Original Research Article

Effect of hydrophilic-lipophilic balance (HLB) of mixed blend surfactants on physicochemical properties of emulsifiable concentrate formulations of difenoconazole

Abstarct

The aim of this study was to investegate the effect of hydrophilic-lipophilic balance of mixed blend surfactants on the physicochemical properties of emulsifiable concentrate formulations of difenconazole. Physical tests including storage stability studies were performed for the different samples in order to predict the stability of these formulations. Different parameters such as active ingredient content, pH, refractive index, surface tension, viscosity, flash point, effect of centrifugation, persistent foam and emulsion stability were determined for the prepared samples. The results of different tests performed showed that the difenconazole content in the different formulations was within the acceptable limits to FAO Specifications. Difenconazole could be formulated as a stable emulsifiable concentrate using a mixed blend surfactants at different HLB values except at HLB 13.5. The different formulations exhibited good physical characteristics when tested especially when kept at the accelerated storage temperatures (0˚C and 54˚C) for 7 and 14 days and no phase separation or sediment was observed in these formulations after centrifugation.

Keywords: Hydrophilic-lipophilic balance, Surfactants, Difenconazole, Emulsifiable concentrate.

1. Introduction.

Over the last 50 years, farmers and growers in all the main agricultural areas of the world have relied substantially upon fertilisers and crop protection chemicals to help them meet the ever-increasing demand for food and other materials such as natural fibres. To meet these demands simple dusting powders and spray oil formulations have been used for many years to protect growing crops from weeds, pests and diseases. However, since the 1950's, the chemical industry has satisfied consumer demands for increased crop yield, quality and variety by the continuous development and introduction of more effective crop protection chemicals. During this period the population of the world has increased from a bout 2.5 billion in1950 to about 6 billionin 2000. Furthermore, the world's populationis expected to reach about10 billion by the year 2040, placing an even greater pressure on food security and environmental safety (Knowles, 2008; Webb and Buratini, 2016).

The liquid formulation components are active ingredient (technical grade), solvent, co-solvent and surfactants. Furthermore, the 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 or dispersions and give appropriate container design, are usually easy to rinse out of the package and do not cause application problems.

Emulsifiable concentrate formulations comprise the biggest volume of all pesticide formulations representing about 40 % in terms of global volume usage. Emulsifiable concentrate formulations have been very popular for many years and represent the biggest volume of all pesticide formulations in terms of consumption world-wide. Emulsifiable concentrates are made from oily active ingredients or from low melting,waxy solid active ingredients which are soluble in non-polar hydrocarbon solvents, such as xylene,C9–C10 solvents,solvent naphtha, odourless kerosene,or other proprietary hydrocarbon solvents. Emulsifiable concentrates have many advantages, easy to produce, handle, transport, store, little agitation required, not abrasive, do not plug screen or nozzles, little visible residue on treated surface, useful for water-insoluble, good storage stability, easy application, low melting point active ingredients and high efficiency (Kozuks et al., 2009; Vanitha, 2010; Rajmani et al., 2014; Thakur et al., 2014). Surfactant emulsifier blends are added to these formulations to ensure spontaneous emulsification into water in the spray tank. Surfactant suppliers provide advice on the selection of a "balanced pair" emulsifier blend is frequently necessary to ensure good emulsion stability after dilution in water of varying degree of hardness. The correct balance of surfactant emulsifiers can be obtained from knowledge of the Hydrophilic-Lipophilic balance (HLB) values of the surfactants. The HLB stands for hydrophilic-lipophilic balance, surfactants in the HLB range of 8-18 normally give good emulsions (Dennis, 2003).The optimum ratio of mixed blend surfactants is determined by experimentation to give spontaneous emulsification in water (strike or bloom), and to give a stable emulsion with minimum creaming and no oil droplet coalescence. (Knowles, 2005; Magda, 2010). Therefore, the aim of the present study was to prepape several emulsifiable concentrate formulations of difenoconazole and study the effect of hydrophilic-lipophic balance number of mixed blend difffrent surfactants on the stability of the prepared formulations.

