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Stability Analysis of Formulated Emulsion Containing Black Cumin (*Nigella sativa*) Oil

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Abstract: In this present study emulsions were prepared by using black cumin oil. Dry gum method was followed to prepare emulsions formula A & B. Stability tests including organoleptic property evaluation, pH test, accelerated stability studies, water-drop test and peroxide value were determined. In case of organoleptic property evaluation emulsions showed insignificant changes and there were no variation in pH in both preparations up to 3 months. Emulsions were also undergone to different temperature effects and centrifugation at 4000 rpm, but lost the homogenous structure and separated into two phases under the effect of light and centrifugation. The study showed better results for emulsion B comparing with emulsion A. Thus our results showed that emulsion formulation B can be a good semi-solid preparation in Pharmaceutical field and food industry.

Keywords: Black Cumin Oil, Emulsions, Dry Gum Method, Stability Tests, Better Results, Pharmaceutical Field

1. Introduction

An emulsion is defined as a disperse system consisting of two immiscible liquids, one of which is distributed throughout the other in minute globules, the system being stabilized by the presence of a third substance, the emulsifying agent [1]. Emulsifying agents can also be classified into two classes based on the chemical, natural and synthetic agent [2]. The radius of the emulsified droplets in an opaque, usually white emulsion ranges from 0.25 to 10 microns [3].

Common emulsions are inherently unstable and do not tend to form spontaneously. Energy input through shaking, stirring, homogenizing or exposure to power ultrasound is needed to form an emulsion [4]. Emulsions tend to revert to the stable state of the phases comprising the emulsion. There are important exceptions to this rule, micro-emulsions are thermodynamically stable while translucent nano-emulsions are kinetically stable [5]. Therefore the purpose of emulsions preparation is to increase drug solubility, drug stability, drug action, taste and appearance. Moreover, there are some limitations of emulsion preparation such as difficult to prepare as special processing techniques are required and choice of suitable emulsifying agent is a high risk.

Nigella sativa is known as *kalojira* in Bangladesh. It is also known as nigella or *kalonji* used as a spice in Indian and Middle Eastern cuisine. The plant is native to Southern Europe; North Africa and Southwest Asia. It is indigenous to the Mediterranean region but now found widely in India (Jammu, Kashmir, Himachal Pradesh, Bihar, Assam and Punjab). The herb is also cultivated in Bengal and North-east India [6]. Black cumin oil is derived from black cumin seed. It is effective in treating diseases, but only limited numbers of dosage forms are available in market containing black cumin oil. In this present study therefore gave emphasize on the formulation and preparation of a stable emulsion containing black cumin oil.

2. Materials and Methods

2.1. Materials

Black cumin oil sample (Amanah Consumer & Beverage Ltd) purchased from local market; E-cap (400 IU) was the

brand product of Drug International Limited. All ingredients used were of analytical grade.

2.2. Formulation of Emulsion

The emulsions were prepared by following continental or dry gum method. The continental method is also referred to as the 4:2:1 method because for every 4 parts by volume of oil, 2 parts of water and 1 part of gum are added in preparing the initial or primary emulsion [7]. If oil contains volatile compounds the ratio will be 2:2:1 [8]. The composition of two developed formulations is presented in table 1.

Table 1. *Composition of developed formulations.*

Ingredients	Justification	Master formula (100 ml)	
		Formula A	Formula B
Black cumin oil	Therapeutic agent	40 ml	40 ml
Distilled water[1] / Distilled water:Glycerin[2]	External phase	40 ml	40 ml
Acacia	Emulsifying agent	20g	20g
Tragacanth	Emulsion stabilizer	2 g	2 g
2 % Na-CMC	Binding agent	2 ml	2 ml
CH_3-paraben	Preservatives	0.06 gm	0.06 gm
Propylene glycol	Water miscible co-solvents	10 ml	10 ml
2% PVP K 30 solution	Viscosity imparting agent	2 ml	2 ml
Ascorbic acid	Antioxidant	0.1 gm	0.1 gm
E-cap (400 IU)	Antioxidant & emollient	4 pieces	4 pieces
Distilled water[1]/ Distilled water:Glycerin[2]	Solvent	q.s. up to 100 ml	q.s. up to 100 ml

1= For formula A, 2= For formula B

At first Acacia was weighted by weighing machine (Shimadzu corporation, Japan) and taken into dried cleaned mortar. Then measured amount of oil added to acacia and triturated homogenously with pestle until cracking sound produced. When the mixture became sticky, external phase (distilled water for formula A and distilled water: glycerin in 50:50 ratio for formula B) was added and mixed well to form primary emulsion in a ratio of 2:2:1 (As black seed oil contains volatile compounds). Rest of the ingredients added to the primary emulsion successively and mixed well to form final emulsion. Formations of final emulsion looked homogenous and were stored in glass bottles at ambient condition (room temperature) to evaluate the quality of preparations in different Stoppard test tubes.

