

HARUAN (*CHANNA STRIATUS*) INCORPORATED PALM-OIL CREAMS: FORMULATION AND STABILITY STUDIES

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Topical emulsions stabilized with non-ionic emulsifiers have been an attractive alternative as vehicles for drug delivery, particularly for the patients suffering from dermatological problems. Haruan (a natural wound healer) creams were formulated with different types of emulsifiers (Tween 80 and Span 80) using different grades of Malaysian Palm-oleins (DFPL 56, 60, 62 and 65). The stability (at room temperature and accelerated stability testing) of the various creams was evaluated at different temperatures (5, 25 and 45°C) for a period of 6 months by measuring changes in droplet size, viscosity and percentage oil separation. The emulsifier type and concentration showed pronounced effect on the physicochemical properties of the cream, whereas storage time did not. This study suggested that the choice of emulsifiers and concentration of haruan extract are the most important factors in the stability of the haruan creams.

Keywords: Haruan, *Channa striatus* and Malaysian double filtered palm oleins (DFPL)

INTRODUCTION

Haruan (*Channa striatus*), a fresh water fish, has been used to enhance wound healing (Baie and Sheikh, 2000) and is available in the tablet and capsule forms (Major Interest (M) Sdn Bhd). The influence of oil phase on the stability of the resulting emulsion has already been established. Many oil phases like liquid paraffin and vegetable oils have been used in the past. Malaysian Palm stearin has previously been used in the formulation of ointments but oleins have not yet been used for the formulation of creams (Ayesha, 1998). This fact made this study more significant. Palm-oleins differ in their characteristics on the basis of iodine and saponification values. Selection of palm-oleins is based on the amount of fatty acids, carotenoids, tocopherols and potent triglycerides, which are helpful in wound healing (Berger, 1975). An emulsifying agent that is predominantly hydrophilic like Tweens, tends to form an o/w emulsion and lipophilic agent like Spans, tend to form w/o emulsions (Jiao and Burgess, 2003). In addition to solubilization of poorly water-soluble drugs, the topical emulsions are used to stabilize hydrolytically susceptible drugs (Pranker and Stella, 1990), reduction of irritation or toxicity (Mbela *et al.*, 1994), possible targeted delivery of drugs to various organs (Friedman and Benita, 1987), and possible enhanced pharmacological effect (Levy and Benita, 1988). The aim of this study was to evaluate the different grades of Palm-oleins of Malaysian origin for the formulation of haruan cream and to observe the stability of those creams under different storage conditions (at room temperature and accelerated stability testing) at 5, 25 and 45°C for up to 6 months.

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MATERIAL AND METHODS

Haruan fish extract was a generous gift from Major Interest (M) Sdn Bhd. Double fractionated palm-oleins (DFPL 56, 60, 62 and 65) were purchased from Lam Soon Huat (M) Sdn Bhd. Span 80 and Tween 80 were obtained from Sigma Chemical Co. (St. Louis, USA). All materials were used as received without further purification.

FORMULATION OF CREAMS

Selection of oil phase

Four types of DFPL (56, 60, 62 and 65) were used as the oil phase in different experiments. Studies were carried out to select the best palm-olein, which gives the most stable formulation.

Selection of emulsifier

Spans and Tweens were selected as the HLB of Span 80 is 4.3 while of Tween 80 is 15. Their combination results in the stable emulsions at HLB over 10. The HLB system, however, provided no information about the correct amount of emulsifier to be used. The minimum amounts of emulsifiers alone and their combinations need to be determined to achieve a stable emulsion. Various concentrations of Span 80 and Tween 80 alone, and concentrations of their mixtures (Tween 80 and Span 80) were used to determine the interfacial tensions using du Nouy Torsion Balance.

Preparation of creams

In the formulation of creams, Palm-oleins were used as the oil phase, Tween 80 and Span 80 were used as emulsifiers in different proportions. One of the formulations contains Tween 80 only, the other Span 80 only and the third one containing mixture of Tween 80 and Span 80 (7:3 v/v)

respectively). Methyl paraben and propyl paraben were used as preservatives. In the preparation of creams Span 80 and propyl parabens were dissolved in Palm-oleins, whereas methyl parabens and Tween 80 were dissolved in water. Both the aqueous and oil phases were heated separately to 70°C and then inter-dispersed. Coarse emulsions were cooled to 45°C and homogenized using Silverson homogenizer at 1500 rpm for 40 minutes. The resulting cream base was cooled to room temperature and various concentrations of haruan extract (1-15%) were added to obtain the most stable cream with haruan extract. The pH of creams was adjusted to 8.5 with 0.1 N sodium hydroxide solutions.