2. Materials and Methods.

2.1. Materials

2.1.1. The tested active compound : Difenoconazole (technical grade, purity 97 %) was purchased from China

IUPAC name: Cis, trans-3-chloro-4-[4-methyl-2-(1H-1, 2, 4-triazol-1-ylmethyl)-1, 3-dioxolan-2-yl] phenyl 4-chlorophenyl ether

Empirical formula:$C_{19}H_{17}Cl_2N_3O_3$

Structural formula:

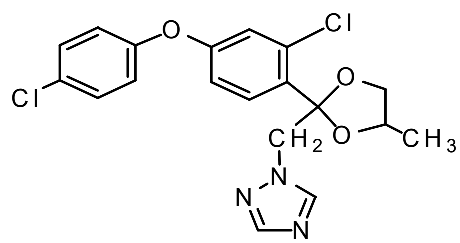

Fig 1. Sturucture of Difenconazole

2.1.2. 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; nonylphenol polyglycoether (10EO), HLB 13.5; Anionic Surfactnts:Rodacal 60/BE (dodecyl benzene sulfonate, calcium salt, HB 8.3); 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); was purchased from Sigma-Aldrich Chemie GmbH, Riedstr, Steinheim, Germany. Dimethyl formamide and polyethylene glycol were purchased from ADWIC. El Nasr Pharmaceutical Chemical Co., Egypt. Calcium carbonate and Solvesso 100 were purchased from Sigma - Aldrich Chemie GmbH Steinheim, Germany, Magnesium oxide and methyl red were purchased from Qualikems Fine Chemicals PVT Ltd.India. Ammonia solution was purchased from Prolabo. All materials were used as received without further purification.Water used in all preparations obtained from Water distiller LABCONCO water PROT.M PS LABCONCO Corporation, Kansas City, Missouri 64132-USA.

2.2. Methods

2.2.1. Preparation of emulsifiable concentrate formulations (EC) of difenconazole

Emulsifiable Concentrate formulation of difenconacole (EC) was prepared by simple mixing method. Difenconazole tecgnical grade, solvent, co-solvent, emulsifiers and freezing agent were weighed into a beaker and placed on a laboratory mixing until clear solution was obtained. The resulting formulation was stored in glass bottle at room temperature (25°C ± 2) and protected from light.

2.2.2. Preparation of difenconazole emulsifiable concentrate formulations using a mixed blend surfactants.

Several emulsifiable concentrates were prepared by using a combination of surfactants.The proportion between the two surfactants was calculated in order to obtain HLB values.

Table 1. Composition of Difenconazole EC Formulation.

Formulation Code	Mixed blend surfactant	HLB number
F1	Alkamuls RC:Rodacal 60/BE	9.7
F2	Soprophor Cy/8:Rodacal 60/BE	11.9
F3	Arkapol N 100:Alkamuls RC	12.5
F4	Alkamuls 14/R:Geronol FF4	13.1
F5	Tween 80:Alkamuls RC	13.5

2.2.3. Characterization of the prepared emulsifiable concentrate formulations of difenconazole

2.2.3.1. Active Ingredient Content

The content of active ingredient of difenconazole 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, was used. Gas Chromatography with the following conditions: Oven: 260℃, Injector: 280℃, Detector (FID): 290℃.

2.2.3.2. Formulation Stability Test.

2.2.3.2.1. Emulsion stabilityand re-emulsification[CIPAC MT 36.3].

The formulation was diluted at 30 ± 2°C with CIPAC Standard Waters A and D [CIPAC MT 18]. In the emulsion characteristics experiment, 5 ml of the formulation sample was separately mixed with 95 ml standard water: (CIPAC A, 20 ppm hardness, pH 5.00 - 6.00,Ca^{2+}: Mg^{2+}=1:1 and CIPAC D, 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 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. The amount of free oil or cream that separated at the top or the bottom of the emulsion was determined after the emulsion was allowed to stand undisturbed for various time intervals (0.5, 2 and 24h and 24.5h :30 min after re-emulsification).

2.2.3.2.2. 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 in an attempt 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.

Liquid formulations may be adversely affected by storage at low temperatures according to the Food and Agriculture Organization (FAO) of Pesticides. Thr change in physical apparence of samples was visually observed. that recommend testing of the relevant product parameters before and after storage. Accelerated storage at elevated temperatures [CIPAC MT 46.3] is performed by placing 50 ml of the product in a tightly capped bottle in an incubatorat at 54°C ±2°C for 14 days. The volume of any separated material at the bottom of the tube was then recorded. Storage at low temperatures may result in crystallization of active constituent, significant changes in viscosity or separation of formulation. The liquid formulations was also be tested at 0 ± 2°C or lower for 7 days [CIPAC MT 39.3]. A 100 ml of the the sample was transferred to a glass tube and cooled in a refrigerator at 0 ± 2°C or lower 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.

