ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Investigation of Oil in Water Emulsion properties depending on the change of Emulsifiers combinations

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ABSTRACT:
Oil in water emulsion formulations as dosage forms for topical application are intended to produce the therapeutic effect in the skin. Their quality features mainly depend on the choice of emulsifying combination composition. This work aimed at establishing a mixture of oil phase, water and combinations of different kinds of emulsifiers to screen factors that affect the quality features of oil in water emulsion cream`s formulations. Eleven formulations were prepared with different ratio of different kinds of emulsifiers by a high temperature method. The emulsions were prepared with oil phase having calculated HLB value range 10.96 and emulsifiers having HLB values ranged from 0.20 to 14.60. The formulated oil in water emulsion compositions were evaluated for physical stability, rheological properties, colloidal and thermodynamic stability, microscopy observation. The results showed that not all formulations were physically stable after 24h storage period. Stable formulations were subjected to rheological properties studies. Some of samples presented the flow which was close to the Newtonian fluid, others showed pseudo-plastic flow type. The results of colloidal and thermodynamic stability evaluations allowed to establish the regularities between the type of the flow and formulations` stability. When varying the ratio of emulsifiers, the total HLB range of the mixture changed, which also affected the dispersion properties of emulsion systems. With certain ratios of emulsifiers the dispersion of the system increased.

KEYWORDS: Pharmaceutical Technology, Cream, Emulsion, Rheology, Stability, Oil in water Emulsion, Emulsifiers, Stability, Casson Rheological Model, Microscopy Observation.

INTRODUCTION:
Emulsion cream formulations are intended to produce the therapeutic effect at specific targets in the skin (softening, nourishing, protective effect of the semi-solid base in combination with the pharmacological actions of active pharmaceutical ingredients). The cream is based on biphasic systems, consisting of two immiscible liquids where one of which is dispersed throughout the other to form oil in water or water in oil emulsions[1]. Their pharmaceutical development need to be based on Quality by Design strategy and is aimed to obtain product with intended quality performance. That is why during emulsion cream development, a detailed process identification and control of critical variables is needed to yield a final product with the required quality profile[2].

Obtaining of emulsion cream formulation is a complex process due to the thermodynamically instability of emulsion systems[3,4,5]. Emulsion stability refers to it`s ability to resist changes in its physico-chemical properties over time[6]. The stability of emulsion systems depends on many factors: quantitative ratio of all components, compositional materials (concentration, size, oil composition, density, viscosity of oil, water droplet diameter, water droplet electrification, pH), processing conditions (preparation methods) and using proper emulsifying or surface stabilizing agents[4-12]. Selection of an appropriate emulsifier or their combinations is one of the most important decisions when formulating emulsion-based products[3]. This work aimed at establishing a mixture of oil phase, water and combinations of different kinds of emulsifiers to screen factors that affect the quality features of oil in water emulsion cream`s formulations.

Received on 23.05.2023 Modified on 29.07.2023
Accepted on 19.10.2023
Research J. Pharm. and Tech 2023; 16(12):5603-5609.
DOI: 10.52711/0974-360X.2023.00906

MATERIALS AND METHODS:

Materials:

Silver citrate solution (Nanomaterials and Nanotechnologies LLC, Ukraine), Paraffinum liguidum (Sfera Sim, Ukraine), Isopropyl Myristate (SAFC Merck), Isopropyl palmitate (Jinan Future Chemical Industry Co. Ltd, China), Eutanol G (Octyldodecanol) (BASF, Germany), Dimethicone (Sigma-Aldrich Chemie GmbH, Germany), LASEMUL 92 N 40 PH (Glyceryl monostearate) (IQL s.a.u., Spain), LANETTE® O OR (Cetyl Stearyl Alcohol) (BASF, Germany), Emulsifier No. 1 (Electrogazokhim LLC, Ukraine), Polysorbate 80 (ERCA, Italy), purified water were of analytical/ pharmaceutical grade.

Methods:

Preparation of oil in water emulsion cream:

Oil in water emulsion cream`s formulations were prepared using different kinds of emulsifiers while the composition of the oil phase was unchanged. Eleven formulations were prepared (F1–F11) with different ratio of different kinds of emulsifiers (Line 1-Line 4). The composition is shown in tab. 1.

Formulations were prepared by a high temperature method (oil and water phases were separately heated to (70-75)ºC). The emulsification of the formulations was carried out with continuous stirring (5000rpm) using a homogenizer Polytron® System RT 2500, Kinematica AG, Switzerland. After cooling to (50-55)°C, a silver citrate solution was added with continuous stirring until uniformly mass achieved. Formulations were kept undisturbed for 24 h before their evaluation.