Stability testing of creams

The stability of the creams was studied at 5, 25 and 45°C for a period of 6 months by monitoring the changes in particle size, percentage oil separation and rheological properties (viscosity). In addition, the creams were tested for stability to freezing and heating by exposing it to a series of *Freeze-thaw cycles* (accelerated stability testing).

Particle size measurements

Particle size of an emulsion is one of its most important characteristics, which determines the appearance of the emulsion and has a strong impact on its stability. The droplet size of all formulations was determined using a Coulter Counter, with a 50 µl orifice tube and reconfirmed employing microscope (using stage micrometer and graticules) for size distribution, every fortnight for 6 months at 5, 25 and 45°C. The results were compared with a patented cream formulation containing haruan and cetrimide (unknown formula). Graphs of percentage cumulative counts against linear diameter were plotted on log-probability graphic papers and the average particle diameters were determined.

Determination of rheological properties

Rheological properties of an emulsion are important in the production and use of emulsions. The ultimate use of emulsions, in turn, dictates the rheological properties needed for an acceptable product (Aulton, 1996). The rheological evaluation (viscosity, yield value and thixotropy) of the creams was performed using Rheomat 115 (Contraves Rheoanalyzer) at room temperature, based on continuous shear procedures. The experimental parameters used included:

Minimum shear rate: 0.5 S⁻¹, ascending curve time: 120 sec, descending curve time: 120 sec, maximum shear rate: 100 S⁻¹ and temperature 25°C.

The rheograms were analyzed and then viscosity, yield value and thixotropy of all formulations were measured to obtain the most stable cream.

Determination of percentage oil separation

Centrifugation is an excellent tool for the evaluation of accelerated deterioration of creams and has from time to time been used to obtain a measure of their stability (Depraetere *et al.*, 1980). Stability of formulated creams to centrifugation was determined in 10 ml graduated tubes at 11,000 g (gravitational force) for 10 minutes using Sigma centrifuge as described by Cockton and Wynn (1952). The percentage of oil separated was calculated to select the most stable cream base and most appropriate palm-olein grade.

Statistical analysis

Changes in the physicochemical properties of creams were also evaluated statistically by using ONE WAY ANOVA followed by Duncan's test. The probability level *P<0.05 and **P<0.01 were considered significant.

RESULTS AND DISCUSSION

Selection of emulsifier

Oils with interfacial tension less than 45 dynes/cm² more rapidly form the o/w emulsions. All grades of Palm-oleins were found to show interfacial tension below 45 dynes/cm², which is in agreement with the findings of Garrette (1965). The results of interfacial tension and HLB values are demonstrated in table 1, which shows that the mixture of Tween 80 and Span 80 (7:3 v/v respectively) provided the required HLB for an o/w emulsion (11.8).

Selection of Palm-olein

According to Stoke's law, creaming is a function of gravity and increasing gravity will accelerate phase separation. However, there is a significant difference between emulsion stability under normal storage conditions and centrifugation, because centrifugation will rupture emulsifier films. Therefore, it only provides information about the final stages of emulsion stability (Opawale and Burgess, 1998). The percentage oil separation after centrifugation showed that, emulsions prepared using DFPL 65 presented less oil separation than the emulsions prepared with DFPL 56, 60 and 62 (fig. 1). Emulsions stabilized with mixture of Tween 80 and Span 80 (7:3), provided more stable emulsions than other concentrations of combinations of emulsifiers and individual emulsifiers. There was no oil separation observed in the freshly prepared creams containing 1% haruan. The creams containing more than 1% haruan showed various levels of oil separation (fig. 2). The minimum oil separation could be attributed to the probable formation of a closely-packed mixed film of Tween 80 and Span 80 at the oil-water interface. This may act as a stabilizer at the earlier stages of the emulsification process by forming a high-energy barrier to cause repulsion of adjacent particles to prevent coalescence. The storage temperature did have a significant impact on oil separation as the creams stored at 45°C showed higher oil separation than the creams at 25°C.