2.2.3.2.3. Centrifugation Test

Laboratory Centrifuge REMI Centrifuge Bombay-India- R32A.4000002 was used to determine the stability of the emulsifiable concentrate formulations against gravity. Each sample was centrifuged for 10 min at 5000 rpm to monitor whether phase separation occurred. The formulation was centrifuged at 25°C.

2.2.3.2.4. Persistent Foam

Persistent foam is a measure the amount of foam likely to be present in a spray tank or other application equipment following dilution of the product with water. 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 was left to stand undisturbed for the specified time. The volume of foam was noted [CIPAC MT 47.2.].

2.2.3.2.5.Physical characterization

2.2.3.2.5.1.pH Measurement

The pH of a 1% solution of the formulations was measured using a pH meter (Jenway model pH 3510). It was recalibrated befor testing [CIPAC MT 75.3].

2.2.3.2.5.2. Refractive index

Refractive index is an optical measurement of a material's ability to bend a beam of light. The refractive index may be used to determine the purity of the material. Refractive index of the prepared formulation was measured using ABBE Refractometer, ATAGO, Co., LTD, Japan. (ASTM,2016).

2.2.3.2.5.3.Viscosity Measurement

Viscosity of the prepared formulation 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 TC-502 USA and each reading was taken after equilibrium of the sample. (ASTM, 2015).

2.2.3.2.5.4. Surface Tension measurment

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

2.2.3.2.5.5. Density measurement.

Density of the prepared formulation was measured using digital density meter model DDM 2910 with touch screen. Rudolph Research Analytical, USA. (ASTM, 2018).

2.2.3.2.5.6. Flash point.

Measurement of flash point of the prepared formulations was carried out by 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 [CIPAC MT12].

3. Results and Discussion.

3.1 Active Ingredient Content.

The data presented in Table (2) showed that the content of difenconazole in 25 % emulsifiable concentrate formulations at different storage conditions was detected by Gas Chromatography. The difenconazole content in freshly prepared and accelerated storage samples (0˚C for 7 days and 54˚C for 14 days) was within the acceptable range of defined specification (+/- 5%).

Table.2. Effect of different storage conditions on active ingredient content in 25 % difenconazole emulsifiable concentrate formulations.

Formulations Code.	Storage Conditions				
	Fresh formulation	After 7 days		After 14 days	
	room temperature	0˚C		54˚C	
	AI	AI	% loss	AI	% loss
F1	25.94	24.91	3.97	24.65	4.97
F2	25.67	25.06	2.37	25.04	2.45
F3	24.37	24.06	1.27	24.03	1.39
F4	24.42	24.09	1.35	24.06	1.47
F5	25.09	24.92	0.67	24.63	1.83

3.2. Stability Study of Emulsion.

The emulsion stability and re-emulsification test states that the formulation when diluted at 30°C±2 with CIPAC standard waters A and D shall comply with the specification of emulsification of emulsifiable concentrate formulation (JMPS FAO/WHO pesticides specifications, 2010). 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 (3-5) showed that all formulations passed successufly through emulsification stability and re-emulsification test before and after storage when diluted with standard CIPAC waters A and D except for formulation number 5 with HLB value 13.5 in the different storage conditions though in CIPAC water D at 0˚C for 7 days it was within the acceptance limit.

3.3.Physical Parameters.

The physical properties of the emulsifiable concentrate formulations of difenconazole are illustrated in Tables (6-8). Performance in accelerated testing and kinetics of pH profiles are key in a formulation's chemical stability (Issa et al., 2000). The pH values of the prepared formulations were in range (5.45-6.61), which indicated that the formulations had acidic character. The prepared 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 plant (Giardino 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 et al., 2005). The prepared formulations in all the storage conditions showed high flash points values (54-56˚C). The variation of refractive index was (1.5044-1.5122) and the viscosity of the prepared formulations ranged from (3.81 to4.96 mPas). Finally, the volume of foam from the samples was low and within the acceptable limits for foam.

