup>	100.00 $\pm$ 0.00 <sup>a,b,c</sup>	88.47 $\pm$ 1.18 <sup>c</sup>

\*Mean value  $\pm$  SD, n = 3 \*\* Emulsions subjected to 14 days shelf storage.

a, b or c: non significantly different from 3% , 4% , 5% respectively at  $p > 0.05$  using one way ANOVA followed by Bonferroni test for multiple comparisons.



**Figure (4): Effect of emulsifier concentration on the turbidity of 20 and 30% w/w jojoba oil emulsions after 7 days shelf storage.**

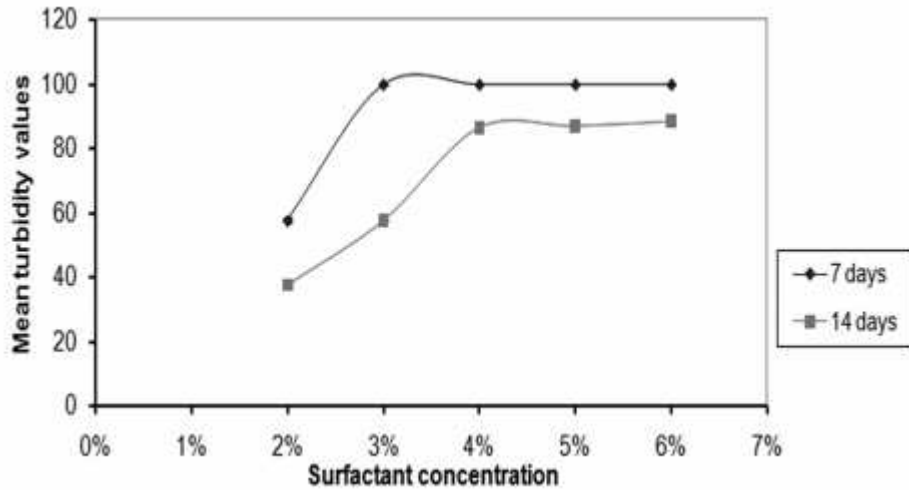


Figure (5): Effect of emulsifier concentration on the turbidity of 40% w/w jojoba oil emulsions after 7 and 14 days shelf storage.

Table IV: Effect of emulsifier concentration on the turbidity of liquid paraffin emulsions after 7 and 14 days shelf storage.

surfactant concentration	*Mean turbidity values			
	20% oil	30% oil	40% oil	40% oil**
2%	23.03 ± 0.23	29.10 ± 0.17	35.13 ± 0.63	30.73 ± 0.15
3%	50.67 ± 0.75	67.37 ± 0.92	100.00 ± 0.00	71.63 ± 1.16
4%	81.50 ± 0.17	90.67 ± 0.75	100.00 ± 0.00 <sup>a</sup>	83.83 ± 0.05
5%	82.47 ± 0.35 <sup>b</sup>	92.27 ± 0.35 <sup>b</sup>	100.00 ± 0.00 <sup>a,b</sup>	84.40 ± 0.20 <sup>b</sup>
6%	83.60 ± 0.45 <sup>c</sup>	93.50 ± 0.10 <sup>c</sup>	100.00 ± 0.00 <sup>a,b,c</sup>	84.70 ± 0.10 <sup>b,c</sup>

\*Mean value ± SD, n = 3

\*\*Emulsions subjected to 14 days shelf storage.

a, b or c: non significantly different from 3%, 4%, 5% respectively at p> 0.05 using one way ANOVA followed by Bonferroni test for multiple comparisons.

