Original Research Article

Effect of Hydrophilic-Lipophilic Balance (HLB) Values of Surfactant Mixtures on the Physicochemical Properties of Emulsifiable Concentrate Formulations of Difenoconazole

Abstract

This study was designed to investigate the effect of hydrophilic-lipophilic balance (HLB) values of different surfactant mixtures on the physicochemical properties of emulsifiable concentrate formulations of difenoconazole. Physical tests of emulsion characteristics and storage stability studies were performed for the different samples to predict the stability of these formulations. Different parameters such as active ingredient content, pH, refractive index, surface tension, viscosity, flash point, persistent foam was determined for the prepared samples. The results showed that difenoconazole could be formulated as a stable emulsifiable concentrate by using a mixture of surfactants at HLB values 9.7, 11.9, 12.5 and 13.1. The storage stability test showed that the decomposition rate of the active ingredient content of difenoconazole in different stable formulations was within the acceptable limits of FAO Specifications. The physical and chemical properties of the stable formulations fulfilled the requirements of EC formulation.

Keywords: Formulation, Hydrophilic-Lipophilic Balance, Surfactants, Difenoconazole, Emulsifiable concentrate.

1. Introduction.

Pesticides are important agrochemicals that are widely used to control pests, preventing crop loss caused by major diseases and pests and increases crop yield. (Qin, Zhang et al. 2017; Wang, Wang et al. 2017; Cui, Lv et al. 2019; Wang, Cui et al., 2019). Most pesticides need to be formulated using suitable formulations.

The pesticide formulations play an important role in delivering agrochemicals to target sites and enhancing their efficacy (Shao, Xi et al., 2018). Liquid formulations are preferred by the farmer for preparing spray solutions for several reasons; they can be measured volumetrically, are easy to handle, spontaneously form stable emulsions and provide suitable container design, are usually easy to rinse out of the package and do not cause application problems.

Emulsifiable concentrate formulations of pesticides (also referred to as EC) have been very popular for many years and represent the biggest volume of all pesticide formulations (about 40%) in terms of global volume usage, and are the most commonly used delivery system for increasing the yield and quality of crop production (Encinar, González et al. 2005). In addition this formulation easy to produce, handle, transport, store, possessing excellent thermodynamics and storage stability (Vanitha 2010, Rajmani, Sunita et al. 2014, Thakur, Sunita et al. 2014, Sarwar 2015). The advantages of EC formulations are that they require little agitation , they are not abrasive, do not plug screen or nozzles, leave little visible residue on treated surfaces, are useful for water-insoluble active ingredients and are easy to apply with high efficiency (Kozuks and Ohtsubo. 2009).

EC formulations are typically optically transparent oily liquid formulations that are prepared by dissolving the active ingredient in organic solvents (such as benzene, toluene, xylene, etc.), and they also contain surfactants and other additives. These systems are then diluted with water before utilization, which leads to the spontaneous formation of an oil-in-water emulsion that contains the active ingredients inside oil droplets (Feng, Zhang et al., 2018), (Aguilar, Coronel et al., 2019). The stability of the emulsions may be affected by several factors such as the Hydrophilic-Lipophilic Balance (HLB) value (Losada-Barreiro, Sánchez-Paz et al., 2013), concentration of active ingredients (Hallouard, Dollo et al., 2015), and addition of surfactant type (Feng, Shi et al. 2016), among others (Feng, Chen et al., 2018).

Surfactant emulsifier blends are added to these formulations to ensure spontaneous emulsification into the water in the spray tank. Surfactant suppliers provide advice on the selection of a “balanced pair” emulsifier blend which is frequently necessary to ensure good emulsion stability after dilution in water of varying degrees of hardness. The correct balance of surfactant emulsifiers can be obtained by knowing the Hydrophilic-Lipophilic Balance (HLB) values for the surfactants. Surfactants in the HLB range of 8-18 normally give good emulsions (Dennis 2003, Knowles 2008). The optimum ratio of surfactants is determined by experimentation to give spontaneous emulsification in water (strike or bloom), and a stable emulsion with minimum creaming and no oil droplet coalescence (Knowles 2005, Ferreira, Santiago et al., 2010). Therefore, the present study aimed to prepare several emulsifiable concentrate formulations of difenoconazole and study the effect of HLB values of different surfactants on the stability of the prepared formulations.