Table 1
Effect of various concentrations of Tween 80 and Span 80 on interfacial tension of Palm-olein (DFPL 65) –water interface. Results are presented as means \pm SD

Ratio of palm-olein and water (ml)	Conc. of Tween 80 (ml)	Interfacial tension (dynes/cm ²)	Conc. of Span 80 (ml)	Interfacial tension (dynes/cm ²)	Ratio of Tween 80 & Span 80 (ml)	Interfacial tension (dynes/cm ²)	HLB value
15:15	0.0	45.0 \pm 0.5	0.0	45 \pm 0.1	9.5:0.5	7.3 \pm 0.5	14.47
15:15	0.5	17.5 \pm 0.4	0.5	27.5 \pm 0.2	9:1	7.9 \pm 0.5	13.93
15:15	1.0	16.4 \pm 0.2	1.0	26.4 \pm 0.1	8:2	8.0 \pm 0.2	12.9
15:15	2.0	15.3 \pm 0.3	2.0	25.3 \pm 0.3	7:3	8.5 \pm 0.1	11.8
15:15	3.0	15.1 \pm 0.1	3.0	25.1 \pm 0.1	6:4	10.3 \pm 0.4	10.72
15:15	4.0	14.0 \pm 0.3	4.0	24.0 \pm 0.1	5:5	11.4 \pm 0.3	9.65
15:15	5.0	10.5 \pm 0.2	5.0	23.5 \pm 0.5	4:6	11.7 \pm 0.4	8.58
15:15	6.0	9.7 \pm 0.3	6.0	22.7 \pm 0.5	3:7	12.5 \pm 0.1	7.51
15:15	7.0	9.5 \pm 0.1	7.0	21.5 \pm 0.4	2:8	12.8 \pm 0.2	6.44
15:15	8.0	9.1 \pm 0.3	8.0	21.1 \pm 0.4	1:9	13.0 \pm 0.3	5.37
15:15	9.0	8.0 \pm 0.5	9.0	20.0 \pm 0.2	0.5:9.5	13.2 \pm 0.4	4.83

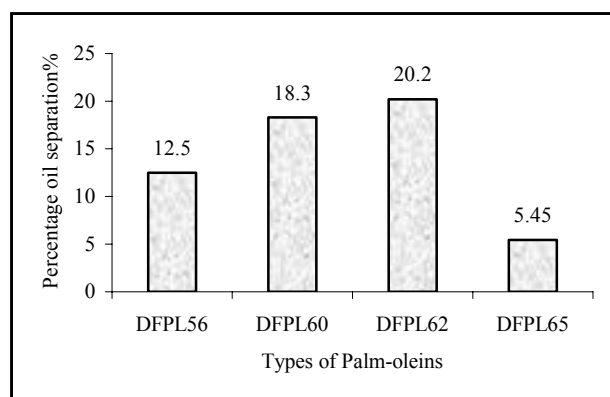


Fig. 1: Effect of centrifugation on the percentage oil separation of cream bases formulated, with different Palm-olein grades.

Particle size

Mean particle size and particle size distribution are the most important physicochemical characteristics of disperse systems such as emulsions, because they determine the stability and toxicity of the products (Washington *et al.*, 1993). In this study, the average particle size was observed to be the smaller in DFPL 65 formulation containing 1% haruan and Tween 80 and Span 80 mixture (7:3) than the creams formulated with DFPL 56, 60 and 62 with Tween 80 and Span 80 combination or individual concentration of each of Tween 80 and Span 80, and comparable to the patent cream at different temperatures for first 3 months (figs.3-5). Storage temperature on the other hand, did not significantly affect the particle size. This result agrees with those of Santos *et al.* (1991), who demonstrated that storage temperature from 25°C to 40°C only begin to affect emulsion stability after a storage period of at least 12 months.

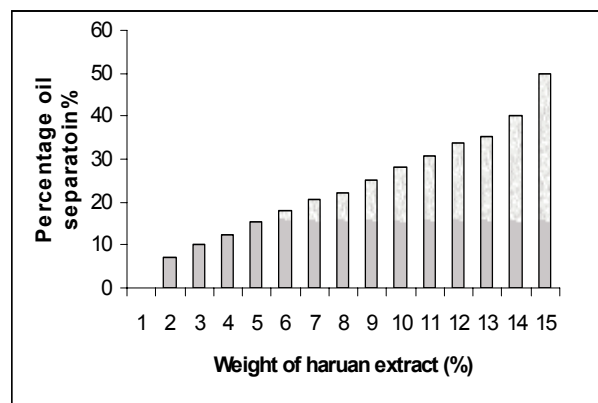


Fig. 2: Effect of haruan weight (%) on oil separation of various formulations.