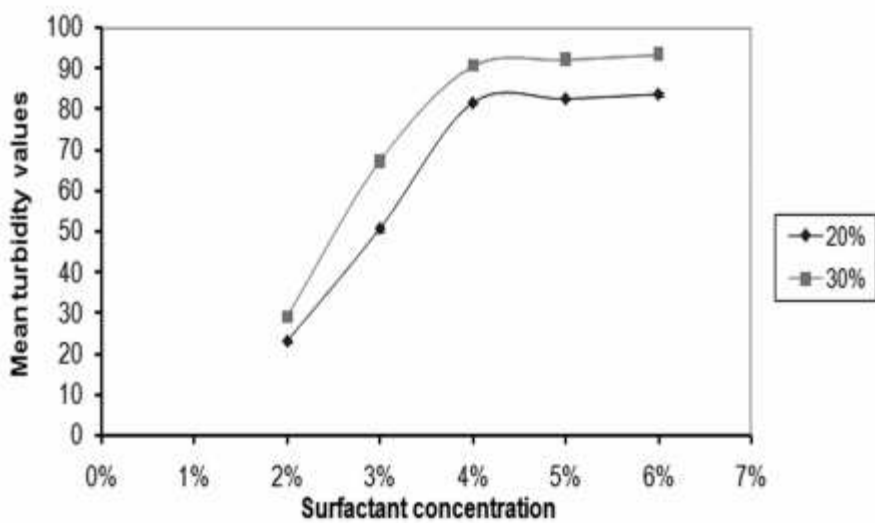
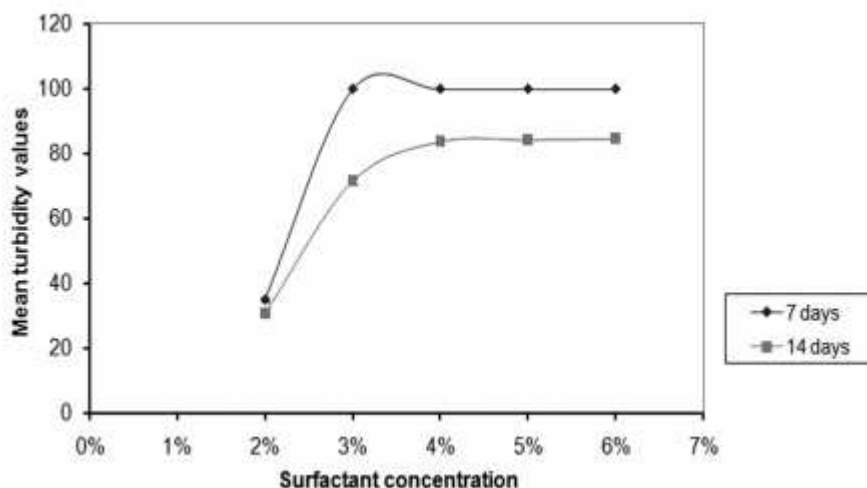


Figure (6): Effect of emulsifier concentration on the turbidity of 20 and 30% w/w liquid paraffin emulsions after 7 days shelf storage.



**Figure (7): Effect of emulsifier concentration for 40% liquid paraffin emulsions (Turbidimetric method) after 7 and 14 days storage.**

These emulsions were subjected to 14 days shelf storage for successful measurement. The plateau appeared only when the emulsifier was  $\geq 4\%$  w/w. By carrying out one way ANOVA, results revealed a non significant difference ( $P > 0.05$ ) in the mean turbidity value after 4% w/w. Consequently, a concentration of 4% w/w span 60/brij 35 (52:48) would be satisfactory for emulsification of liquid paraffin. The high viscosity grade of liquid paraffin used in this study behind the results that no difference in emulsion stability was observed whatever the concentration of the oily phase.

#### Isopropyl myristate

From figure 8 it could be depicted that a linear relationship ( $r^2 = 0.9952$ ) existed between the mean turbidity value and the surfactant concentration over the concentration range of 2-6 %w/w for emulsions containing 20% w/w oily phase. Attempts to increase the surfactant concentration were not approached for irritancy

and safety purposes [24, 25]. Hall- Manning et al. [26] studied the skin irritation potential of mixed surfactant systems and attributed this irritation potential to the binding of these systems with the skin protein and to the interaction of the surfactant micelles with the lipids which form the packing between squams. On the other hand, when the oily phase was increased to 30 or 40% w/w, a plateau appeared at 100% turbidity after an emulsifier concentration 5 and 4% w/w respectively as shown in table V, figures 9 and 10. For emulsions containing 30% w/w oil and were subjected to 14 days shelf storage, one way ANOVA revealed a non significant increase ( $P > 0.05$ ) in the mean turbidity value after 5% w/w. On the other hand, for the emulsions containing 40% w/w oil and were subjected to 14 days shelf storage, the difference in the turbidity values after 4% w/w emulsifier were not significant ( $P > 0.05$ ) as verified by one way ANOVA.