2. Materials and Methods

2.1. The Test Active Compound

Difenoconazole (Technical grade, purity 97%) was purchased from China. Difenoconazole (Cis, trans-3-chloro-4- [4-methyl-2-(1H-1, 2, 4-triazol-1-ylmethyl)-1, 3dioxolan-2-yl] phenyl 4-chlorophenyl ether), CAS Registry No. [119446 - 68-3] chemical structure is shown in Figure 1. The substance is white to light beige crystals with a melting point 82.0 - 83.0 °C. The value of Kow logP (the octanol/water partition coefficient) is 4.4 (at 25°C). The solubility is 15 mg/l in water at 25°C and >500 g/l in acetone, dichloromethane, toluene, methanol and ethyl acetate, 3 g/l in hexane, 110 g/l in octanol at 25°C, and it is stable up to 150 °C.

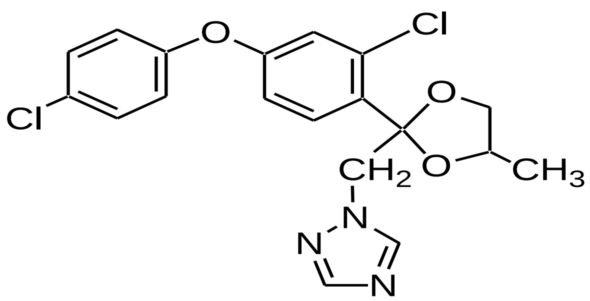

Fig 1. Structure of difenoconazole

2.2. Chemicals

Nonionic Surfactants: Soprophor CY/8 (Ethoxylated tristyryl phenol, HLB 13.7); Alkamuls RC (ethoxylated Castor oil, HLB 10.5); Alkamuls 14/R (Ethoxylated Castor oil, HLB 14.9); Arkapol N 100; nonylphenolpolyglycoether (10EO), HLB 13.5 were kindly supplied by Rhodia-Home, Personal Care &Industrial Ingredients, Milano, Italy. Anionic Surfactants: Rodacal 60/BE (dodecyl benzene sulfonate, calcium salt, HB 8.3) and Geronol FF4, mixture of anionic and non-ionic derivatives, HLB12.7 were kindly supplied by Rhodia-Home, Personal Care &Industrial Ingredients, Milano, Italy. Tween 80 (Polyoxyethylene (20) Sorbian monooleate, HLB 15.00); Tween 20 (Polyoxyethylene(20) sorbitanmonolaurate, HLB 16.7), Span 20 (Sorbitanmonolaurate, HLB 8.6) and Span 80 (Sorbitanmonooleate, HLB 4.3) and calcium carbonate were purchased from Sigma-Aldrich Chemie GmbH, Riedstr, Steinheim, Germany. Dimethyl formamide and polyethylene glycol were purchased from ADWIC. El Nasr Pharmaceutical Chemical Co., Egypt. Magnesium oxide, methyl red and Solvesso 100 were purchased from Qualikems Fine Chemicals PVT Ltd. India. Ammonia solution was purchased from Prolabo. All materials were used as received without further purification. Deionized water used to prepare the solutions was obtained from a Milli-Q-system (Millipore).

2.3. Preparation of Difenoconazole EC

Emulsifiable concentrate (EC) formulation of difenoconazole was prepared by simple mixing method. Difenoconazole technical grade, solvent, cosolvent, anti-freezing agent and mixed surfactants. The proportion between the two surfactants was calculated to obtain HLB values in TableA. The resulting formulation was stored in a glass bottle at room temperature (25 ± 2°C) and protected from light.