2.3. Procedure of the Performed Tests

Stability testing is an integral part of emulsion development work. Table 2 lists experimental condition commonly used to evaluate emulsion stability.

Table 2. *Stability testing condition and observation period.*

Test	Storage condition	Storage period
Organoleptic property	Ambient temperature	90 days
Determination of pH	Ambient temperature	90 days
Accelerated stability studies (Temperature)	Exposed to sunlight and 4°C	5 days
Accelerated stability studies (centrifugation)	For 5 hours with 30 minutes interval, at 4000 rpm.	Done at first day
Water-drop test	Ambient temperature	90 days
Peroxide value	Ambient temperature	90 days

2.3.1. Organoleptic Property Evaluation

For evaluation of organoleptic property five volunteers were chosen to perform the study for 3 months. Recorded data were compared with each other by a typical thermometer scale (figure 1) as described in Lachman *et al.,* 1990 and results were calculated by statistical application using the formula of Z test [9]. The normal approximation test is

$$Z = \frac{P - 0.5 - 1/(2N)}{0.5/\sqrt{N}}$$

Where P is the observed proportion and N is the sample size. If Z is greater than 1.96 (significant), the treatment differs at the 5% level (two-sided test). The calculation can be simplified as follows

$$Z = \frac{\text{number of} +'S - \text{number of} -'S - 1}{\sqrt{\text{number of} +'S + \text{number of} -'S}}$$

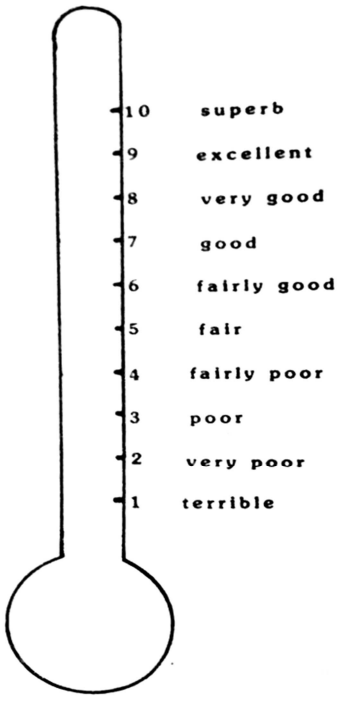

Figure 1. *Typical thermometer scale for evaluating consumer products.*

2.3.2. pH Test

pH test is the most important parts of chemical stability [10]. It is a numeric scale used to specify the acidity or alkalinity of an aqueous solution. pH Indicators was used to measure pH, by making use of the fact that their color changes with pH. Soap type emulsions usually have a pH of 8 or more and will separate if the pH is reduced below 8. Non-ionic emulsified products may be used in a pH range from 3 to 10, depending on the nature of the emulsifier. Cationic emulsions shows generally range in pH from 3 to 7 [11].

2.3.3. Accelerated Stability Studies

Accelerated stability studies normally employed for evaluating the stability of emulsions.

I. Effect of temperature:

The most widely used stress in emulsion testing is temperature. A particularly useful means of evaluating shelf life is cycling between two temperatures and this should be conducted between 4 and 45°C [11]. Care must be taken to protect emulsions against extremes of cold and heat. Freezing and thawing coarsen an emulsion and sometimes break it. However, excessive heat has the same effect [7]. In our study, the emulsions were undergone to different temperature for 5 days. Duration of exposure to sunlight was 4 hours/day and the freezing temperature was 4°C. The effect of both sunlight and freeze were observed and the changes were noted done.

II. Effect of centrifugation:

It is commonly accepted that shelf life under normal storage condition can be predicted rapidly by observing the separation of the dispersed phase due to either creaming or coalescence when the emulsion is exposed to centrifugation. Stokes' law shows that creaming is a function of gravity and an increase in gravity therefore accelerates separation. Becher indicates that centrifugation at 3750 rpm in a 10 cm radius centrifuge for a period of 5 hours is equivalent to the effect of gravity for about 1 year [12]. In our study the preparations were undergone to centrifugation (Laboratory centrifuge, Model 800, China) for 5 hours with 30 minutes interval, at 4000 rpm. At the end of centrifugation the samples were checked to see whether there was any change.