**Table V: Effect of emulsifier concentration on the turbidity of isopropyl myristate emulsions after 7 and 14 days shelf storage.**

surfactant concentration	*Mean turbidity values				
	20% oil	30% oil	40% oil	30% oil**	40% oil**
2%	6.50 ± 0.90	9.10 ± 0.51	13.60 ± 0.62	6.10 ± 0.51	9.77 ± 0.057
3%	22.53 ± 0.97	27.73 ± 0.15	37.13 ± 0.60	24.73 ± 0.15	29.83 ± 0.057
4%	48.77 ± 1.05	65.30 ± 0.10	99.30 ± 0.26	60.30 ± 0.1	93.43 ± 0.56
5%	66.30 ± 1.05	99.10 ± 0.45	100.00 ± 0.00 <sup>b</sup>	93.37 ± 1.55	94.17 ± 0.94 <sup>b</sup>
6%	90.30 ± 0.86	100.00 ± 0.00 <sup>c</sup>	100.00 ± 0.00 <sup>b,c</sup>	93.70 ± 0.72 <sup>c</sup>	95.37 ± 1.41 <sup>b,c</sup>

\*Mean value ± SD, n = 3

\*\* Emulsions subjected to 14 days shelf storage.

a, b or c: non significantly different from 3%, 4%, 5% respectively at  $p > 0.05$  using one way ANOVA followed by Bonferroni test for multiple comparisons.



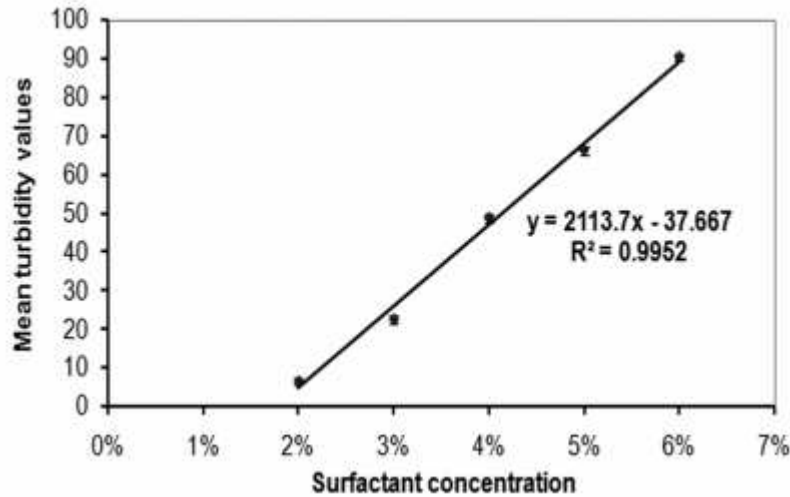


Figure (8): Effect of emulsifier concentration on the turbidity of 20% w/w isopropyl myristate emulsions after 7 days shelf storage.

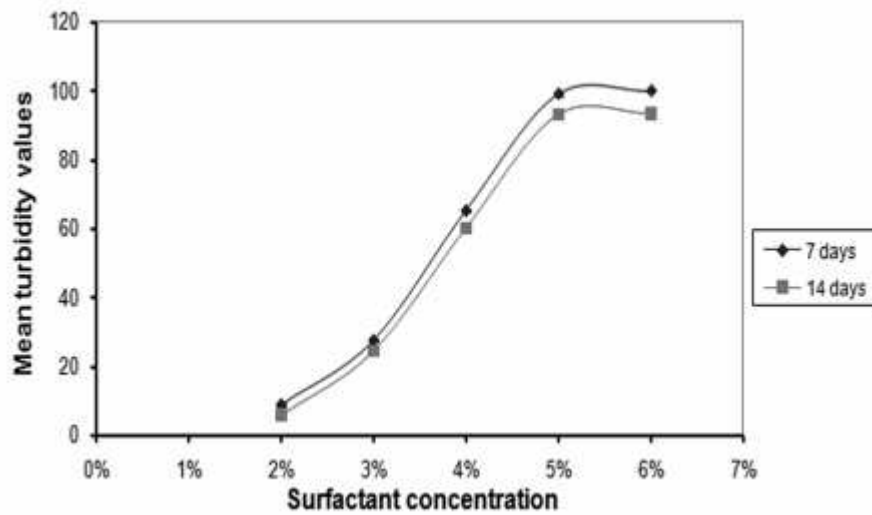


Figure (9): Effect of emulsifier concentration on the turbidity of 30% w/w isopropyl myristate emulsions after 7 and 14 days shelf storage.