Table A. Composition of Difenoconazole EC

Formulations Code	Mixed surfactants	HLB value
F1	Alkamuls RC: Rodacal 60/BE	9.7
F2	Tween 80: Span 80	11.4
F3	Soprophor Cy/8: Rodacal 60/BE	11.9
F4	Arkapol N 100: Alkamuls RC	12.5
F5	Alkamuls 14/R: Geronol FF4	13.1
F6	Tween 80: Alkamuls RC	13.5
F7	Tween 20: Span 20	14

2.4. Determination of Difenoconazole Content

The content of active ingredient (AI) of difenoconazole in the prepared formulations was detected using (Unicam pro GC with FID) equipped with Electron Capture Detector (ECD) programmed for external standardization using peak area. Gas Chromatography with the following conditions: Oven: 260°C, Injector: 280°C, Detector (FID): 290°C.

2.5. Stability Tests

Pesticides chemical stability is determined by its ability to withstand (a) the deteriorating effects encountered during storage formulation and (b) the environment to which it is subjected after application (Allawzi, Allaboun et al., 2016). The formulation stability tests included emulsion characteristics, stability at 0°C, accelerated storage procedure at 54°C, and persistent foaming.

Emulsion Characteristics and Re-emulsification:

In the emulsion characteristics test, the emulsion and re-emulsion stability tests were performed according to CIPAC MT 36.3. (CIPAC, 2016). The formulation was diluted at 30 ± 2°C with CIPAC Standard A and D waters prepared as per CIPAC MT 18 (CIPAC, 1995). In the emulsion characteristics experiment, 5 ml of the formulation sample was separately mixed with 95 ml standard CIPAC A water (20 ppm hardness, pH 5.00- 6.00,Ca^{2+}: Mg^{2+}=1:1) and CIPAC D water (342 ppm hardness, pH 6.00- 7.00,Ca^{2+}:Mg^{2+}= 4:1) in a 100 milliliter measuring cylinder to produce 100 milliliter of aqueous emulsion. The cylinder was then stoppered and inverted once then it was noted if the emulsion was homogenous or not, after 30 sec it was inverted 10 times and left to stand. Emulsion stability was determined according to CIPAC MT 36.3 (CIPAC, 2016) by recording traces of oil and cream < 2 ml at 0, 0.5, 1, 2, 24, and 24.5 h.

Storage Stability

Accelerated stability tests at elevated temperatures are designed to increase the rate of chemical degradation or physical change of a product. Accelerated testing was performed at elevated temperatures to obtain information on the shelf life of a product in a relatively short time. Accelerated testing involves extrapolations from higher to lower temperatures and from shorter to longer storage periods. The accelerated storage test at elevated temperatures is performed by placing 50 ml of the product in a tightly capped bottle in the oven at 54 ±2°C for 14 days. The volume of any separated material at the bottom of the tube was then recorded CIPAC MT 46.3 (CIPAC, 2016).

Storage at low temperatures may result in crystallization of active constituent, significant changes in viscosity or separation of the formulation. The liquid formulation was tested at 0 ± 2°C for 7 days as per CIPAC MT 39.39 (CIPAC, 2016). A 100 ml of the sample was transferred to a glass tube and cooled in a refrigerator at 0 ± 2°C for 7 days, the tube was left to stand at room temperature for 3 hours. The volume of any separated material at the bottom of the tube was recorded.

Persistent Foam

Persistent foam is a measure of the amount of foam likely to be present in a spray tank or other application equipment following dilution of the product with water. A specified amount of the prepared formulation is added to CIPAC standard waters (95 ml) in the measuring cylinder and made up to the mark. The cylinder is stoppered and inverted 30 times. The cylinder is left to stand undisturbed for the specified time. The volume of foam was noted at 0, 10 s, and 1, 3, and 12 min according to CIPAC MT 47.2 (CIPAC, 2016).

2.6. Physical Characterization

The pH of a 1% solution of the formulations was measured using a pH meter (Jenway model pH 3510) which was recalibrated before testing; the measurements were carried at 25°C by direct immersion of pH glass electrode into EC samples as per CIPAC MT 75.3 (CIPAC, 2016).