Determination of rheological properties

The viscosity, yield value and thixotropy of emulsions play an important role in their flow properties. However, most emulsions are normally polydispersed and, as a result, particle size distribution may have an important influence on viscosity. Evaluation of changes in stability will be meaningful when rheological properties are combined with changes in particle size (Sepulveda *et al.*, 2003). Particle size distribution, which was found to be higher for formulations stabilized with either Tween 80 or Span 80, exhibited a pronounced fall in viscosity compared to formulations stabilized with the combination of Tween 80 and Span 80 (7:3) (figs.6-8).

In the present study, the rheograms of all the cream formulations were of hysteresis loop type and exhibit a yield point. This means that there is some definite minimum shear stress required for materials to flow and below this point the materials will behave as solids. From the results (table 2),

the viscosity yield value and thixotropy of the creams prepared with DFPL 56, 60 and 62 were very low and hence unstable as compared to the formulations containing DFPL 65 and stabilized by Tween 80 and Span 80 mixture (7:3). The stability of creams may be attributed to the most refined form of oleins (DFPL 65) with higher amounts of β -carotenes and tocopherols compared to other grades of oleins (Ooi, 1982; Baie and Sheikh, 1999). It is well established that maximal stability of emulsion occurs at the HLB value at which particle size is at minimum, which is in agreement of the present study.

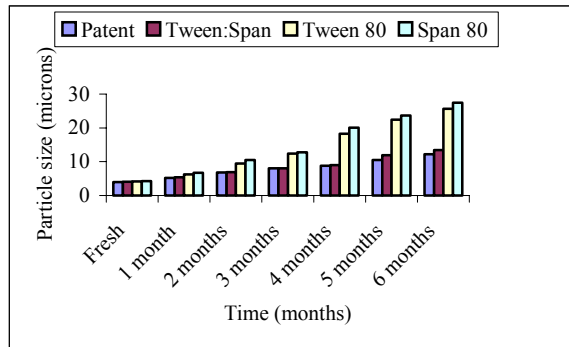


Fig. 3: Effect of storage time on particle size of different creams at 25°C.

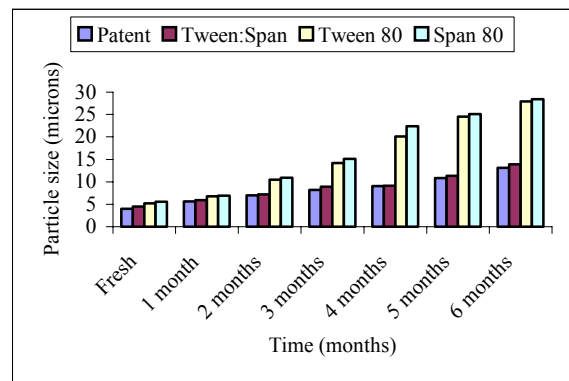


Fig. 4: Effect of storage time on particle size of different creams at 45°C.

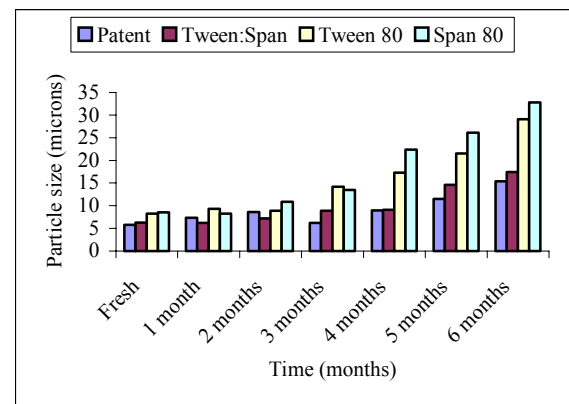


Fig. 5: Effect of storage time on particle size of different creams at 5°C.