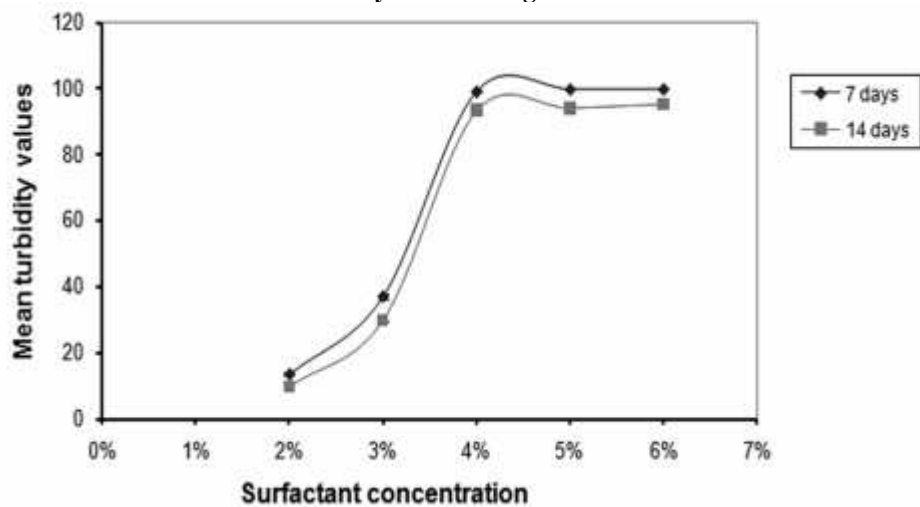


Figure (10): Effect of emulsifier concentration on the turbidity of 40% w/w isopropyl myristate emulsions after 7 and 14 days shelf storage.

From the previous finding, it could be deduced that an inverse relationship existed between the oily phase concentration and the emulsifier concentration; i.e. to obtain a stable emulsion with isopropyl myristate containing 20, 30 or 40 w/w oily phase, a surfactant blend composed of 6, 5 or 4 % w/w span 60 and brij 35 in a ratio of 43:57 respectively, would be appropriate. The previous observation could be attributed to the composition of the oily phase, isopropyl myristate, which is a self emulsifying oil [27]. The relatively higher stability of emulsions containing larger proportions of the oil phase could be ascribed to the decrease in the creaming velocity of emulsions with increasing droplet concentrations as well as the increase in the apparent viscosity of the emulsion by increasing the internal phase concentration [28].

### CONCLUSION

The RHLB of jojoba oil was determined experimentally using different methods and it was around 12.5. The best non ionic surfactant chemical type to be used with the three studied oils (jojoba oil, liquid paraffin, isopropyl myristate) was the mixture of span 60 and brij35. For jojoba oil emulsions containing either 20 or 30%w/w oily phase, a surfactant blend composed of 3 % w/w span 60 and brij 35 in a ratio of 36:64 would be appropriate to obtain a stable emulsion. While a blend of 4% w/w of the same emulsifier blend was suitable for 40% w/w oily phase emulsions. However, liquid paraffin emulsions containing either 20, 30 or 40 %w/w oily phase, a surfactant blend composed of 4 % w/w span 60 and brij 35 in a ratio of 52:48 would be suitable. On the other hand, isopropyl myristate emulsions containing 20, 30 or 40 w/w oily phase, a surfactant blend composed of 6, 5 or 4 % w/w span 60 and brij 35 in a ratio of 43:57 respectively, would be satisfactory to get an emulsion with good stability.

### CONFLICT OF INTEREST DECLARATION

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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