The Refractive index is an optical measurement of a material's ability to bend a beam of light. Also, the refractive index may be used to determine the purity of the material; the refractive index of the EC samples was measured using a digital ABBE Refractometer, ATAGO, Co., LTD, Japan by placing one drop of the EC formulation on the slide at 25°C. Distilled water was used as a standard, it had a refractive index of 1.3330 (ASTM, 2016).

The viscosity was measured using ″Brookfield DV II+ Pro″ digital Viscometer (Brookfield, USA) UL rotational adaptor, the temperature was kept at 25°C during the measurement by water bath (Model: TC-502 USA) and each reading was taken after equilibrating the sample .The data were acquired via a personal computer using Rheolac software developed by Brookfield Engineering Lab. Five replicates were conducted for the sample and the average was reported and expressed as millipascal-second (mPa.s) (ASTM, 2018).

Surface tension was measured using “Sigma 700” by Wilhelmy plate method, the instrument was recalibrated before testing and the sample measured was clean, homogenous, and free from any bubbles and has a stable surface. The surface tension of the EC samples was recorded (ASTM, 2014).

Density was measured using a digital density meter model DDM2910 with a touch screen (Rudolph Research Analytical, USA) (ASTM, 2017).

Measurement of the flash point of the prepared EC was carried out by a tag open cup method by Koehler instrument company, INC, USA. The flash point was recorded as the temperature at the thermometer when a flash appeared (ASTM, 2016).

3. Results and Discussion

3.1. Storage stability

Storage stability is an effective factor in determining the shelf-life of pesticide formulations. Delamination and degradation of AIs in pesticides occurred particularly during long-term storage, leading to reduced effectiveness; hence, whether agrochemical formulations can maintain excellent stability is important for practical applications (Aguilar, Coronel et al., 2019).

We investigated the stabilities of difenoconazole samples including stability at 0 ± 2°C for 7 days and accelerated storage in the oven at 54 ± 2°C for 14 days, and the experimental results are summarized in Table 1. The AI contents of difenoconazole in freshly prepared, 0°C for 7 days and 54°C for 14 days was within the acceptable range of defined specification (+/- 5%) (FAO/WHO specifications, 2018) and the stable formulations had no phase separation and no sedimentation, which indicated that formulated EC is thermodynamically stable.

Table.1. Active ingredient content of difenoconazole EC formulations

Test Period	Fresh Form.	7 days		14 days	
Formulations Code	AI	AI	% loss	AI	% loss
F1	25.94	24.91	3.97	24.65	4.97
F3	25.67	25.06	2.37	25.04	2.45
F4	24.37	24.06	1.27	24.03	1.39
F5	25.09	24.92	0.67	24.63	1.83
F6	24.89	24.76	0.52	24.52	1.48
F7	25.03	24.92	0.44	24.30	2.92

3.2. Emulsion stability

The most important factor in emulsion preparation is the choice of appropriate surfactants that will emulsify the selected ingredients satisfactorily and preserve their stability. Surfactant mixtures play a promising role in surface chemical applications and often exhibit interfacial properties more pronounced than those of the individual surface-active components of the mixture (Myers, 1998; Rai and Pandey, 2013). The hydrophilic-lipophilic balance (HLB) value of surfactants plays an important role in the determination of its functionality. Emulsions with surfactants of HLB < 6 tend to be oil soluble and stabilize water-in-oil, whereas emulsions with surfactants of HLB >10 tend to be water soluble and stabilize oil-in-water (Feng, Zhang et al., 2018)

The emulsion and re-emulsification stability test state that the formulation when diluted at 30 ± 2°C with CIPAC standard waters A and D shall comply with the specification of emulsification of emulsifiable concentrate formulation (FAO/WHO, 2018). The maximum level of cream and precipitate layer didn't exceed 2 ml after 0.5, 2 and 24.5 hrs from dilution.