Table 1: Ingredients used in the preparation of oil in water emulsion cream

Ingredient (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Silver citrate solution	0,5										
Paraffinum liquidum	5										
Isopropyl Myristate	5										
Isopropyl palmitate	5										
Octyldodecanol	5										
Dimethicone	1										
Purified water	up to 100										

Combinations of different emulsifiers	Line 1 Glyceryl monostearate: Cetyl Stearyl Alcohol	Line 2 Emulsifier No. 1: Polysorbate 80	Line 3 Glyceryl monostearate: Polysorbate 80	Line 4 Emulsifier No. 1: Cetyl Stearyl Alcohol
F1	0:10			
F2	1:9			
F3	2:8			
F4	3:7			
F5	4:6			
F6	5:5			
F7	6:4			
F8	7:3			
F9	8:2			
F10	9:1			
F11	10:0			

Hydrophilic–lipophilic balance (HLB) plays a crucial role to balance the interfacial tension between the two immiscible liquids in emulsion systems [13, 14]. HLB value of oil phase was calculated as 10.96. As the HLB value increases, the emulsifiers become more soluble in water and their action transfer to be oil in water emulsifiers [15]. HLB values of emulsifiers in the range of 8 to 18 stabilize oil in water emulsions [13]. The HLB range of emulsifiers` combinations was calculated in order to determine the ratio of different kinds of emulsifiers in each formulation with the same content of the oil phase and the total concentration of emulsifiers, according to the formula:

HLB range of the emulsifiers` combination =
$(x_1 * HLB + x_2 * HLB)/100$,
where

x_1 and x_2 are the quantitative contents of different kinds of emulsifiers. The results are shown in tab. 2.

Table 2: HLB range of the emulsifiers` combination

Formulations / Lines	Line 1	Line 2	Line 3	Line 4
F1	0.20	14.60	14.60	0.20
F2	0.70	14.17	13.69	1.21
F3	1.26	13.74	12.78	2.22
F4	1.77	13.31	11.87	2.32
F5	2.32	12.88	10.96	4.24
F6	2.47	12.45	10.05	5.25
F7	3.38	12.02	9.14	6.26
F8	3.91	11.59	08.23	7.27
F9	4.44	11.16	7.32	8.28
F10	4.97	10.73	6.41	9.29
F11	5.50	10.30	5.50	10.30

Evaluation of oil in water emulsion cream:
Visual inspection
The physical stability of the formulations was evaluated visually after 24h storage period.

Rheological Properties
Rheological properties were determined by rheometer Rheolab QC, Anton Paar, Austria using the C-CC27/SS Coaxial Cylinder System at 25ºC.
The Casson model was used. It is expressed as[16]:

$$\tau^{\frac{1}{2}} = \tau_0^{\frac{1}{2}} + (\mu_0 \cdot \gamma)^{\frac{1}{2}}$$

Where τ is shear stress, τ_0 is Casson yield stress, μ_0 is Casson plastic viscosity, γ is shear rate.

Colloidal Stability:
The colloidal stability test was carried out using laboratory centrifuge with a set of test tubes, mercury thermometer with an interval of measured temperatures from 0 to 100°C (division value 1ºC), stopwatch and water bath. The test tubes were filled with 2/3 of their volume (approximately 9g) by oil in water emulsion cream`s formulations. The test tubes were heated to (42.5±2.5)°C for 20 min using water bath, than wiped from the outside and placed into centrifugal sockets. Centrifugation duration was 5min at 6,000rpm. Formulations were considered stable if after centrifugation no separation was observed. The analysis was repeated if at least in one test tube separation or sedimentation were observed. The formulation was considered unstable if retest showed at least in one test tube separation or sedimentation.

Thermodynamic stability:
5-6 glass test tubes with a diameter of 15mm and a height of 150mm were filled with 8-10ml of formulations and placed in the TC-80M-2 thermostat at (42.5±2.5)°C for 7 days. Then formulations were stored for 7 days in a fridge at (6±2)°C, and then kept at (25±2)°C for 3 days. Stability was determined visually: the sample was considered stable if there was no separation in any of the test tubes.