2.3.4. Water-Drop Test

Water-drop test was mainly performed after the phase separation of the emulsion to identify the separated layers. A particularly simple means of determining phase separation due to creaming or coalescence is apparently so trivial that it has evidently not been described in the literature. It involves withdrawing small specimens of the emulsion from the top and the bottom of the preparation after some period of storage and comparing the composition of the two samples by appropriate analysis of water content, oil content or any suitable constituent [3]. In our study water-drop test was performed by adding one drop from different layers of the preparation to a water filled beaker with the aid of a dropper and different layer was detected.

2.3.5. Determination of Peroxide Value

It is a stability testing, variations in peroxide value indicate the unstable condition of the preparation. The test was performed by AOCS method as described in Lubrizol standard test procedure [13].

1. Conducted a blank determination of the reagents each day.
2. Weighed 5 (±0.05) gm of sample into a 250 ml glass Stoppard Erlenmeyer flask. Recorded weight to the nearest 0.01g.
3. By graduated cylinder, added 30 ml of the acetic acid-chloroform solution.
4. Swirled the flask until the sample was completely dissolved (careful warming on a hot plate might be necessary)
5. Using 1 ml Mohr pipette, added 0.5 ml of saturated potassium iodide solution.
6. Stopped the flask and swirled the contents of the flask for exactly one minute.
7. Immediately added by graduated cylinder, 30 ml of either distilled or de-ionized water, stopperd and shaked vigorously to liberate the iodine from the chloroform layer.
8. Filled the burette with 0.05N sodium thiosulfate.
9. If the starting color of the solution is deep red orange, titrate slowly with mixing until the color lightens. If the solution is initially a light amber color, went to step 10.
10. Using a dispensing device, added 1 ml of starch solution as indicator.
11. Titrate until the blue gray color disappeared in the aqueous (upper layer).
12. Accurately recorded the mls of titrant used to two decimal places.

$$\text{Peroxide Value (PV)} = \frac{(\text{S} - \text{B}) \times \text{N thiosulfate} \times 1000}{\text{Weight of sample}}$$

Where, S= Titration of sample and B= Titration of blank

3. Results and Discussion

3.1. Organoleptic Property Evaluation

General appearance of the emulsions formula A & B is opaque. Noticeable change was found in the organoleptic properties of both preparations, color became slightly brownish and dark. At the same time, the odor was changed but there was no significant change in the parameters up to 3 months (table 3). The Z value of emulsions was less than 1.96 for every parameters and this reflects that there was no significant change of color, odor, feel and taste parameters.

Table 3. *Organoleptic property test results.*

Organoleptic Parameters	Results	
	Formula A	Formula B
Color	Insignificant	Insignificant
Odor	Insignificant	Insignificant
Feel	Insignificant	Insignificant
Taste	Insignificant	Insignificant

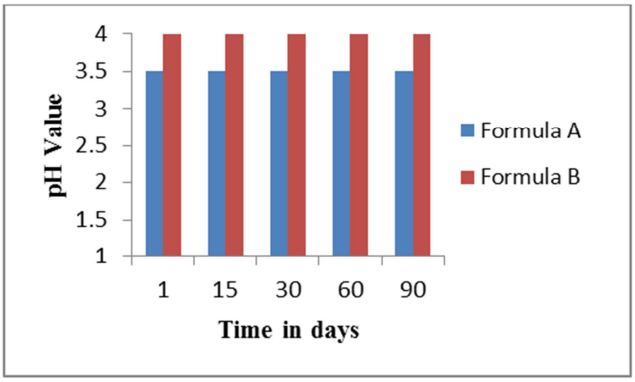

Figure 2. *pH value of emulsions containing black cumin oil.*

3.2. pH Test

No variation in pH value was noticed in emulsions from day 1 to day 90 (figure 2). For both emulsions the value was constant and it was 3.5 and 4 for formula A and B respectively. By analyzing the pH, it can be determined that both emulsions can be categorized as cationic emulsion.