Results in Tables (2-4) showed that the effect of HLB values of mixed surfactants on the emulsification and re-emulsification stability test before and after storage when diluted with CIPAC Standard Waters A and D, for the seven EC formulations of difenoconazole, the formulations code F2, F6 and F7 which have HLB values 11.4, 13.5 and 14 respectively in CIPAC standard waters A and D at room temperature presented some signals of instability by exhibited creaming layer exceeding the acceptable range of defined specification. Creaming can be defined as a destabilization process due to the upward movement of oil droplets since its density is lower than the continuous phase (water) (McClements, 2005; Tadros, 2010). Generally, this process occurred due to gravitational forces, which cause droplets to agglomerate, increase in size and accumulate at the top of the system. Meanwhile the formulations code F1, F3, F4 and F5 which have HLB values 9.7, 11.9,12.5 and 13.1 respectively presented appropriate bloom, formed milky white emulsions, no change in color of the emulsions were noticed when stored at 0 ± 2°C for 7 days and accelerated storage in the oven at 54 ± 2°C for 14 days.

Finally, the volume of foam from the samples was low and within the acceptable limits for foam, the formulation could be applied in the field without any foam problems. Table (5).

Table 2. Emulsion Characteristics at ambient temperature-

Measurement of Creamy Layer – CL in ml for samples made with CIPAC water A & D

Time	F1		F2		F3		F4		F5		F6		F7	
	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D
0.0 h	0	0	0	0	0	0	0	0	0	0	0	0	1	0
0.5h	0	0.1	0	0	0	0	0	0	0.1	0	0.1	0.5	5	0.5
1h	0	0.3	6	0	0	0	0	0	0.5	0	0.2	1	7	1
2h	0.5	0.5	8	2	0	0	0	0	1	0	2	2	9	2
24h	1	1	15	6	0.1	0	1	0	1	0	5	9	11	7
REE	0	1	0	9	0	0.2	0	0	0.5	0	5	5	14	9

***CL: Creamy layer (ml)**

Based on the above results, samples F2, F6 & F7 showed creaming more than 2 ml, which excluded them from further testing as their results didn't conform to required specifications (cream layer <2 ml).

Table 3. Emulsion Characteristics at 0-±2 °C after 7 days- Measurement of Creamy Layer - CL in ml for samples made with CIPAC water A & D

Time	F1		F3		F4		F5	
	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D
0.0 h	0	0	0	0	0	0	0	0
0.5h	0	0	0	0	0	0	0	0
1h	0	0	0	0	0	0	0	0
2h	0.5	0	0	0	0	0	0	0
24h	0	0	0	0	0.1	0	1	1
REE	0	0	0	0	0	0	0	0

***CL: Creamy layer (ml)**

Table 4. Emulsion Characteristics at 54-±2 °C after 14 days Measurement of Creamy Layer CL in ml for samples made with CIPAC water A & D

Time	F1		F3		F4		F5	
	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D	CL*/ml in CIPAC water A	CL*/ ml in CIPAC water D
0.0 h	0	0	0	0	0	0	0	0
0.5h	0	0	0	0	0	0	0	0
1h	0.1	0.1	Trace	0	0	0	0	0
2h	0.5	0.3	0	0	0	Trace	Trace	0
24h	1	0	0	0	0.1	0.1	0.1	0

REE	0	0	0	0	0	0	0	0

***CL: Creamy layer (ml)**

Table 5. Persistent Foam difenoconazole EC formulations made with CIPAC water A & D