Microscopy observation:
The oil in water emulsion creams formulations were evaluated to their microscopy using Lumam P1 microscope, equipped with a DMC 300 digital camera. A small drop of emulsion, which was taken with a glass rod from the middle part of prepared formulations, was placed on the slide glass and covered with a cover glass in form to obtain a homogeneous thin layer. A lens with magnification of 20x and an intermediate magnification of 1.6 times were used to view formulations. Photomicrographs were obtained using the Scope Photo software. Comparison scale (50μm) was applied using a calibrated system.

RESULTS AND DISCUSSION:

Different formulations of oil in water emulsions were made with the ration of the oil phase 21%. Paraffinum liquidum, isopropyl myristate, isopropyl palmitate and octyldodecanol formed oil phase. Dimethicone was used as emollient/skin conditioning agent[13]. Combination of emulsifiers were used in different proportions with the ration of 10% concentration. Glyceryl monostearate, cetyl stearyl alcohol, emulsifier No. 1 and polysorbate 80 were used. Purified water was added as water phase up to 100%. The emulsions were prepared with oil phase having calculated HLB value range 10.96. Cetyl stearyl alcohol has an HLB value of 0.2, which is very lipophilic. Glyceryl monostearate has an HLB value of 5.5 and is lipophilic. Polysorbate 80 has an HLB value of 14,6 and is highly hydrophilic. Emulsifier No. 1 has an HLB value of 10.3 and is between the two: lipophilic and hydrophilic[14]. Emulsifiers combinations used had HLB values ranged from 0.20 to 14.60.

Not all formulations formed satisfactory emulsion systems in their physical appearance. Thus, Line 1 F11 and Line 3 F11 formulations showed a complete separation of the oil and water phases immediately during preparation. This can be explained by the fact that the HLB value from the emulsifiers combinations was less than the HLB value of the oil phase[13]. After 24 h storage period at (25±2)ºC Line 2 F1-F6 and Line 3 F1-F3 formulations showed separation or sedimentation of phases. The HLB value of emulsifiers was greater than 12.45, the combination exhibits hydrophilic character[14].

All formulations that retained physical stability were subjected to rheological properties studies. Rheological properties allow to characterize the formulations by the type of flow, yield stress, and viscosity value. Yield stress allows the investigation of the amount of applied force that is needed to cause the semi solid dosage form to flow out of a tube[17]. The used software automatically builded shear stress-shear rate graphs and performed calculations using the Casson rheological model, as one of used to describe the rheology of visco-elastic non-Newtonian fluids[16].

Fig. 1-4 shows shear stress-shear rate graphs of formulations Lines 1-4. Formulations can be divided into two groups by tracking the behavior of rheological graphs. First group include formulations whose flow begins at a slight yield stress (τ_0 <2.04 Pa) – Line 1: F1-F3, F10; Line 2: F7; Line 3: F4-F10; Line 4: F1-F4. This formulations presented the flow which was close to the Newtonian fluid. Therefore, it can be assumed that the emulsion formed was of the type water in oil, rather than oil in water as required. Second group included formulations whose flow began at a certain yield stress

exceeding 2.04 Pa. Shear stress-shear rate graphs described the pseudo-plastic flow type. The viscosity decreases with an increase in shear stress[13].