3.3. Accelerated Stability Studies

I. Effect of Temperature

Both preparations of emulsions were undergone to phase separation from day 1 to day 5 during exposure to sunlight and refrigeration in 4°C temperature (table 4).

Table 4. *Accelerated stability study of emulsion by temperature.*

Effect	Layer	Height of layer		Total height	% Ratio (Height of layer/total height)	
		A	B		A	B
Sunlight	Upper layer	7.6 cm	7.9 cm	A= 8.4 cm	90	94
	Lower layer	0.8 cm	0.5 cm	B= 8.4 cm	10	6
Refrigeration	Upper layer	7 cm	8.1 cm	A= 8.4 cm	83	96
	Lower layer	1.4 cm	0.3 cm	B= 8.4 cm	17	4

II. Effect of Centrifugation

After centrifugation, both preparations separated into different layers (table 5). The separation was clear, applied centrifugal force clearly separated the homogenous mixture of emulsion within 5 hours.

Table 5. *Effect of centrifugation on emulsion formulations.*

Layers	Height of layer		Total height	% Ratio (Height of layer/total height)	
	A	B		A	B
Bottom layer	0.8 cm	1.1 cm	A= 8.4 cm B= 8.4 cm	9	13
Next to middle layer	4.1 cm	---		50	---
Middle layer	0.5 cm	4.5 cm		6	54
Next to the upper layer	2.2 cm	---		26	---
Upper layer	0.8 cm	2.8 cm		9	33

3.4. Water-Drop Test

The aqueous and oil layer was identified by drop test (table 6). Formulations were separated into two layers, one of which is aqueous and another is oil layer.

Table 6. *Identification of oil and water layer by drop test.*

Layers	Formula A		Formula B	
	Observation	Comments	Observation	Comments
Upper layer	Drop remains as droplet at the bottom but doesn't spread.	Aqueous layer.	Drop disseminates at the bottom.	Aqueous layer.
Lower layer	Drop divided into two parts, one part raised from bottom to the top (oily) and another part spreads at the bottom.	This layer is a mixture of oil and aqueous part.	Drop spreads at the bottom but some part rose to the top from bottom.	Due to having glycerin, it acts as viscosity enhancer in the preparation and bridge to both oil and water. So, oil and water part are too close of each other. This layer is of oil and water mixture.

3.5. Determination of Peroxide Value

The results of peroxide value determination of black cumin oil emulsions are very much confusing, and no complete conclusion can be drawn due the fluctuating results after 90 days evaluation (table 7, figure 3).

Table 7. *Peroxide value of Black cumin oil, E-cap, Emulsion A and Emulsion B.*

Day	Peroxide value (milliequivalents peroxide/1000 gm)					
	1	7	15	30	60	90
Black cumin oil	36.76	40.34	40.17	44.92	33.56	26.78
E-Cap	1.6	3.98	3.98	11.98	8.99	13.55
Emulsion A	976.93	971.58	789.17	49.98	41.56	21.96
Emulsion B	255.87	409.26	424.54	255.47	46.2	6

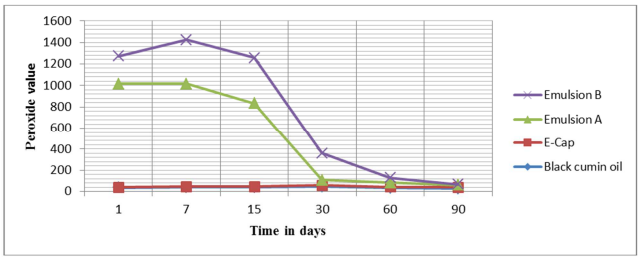

Figure 3. *Peroxide value versus time curve of Black cumin oil, E-cap, Emulsion A and Emulsion B.*

4. Conclusion

Though, the preparations were separated into two distinct layers on their different stage of test, but the separation was reversible and after re-shaking it retains its homogenous look. It may occur because of improper storage condition that can affect the quality of these preparations. Mechanical error and the reagents used may also be responsible for this.

Comparing with all performed tests on emulsions, it is satisfactory that Emulsion formula B shows much better result than formula A. It shows lower peroxide value than formula A, and the aesthetic value of formula B is also higher than formula A, it may be due to the presence of glycerin on formula B. Glycerin acts as viscosity enhancer and links two layers like a bridge, so tendency of phase separation is much lower. So, further research in this sector is required to find out the causes of phase separation. In that case, formula B can be a good semi-solid dosage form of black cumin oil.

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