Time	Persistent foam in CIPAC water A																				
	room temperature							0-±2 °C for 7 days							54-±2 °C for 14 days						
	F1 Foam in ml	F2 Foam in ml	F3 Foam in ml	F4 Foam in ml	F5 Foam in ml	F6 Foam in ml	F7 Foam in ml	F1 Foam in ml	F2 Foam in ml	F3 Foam in ml	F4 Foam in ml	F5 Foam in ml	F6 Foam in ml	F7 Foam in ml	F1 Foam in ml	F2 Foam in ml	F3 Foam in ml	F4 Foam in ml	F5 Foam in ml	F6 Foam in ml	F7 Foam in ml
0s	0	3	3	2	2	3	3	0	3	3	2	2	2	4	2	2	3	3	3	3	4
10s	0	2	2	1	2	2	3	0	3	2	1	1	1	4	0	1	3	2	1	3	3
1 min	0	2	1	0	1	1	3	0	2	2	1	0	1	3	0	0	2	1	1	2	2
3 min	0	1	1	0	1	1	2	0	2	1	0	0	0	3	0	0	1	1	1	1	2
12 min	0	1	0	0	0	0	1	0	1	0	0	0	0	2	0	0	1	0	0	1	1
Time	Persistent foam in CIPAC water D																				
	room temperature							0-±2 °C for 7 days							54-±2 °C for 14 days						
	F1 Foam in ml	F2 Foam in ml	F3 Foam in ml	F4 Foam in ml	F5 Foam in ml	F6 Foam in ml	F7 Foam in ml	F1 Foam in ml	F2 Foam in ml	F3 Foam in ml	F4 Foam in ml	F5 Foam in ml	F6 Foam in ml	F7 Foam in ml	F1 Foam in ml	F2 Foam in ml	F3 Foam in ml	F4 Foam in ml	F5 Foam in ml	F6 Foam in ml	F7 Foam in ml
0s	0	4	4	2	3	4	3	0	4	3	2	2	3	5	3	3	3	4	4	4	5
10s	0	3	3	1	2	3	3	0	4	3	2	2	2	4	1	2	3	3	3	3	4
1 min	0	3	2	0	1	2	3	0	3	2	1	1	1	3	0	1	2	3	3	2	3
3 min	0	2	1	0	1	1	2	0	2	2	0	0	1	3	0	0	1	2	2	1	2
12 min	0	1	0	0	0	0	1	0	1	0	0	0	0	2	0	0	1	1	1	0	1

3.3. Physical Properties.

The physical properties of the emulsifiable concentrate formulations of difenoconazole are illustrated in Tables (6-8).

The pH is a very important parameter because it can reflect a chemical change in the components present in the formulation. Also, the changes in pH over long storage periods can indicate degradation of the active component, a proliferation of bacteria, instability or incompatibility of certain compounds (Campelo, Junqueira et al. 2017). The pH values of the EC formulations were in the range of 5.45 to 6.61, which indicated that the formulations had acidic character.

The prepared EC formulations had a surface tension range (32.58-33.98 mN/m); lower surface tension is a desirable characteristic for most agricultural sprays because it facilitates the spreading of droplets upon impact on leaves or other target surfaces, to increase the surface-active area and improve penetration and uptake of the product into the plants (Giardino, Ambu et al., 2006). The variation of density was (0.98-1.0042 g/cm^3).

The flash point is a measure of the tendency of a sample to form flammable mixtures with air in controlled laboratory conditions and is a parameter for storage and handling when considering as flammable materials (Encinar, González et al., 2005).The prepared formulations in all the storage conditions showed high flash points values (54-55°C).

The variation of refractive index was in the range of 1.5044 to 1.5122, it reflects the EC formulations appear nearly transparent in the visible spectrum and the viscosity of the prepared formulations ranged from 3.81 to 4.96 mPa.

Table 6. Physical properties of difenoconazole EC formulations at room temperature

Physical properties	**Formulation Sample Code**			
	F1	**F3**	**F4**	**F5**
pH (1%)	6.06	5.88	6.36	5.75
Surface Tension (mN/m)	33.51	32.92	32.72	33.98
Density (g/cm^3)	0.9800	1.0036	1.0014	1.0033
Flash point (°C)	54	55	55	54
Refractive Index	1.5115	1.5097	1.5077	1.5077
Viscosity (mPa)	4.95	4.65	4.13	4.96

Table 7. Physical properties of difenoconazole EC formulations at 0-±2 °C for 7 days