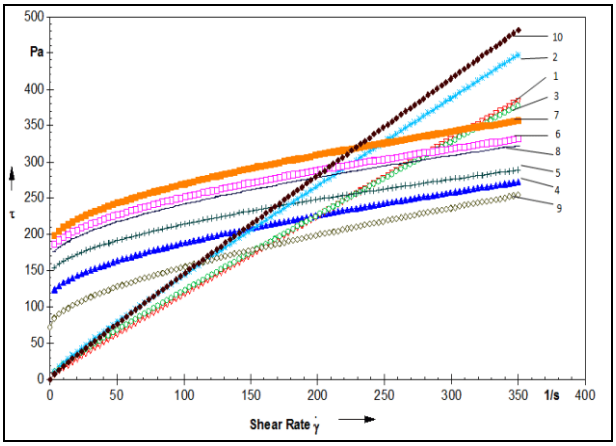


Fig. 1: Casson Shear Stress-Shear Rate Graph of Line 1 F1-F10 formulations

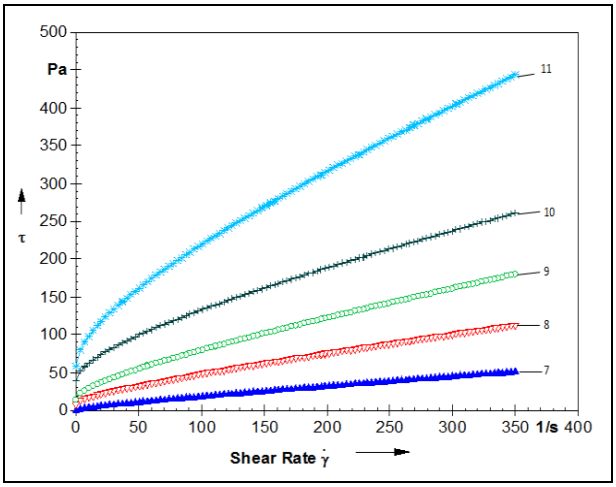


Fig. 2: Casson Shear Stress-Shear Rate Graph of Line 2 F1-F10 formulations

Table-3 shows data of shear stress calculated by Casson`s mathematical model.

Colloidal and thermodynamic stability were studied to establish the regularities between the type of the flow and formulations` stability. The results are shown in table- 4. The difference in the contributed HLB value of the oil phase and HLB value that was achieved by different emulsifier`s combinations is the reason for the instability of emulsion system[13].

- unstable formulation, rheological properties were not studied

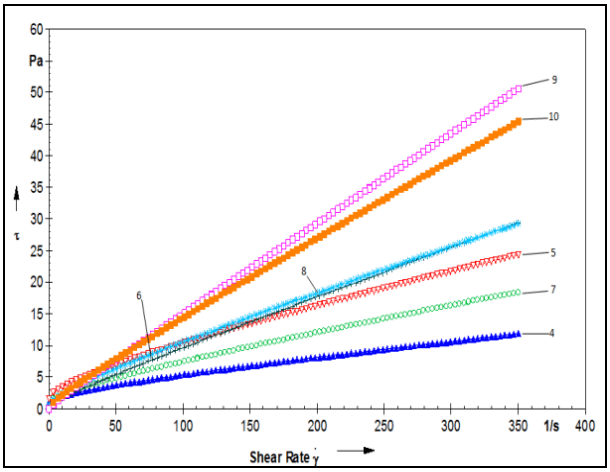


Fig. 3: Casson Shear Stress-Shear Rate Graph of Line 3 F1-F10 formulations

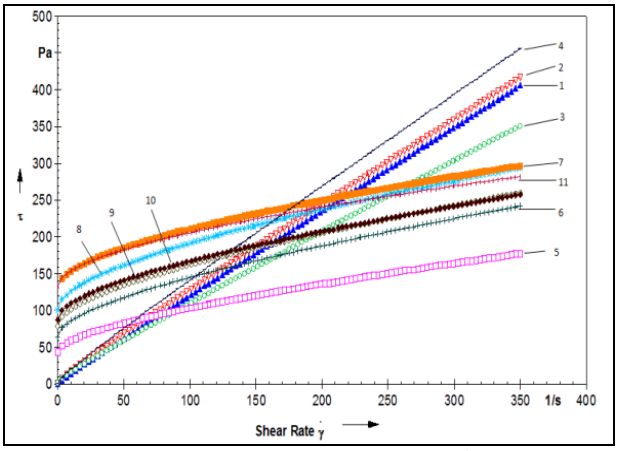


Fig. 4: Casson Shear Stress-Shear Rate Graph of Line 4 F1-F10 formulations

Table 3. Oil in water emulsion cream formulations` shear stress

Line1:	τ, Pa	Line2:	τ, Pa	Line3:	τ, Pa	Line4:	τ, Pa
F1	0.60	F1	-	F1	-	F1	0.20
F2	1.96	F2		F2		F2	0.75
F3	2.04	F3		F3		F3	0.89
F4	110.87	F4		F4	1.03	F4	1.053
F5	141.89	F5		F5	1.55	F5	42.69
F6	172.89	F6		F6	1.20	F6	63.69
F7	184.24	F7	1.45	F7	0.85	F7	129.87
F8	163.17	F8	6.83	F8	0.58	F8	100.33
F9	71.63	F9	14.16	F9	0.01	F9	87.25
F10	0.51	F10	38.58	F10	0.17	F10	78.12
F11	-	F11	132.75	F11	-	F11	132.75

Table 4. Oil in water emulsion cream formulations` colloidal and thermodynamic stability

Line 1	Colloidal stability	Thermo-dynamic stability	Line 2	Colloidal stability	Thermo-dynamic stability	Line 3	Colloidal stability	Thermo-dynamic stability	Line 4	Colloidal stability	Thermo-dynamic stability
F1	-		F1	-	-	F1	-		F1	-	
F2			F2			F2			F2	+	
F3			F3			F3			F3		
F4	+		F4			F4			F4		
F5			F5			F5			F5		
F6			F6			F6			F6		
F7			F7			F7			F7		
F8			F8	+		F8			F8		
F9			F9			F9			F9		
F10	-		F10			F10			F10		
F11			F11	+		F11			F11		

+ stable formulation, - unstable formulation

When varying the ratio of emulsifiers, the total HLB range of the combination changed, which also affected the dispersion properties of emulsion systems. Fig. 5-6 show microphotographs of formulations that were stable. With certain ratios of emulsifiers the dispersion of the system increases.