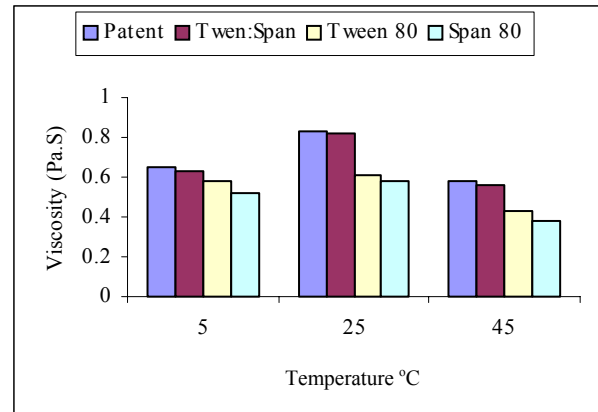


Fig. 6: Effect of temperature changes on the viscosity of cream bases stabilized with various emulsifiers after one day.

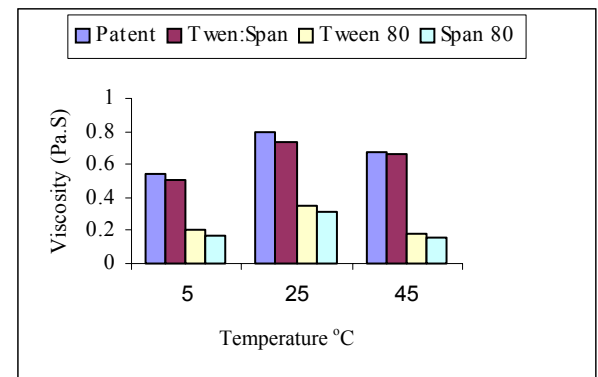


Fig. 7: Effect of temperature changes on the viscosity of cream bases stabilized with various emulsifiers after a period of 6 months.

Table 2

Effect of Palm-olein grades on viscosity, yield value and thixotropy of formulated creams

Types of Palm-olein	Viscosity (Pa.S)	Yield value (Pa.S)	Thixotropy (Pa)
DFPL 56	0.94	12.8	235.3
DFPL60	0.35	5.6	107.4
DFPL62	0.23	3.23	55.04
DFPL65	0.98	16.9	623.5

In the present study, the rheograms of all the cream formulations were of hysteresis loop type and exhibit a yield point. This means that there is some definite minimum shear stress required for materials to flow and below this point the materials will behave as solids. From the results (table 2), the viscosity yield value and thixotropy of the creams prepared with DFPL 56, 60 and 62 were very low and hence unstable as compared to the formulations containing DFPL 65 and stabilized by Tween 80 and Span 80 mixture (7:3). The stability of creams may be attributed to the most refined form of oleins (DFPL 65) with higher amounts of β -

carotenes and tocopherols compared to other grades of oleins (Ooi, 1982; Baie and Sheikh, 1999). It is well established that maximal stability of emulsion occurs at the HLB value at which particle size is at minimum, which is in agreement of the present study.

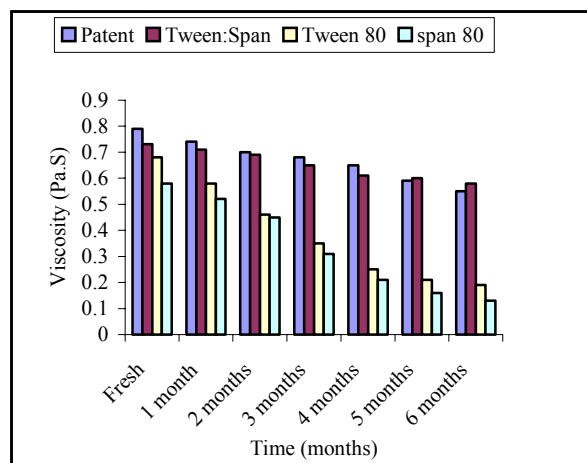


Fig. 8: Effect of storage time on the viscosity of creams stabilized with various emulsifiers.

CONCLUSION

Results of centrifugation, temperature variations, particle size analysis and rheology showed that DFPL 65 was the best among all grades (DFPL 56, 60 and 62), as it provided the most stable creams. Mixture of Tween 80 and Span 80 (7:3 respectively) was determined to be the best combination, which provided creams with smaller particle size, high viscosity and minimum oil separation as compared to the formulations stabilized with, either Span 80 or Tween 80 alone.

Proposed future work

To carry out the microscopic studies of the optimized creams by demonstrating the relationship between microcrystalline structure of the cream and internal phase volume of DFPL 65 grade of Palm-oleins.

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