Physical properties	**Formulation Sample Code**			
	F1	**F3**	**F4**	**F5**

pH (1%)	6.32	6.16	6.29	5.88
Surface Tension (mN/m)	33.62	32.85	32.65	33.86
Density (g/cm^3)	1.0010	1.0038	1.0017	1.0037
Flash point (°C)	54	55	55	55
Refractive Index	1.5044	1.5081	1.5095	1.5075
Viscosity (mPa)	4.34	4.10	3.81	4.96

Table 8. Physical properties of difenoconazole EC formulations at 54-±2 °C for 14 days

Physical properties	Formulation Sample Code			
	F1	**F3**	**F4**	**F5**
pH (1%)	6.00	5.97	6.61	5.45
Surface Tension (mN/m)	33.76	33.10	32.58	33.86
Density (g/cm^3)	1.0014	1.0040	1.0010	1.0042
Flash point (°C)	55	55	55	55
Refractive Index	1.5063	1.5097	1.5054	1.5049
Viscosity (mPa)	4.26	4.15	4.10	4.92

4. Conclusion.

Emulsifiable concentrate formulations of difenoconazole were prepared by mixing surfactants with different HLB values and characterized according to their active ingredient content, pH, refractive index, surface tension, viscosity, flash point, density, persistent foam, and emulsion stability. Difenoconazole could be formulated as a stable emulsifiable concentrate showing good physical characteristics at the different HLB values 9.7, 11.9, 12.5 and 13.1. Further studies could be performed to evaluate the Fungicidal activity of the prepared formulations and compare the activity with commercial formulation available in the market.

References

Aguilar, M., P. Coronel, D. Alvarado, F. Sanchez and R. Alcivar (2019). "oil-in-water (o/w) emulsionable concentrate of ishpink (Ocotea quixos) with thermodynamic STABILITY." Revista Caatinga 32: 590-598.

Allawzi, M. A., H. Allaboun and A. S. Qazaq (2016). "Formulation, emulsion, and thermal stability of emulsifiable malathion concentrate using ethanol as a solvent." International Journal of Applied Engineering Research 12: 221-231.

ASTM (2014). "Standard Test Methods for Surface and Interfacial Tension of Solutions of Paints, Solvents, Solutions of Surface-Active Agents, and Related Materials." American Society for Testing and Materials D 1331

ASTM (2016). “Standard Test Method for Refractive Index and Refractive Dispersion of Hydrocarbon Liquids." American Society of Testing and Materials: D1218 -1212.

ASTM (2016). "Test Methods for flash point by small scale closed cup tester." American Society for Testing and Materials: D 3828.

ASTM (2017). "Standard Test Method for Density, Relative Density, or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method." ASTM D1298.

ASTM (2018). "Standard Test Methods for Rheological Properties of Non-Newtonian Materials by Rotational Viscometer " American Society of Testing and Materials: D2196

Campelo, P. H., L. A. Junqueira, J. V. d. Resende, R. D. Zacarias, R. V. d. B. Fernandes, D. A. Botrel and S. V. Borges (2017). "Stability of lime essential oil emulsion prepared using biopolymers and ultrasound treatment." International Journal of Food Properties 20 (sup1): S564-S579.

CIPAC (1995). " Dobrat W, Martijn A, editors." Preparation of Standard waters A and D. In: CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.: 59-62.

CIPAC, C. I. (2016). "Collaborative International Pesticides Analytical Council. Dyspneal em: <http://www.cipac.org/>. Acesso em.".

Dennis, R. H. (2003). " Encyclopedia of Agricultural, Food, and Biological Engineering." CRC Press.

Encinar, J. M., J. F. González and A. Rodríguez-Reinares (2005). "Biodiesel from Used Frying Oil. Variables Affecting the Yields and Characteristics of the Biodiesel." Industrial & Engineering Chemistry Research 44 (15): 5491-5499.

FAO/WHO, J. and p. specifications (2018). "Food and agriculture organization of the united nations/world health organization - FAO/WHO. Manual on development and use of FAO and WHO specifications for pesticides ".