Table.3. Emulsion stability and re-emulsification of emulsifiable concentrate formulations of difenconazole as function of (HLB) of blend mixed surfactants at room temperatue

Formulation code.	Emulsion Characteristics	
	CIPAC A (CL*)	CIPAC D (CL*)

	0h	**0.5h**	**2h**	**24h**	**24.5**	**0h**	**0.5h**	**1h**	**24h**	**24.5**
1	-	-	0.5	2	-	0.1	0.3	0.5	2	2
2	-	-		0.1	-	-	-	-	-	0.2
3	-	-		2	-	-	-	-	-	-
4	0.1	0.5	1	2	0.5	-	-	-	-	-
5	0.1	0.5	2	5	5	0.5	1	2	9	5

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

Table.4. Emulsion stability and re-emulsification of emulsifiable concentrate formulations of difenconazole as function of (HLB) of blend mixed surfactants at 0°C for 7 days

Formulation no.	**Emulsion Characteristics**									
	CIPAC A(*CL)					**CIPAC D (*CL)**				
	0h	0.5h	2h	24h	24.5	0h	0.5h	1h	24h	24.5
1	-	-	0.5	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	0.1	-	-	-	-	-	-
4	-	-	-	2	-	-	-	-	2	-
5	-	-	0.5	5	0.2	-	-	0.5	2	Trace

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

Table.5. Emulsion stability and re-emulsification of emulsifiable concentrate formulation of difenconazole as function of (HLB) of blend mixed surfactants at 54°C for 14 days

Formulation no.	**Emulsion Characteristics**									
	CIPAC A (*CL)					**CIPAC D (*CL)**				
	0h	0.5h	2h	24h	24.5	0h	0.5h	1h	24h	24.5
1	-	Trace	1	2	-	-	0.5	1	2	-
2	-	Trace	-	-	-	-	-	-	-	-

3	-	-	-	2	-	-	-	Trace	1	-
4	-	-	0.1	2	-	-	-	-	-	-
5	-	3	9	9	9	-	0.5	-	2	0.5

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

Table.6. Physical properties of emulsifiable concentrate formulations of difenconazole at room temperature.

Physical Properties	Different formulations of difenconazole				
	1	2	3	4	5
pH (1%)	6.06	5.88	6.36	6.49	5.75
Surface Tension (mN/m)	33.51	32.92	32.72	32.63	33.98
Density (g/cm^3)	0.9800	1.0036	1.0014	1.0021	1.0033
Flash point ($^{\circ}C$)	54	55	55	54	54
Refractive Index	1.5115	1.5097	1.5077	1.5070	1.5077
Viscosity (mPas)	4.95	4.65	4.13	5.16	4.96
Persistent foam (cm^3)	1	3	3	3	4

Table.7. Physical properties of emulsifiable concentrate formulations of difenconazole at 0°C for 7 days.

Physical Properties	Different formulations of difenconazole				
	1	2	3	4	5
pH (1%)	6.32	6.16	6.29	6.45	5.88
Surface Tension (mN/m)	33.62	32.85	32.65	32.87	33.86
Density (g/cm^3)	1.0010	1.0038	1.0017	1.0024	1.0037
Flash point ($^{\circ}C$)	54	55	55	56	55
Refractive Index	1.5044	1.5081	1.5095	1.5122	1.5075
Viscosity (mPas)	4.34	4.10	3.81	4.03	4.96
Persistent foam (cm^3)	2	3	4	3	5

Table.8. Physical properties of emulsifiable concentrate formulations of difenconazole at 54°C for 14 days.

Physical Properties	Different formulations of difenconazole				
	1	2	3	4	5
pH (1%)	6.00	5.97	6.61	5.67	5.45
Surface Tension (mN/m)	33.76	33.10	32.58	32.91	33.86
Density (g/cm^3)	1.0014	1.0040	1.0010	1.0028	1.0042
Flash point (°C)	55	55	55	54	55
Refractive Index	1.5063	1.5097	1.5054	1.5054	1.5049
Persistent foam (cm^3)	2	2	5	3	3

4. Conclusion.

Emulsifiable concentrate formulations prepared by blend mixed surfactants with different HLB values were characterized according to their active ingredient content, pH, refractive index, surface tension, viscosity, flash point, effect of centrifugation, persistent foam and emulsion stability. Difenconazole could be formulated as a stable emulsifiable concentrate showing good physical characteristics at different HLB values except at an HLB value of 13.5. Further studies could be performed to evaluate the antifungal ctivity of the prepared formulations and also compare the activity with commerical formulation available in the market.

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