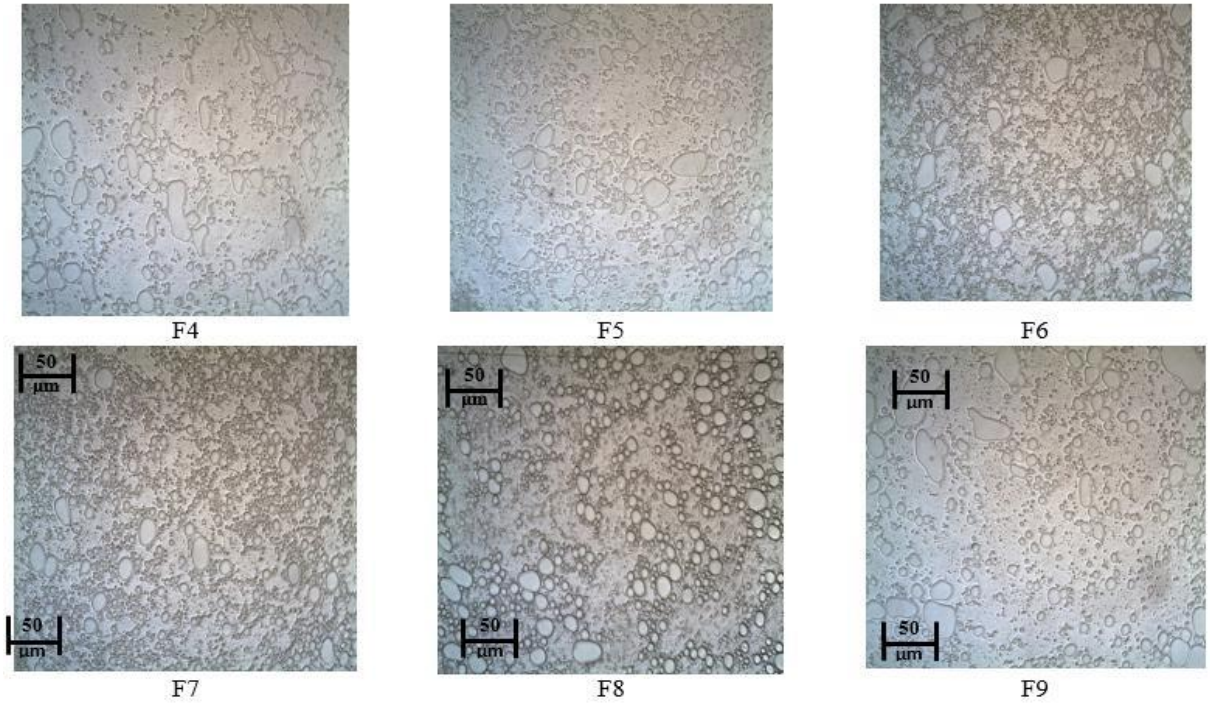
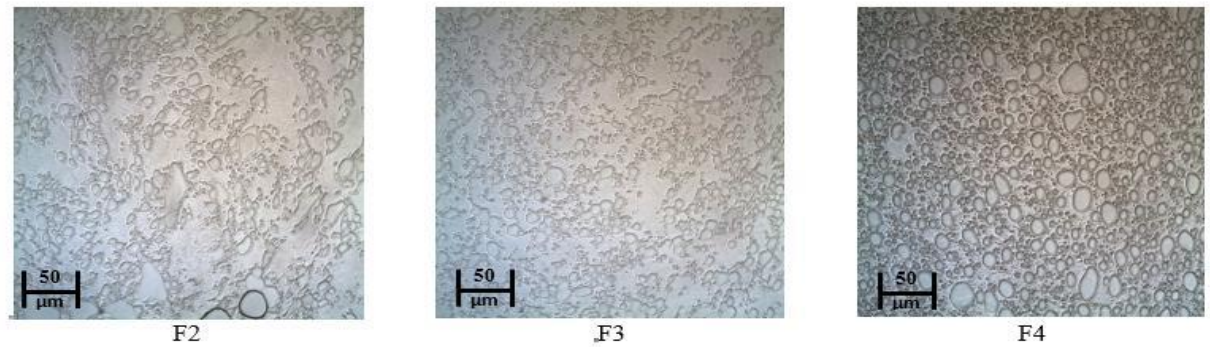