Feng, J., Q. Chen, X. Wu, S. M. Jafari and D. J. McClements (2018). "Formulation of oil-in-water emulsions for pesticide applications: impact of surfactant type and concentration on physical stability." Environmental Science and Pollution Research 25 (22): 21742-21751.

Feng, J., Y. Shi, Q. Yu, C. Sun and G. Yang (2016). "Effect of emulsifying process on stability of pesticide nanoemulsions." Colloids and Surfaces A: Physicochemical and Engineering Aspects 497: 286-292.

Feng, J., Q. Zhang, Q. Liu, Z. Zhu, D. McClements and S. Jafari (2018). Application of Nanoemulsions in Formulation of Pesticides.

Ferreira, M. R., R. R. Santiago, T. P. de Souza, E. S. Egito, E. E. Oliveira and L. A. Soares (2010). "Development and evaluation of emulsions from Carapa guianensis (Andiroba) oil." AAPS PharmSciTech 11(3): 1383-1390.

Giardino, L., E. Ambu, C. Becce, L. Rimondini and M. Morra (2006). "Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic." J Endod 32 (11): 1091-1093.

Hallouard, F., G. Dollo, N. Brandhonneur, F. Grasset and P. L. Corre (2015). "Preparation and characterization of spironolactone-loaded nano-emulsions for extemporaneous applications." International Journal of Pharmaceutics 478 (1): 193-201.

Knowles, A. (2005). "New developments in crop protection product formulation." Agrow Reports UK: Tand F Informa UK Ltd: 153-156.

Knowles, A. (2008). "Recent developments of safer formulations of agrochemicals." The Environmentalist 28 (1): 35-44.

Kozuks, Y. and T. Ohtsubo, A (2009). "Predictive Solubility Tool for Pesticide Emulsifiable Concentrate Formulations." ASTM International mem. of Cross Ref.

Losada-Barreiro, S., V. Sánchez-Paz and C. Bravo-Díaz (2013). "Effects of emulsifier hydrophile-lipophile balance and emulsifier concentration on the distributions of gallic acid, propyl gallate, and α-tocopherol in corn oil emulsions." Journal of Colloid and Interface Science 389 (1): 1-9.

McClements (2005). "Food Emulsion: Principle, Practices, and Techniques. 2nd ed.; CRC Press, Boca Raton, FL,USA."

Myers, D. (1998). "In Surfactant Science and Technology." VCH Publishers, New York.

Rai, N. and I. P. Pandey (2013). "Study of some physiochemical factors determining emulsion stability with mixed Emulsifiers " Journal of Industrial Research & Technology 3(1): 12-16.

Rajmani, P., R. Sunita, M. Sudeep, K. Raza S and K. Thakur L (2014). "Formulation development, standardization and antimicrobial activity of Ageratum Conyzoides extracts and their formulation.International." Journal of Pharmacy and Pharmaceutical Science6.

Sarwar, M. (2015). "Commonly Available Commercial Insecticide Formulations and Their Applications in the Field." nternational Journal of Materials Chemistry and Physics 1 (2): 116-123.

Shao, H., N. Xi and Y. Zhang (2018). "Microemulsion formulation of a new biopesticide to control the diamondback moth (Lepidoptera: Plutellidae)." Scientific Reports 8 (1): 10565.

Tadros (2010). "Rheology of Dispersions: Principles and Applications." First Edit; Rheology of Dispersions: Principles and Applications. Wiley-VCH, Weinheim, Germany.

Thakur, L. K., R. Sunita, P. Rajmani, Mukesh, K. Singh and R. S. K (2014). "Development and Evaluation of Basil emulsifiable concentrates." African Journal of Science and Research 3 (1): 06-09.

Vanitha, S. (2010). "Developing new formulation using plant oils and testing their physical stability and antifungal activity against alternaria chlamydospora causing leaf blight in *solanum nigrum*." RJAS (4): 385-390.