Fig. 5 Microphotographs of Line 1 F4-F9 formulations



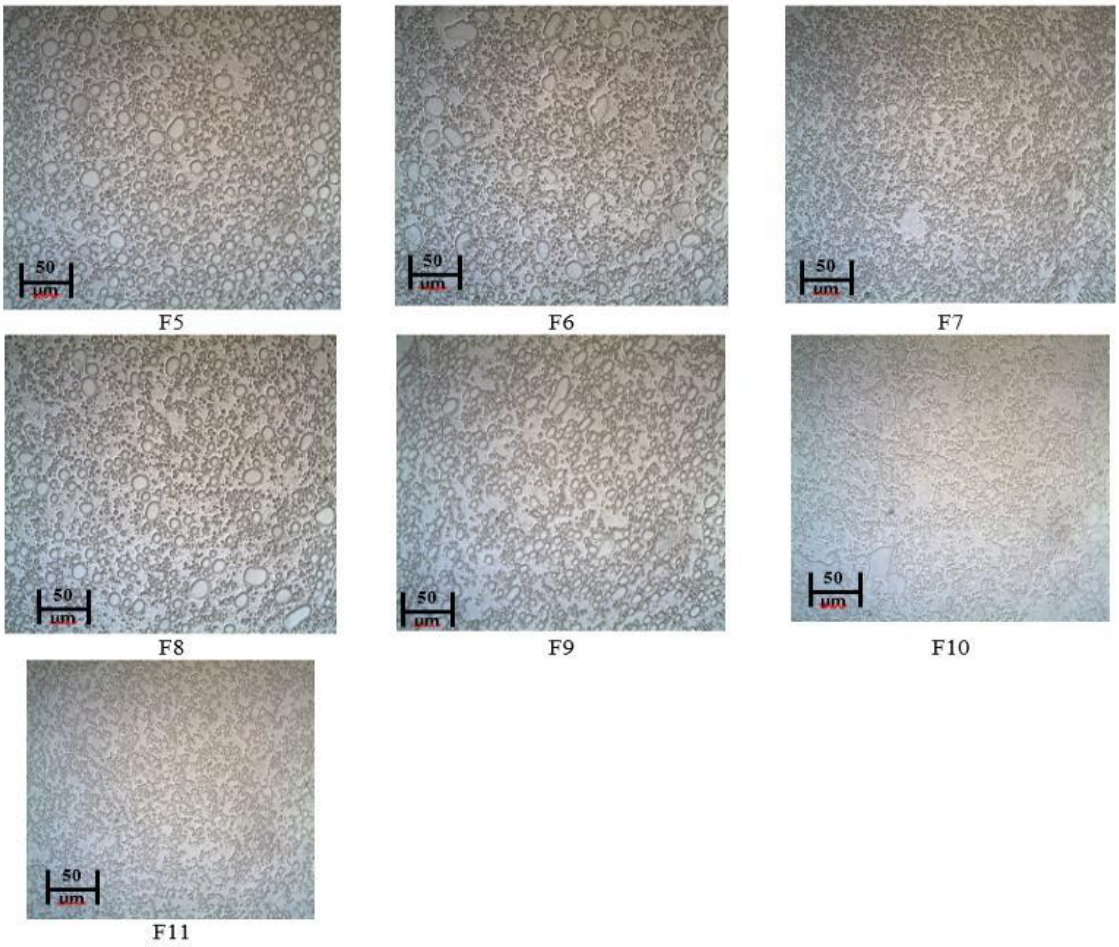


Fig. 5 Microphotographs of Line 4 F2-F11 formulations

CONCLUSION:

Thus, it was investigated that formulation contains emulsifier No. 1 10% with HLB value 10.3 was the most stable oil in water emulsion, had pseudo-plastic flow behavior due to a balanced hydrophilic and lipophilic phases and was selected for further research of emulsion cream`s pharmaceutical development. The stability of this formulation is due to the fact that the HLB contribution of the oil phase is almost equal to the HLB that was achieved by the emulsifier.

CONFLICT OF INTEREST:

No.

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