

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

Abstract

The aim of this study was to investigate 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 about 2.5 billion in 1950 to about 6 billion in 2000. Furthermore, the world's population is expected to



reach about 10 billion by the year 2040, placing an even greater pressure on food security

32

and environmental safety (Knowles, 2008; Webb and Buratini, 2016).

33 The liquid formulation components are active ingredient (technical grade), solvent, co-
34 solvent and surfactants. Furthermore, the liquid formulations are preferred by the farmer for
35 preparing spray solutions for several reasons: they can be measured volumetrically, are easy
36 to handle, spontaneously form stable emulsions or dispersions and give appropriate container
37 design, are usually easy to rinse out of the package and do not cause application problems.

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

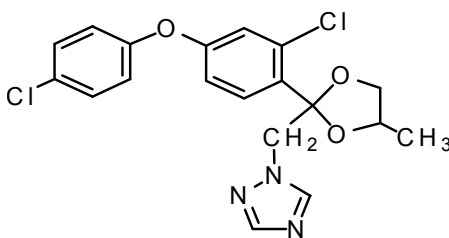
63 **2. Materials and Methods.**64 **2.1. Materials**

65 **2.1.1.** The tested active compound : Difenoconazole (technical grade, purity 97 %) was purchased
66 from China

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

69 **Empirical formula:** C₁₉H₁₇Cl₂N₃O₃

70 **Structural formula:**



74 **Fig 1. Sturcture of Difenoconazole**

75 **2.1.2. Chemicals**

76 Nonionic Surfactants: Soprophor CY/8 (Ethoxylated tristyril phenol, HLB 13.7);
77 Alkamuls RC (ethoxylated Castor oil, HLB 10.5); Alkamuls 14/R (Ethoxylated Castor oil,
78 HLB 14.9); Arkapol N 100; nonylphenol polyglycoether (10EO), HLB 13.5; Anionic
79 Surfactants: Rodacal 60/BE (dodecyl benzene sulfonate, calcium salt, HB 8.3); Geronol FF4,
80 mixture of anionic and non-ionic derivatives, HLB12.7 were kindly supplied by Rhodia-
81 Home, Personal Care & Industrial Ingredients, Milano, Italy. Tween 80 (Polyoxyethylene (20)
82 Sorbian monooleate, HLB 15.00); was purchased from Sigma-Aldrich Chemie GmbH,
83 Riedstr, Steinheim, Germany. Dimethyl formamide and polyethylene glycol were purchased
84 from ADWIC. El Nasr Pharmaceutical Chemical Co., Egypt. Calcium carbonate and
85 Solvesso 100 were purchased from Sigma - Aldrich Chemie GmbH Steinheim, Germany,
86 Magnesium oxide and methyl red were purchased from Qualikems Fine Chemicals PVT
87 Ltd. India. Ammonia solution was purchased from Prolabo. All materials were used as
88 received without further purification. Water used in all preparations obtained from Water
89 distiller LABCONCO water PROT.M PS LABCONCO Corporation, Kansas City,
90 Missouri 64132-USA.

91 **2.2. Methods**

92 **2.2.1. Preparation of emulsifiable concentrate formulations (EC) of difenconazole**

93 Emulsifiable Concentrate formulation of difenconazole (EC) was prepared by
 94 simple mixing method. Difenconazole technical grade, solvent, co-solvent, emulsifiers
 95 and freezing agent were weighed into a beaker and placed on a laboratory mixing until
 96 clear solution was obtained. The resulting formulation was stored in glass bottle at room
 97 temperature ($25^{\circ}\text{C} \pm 2$) and protected from light.

98 **2.2.2. Preparation of difenconazole emulsifiable concentrate formulations using a mixed blend**
 99 **surfactants.**

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

102 **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

103

104 **2.2.3. Characterization of the prepared emulsifiable concentrate formulations of difenconazole**

105 **2.2.3.1. Active Ingredient Content**

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

110 **2.2.3.2. Formulation Stability Test.**

111 **2.2.3.2.1. Emulsion stability and de-emulsification [CIPAC MT 36.3].**

112 The formulation was diluted at $30 \pm 2^\circ\text{C}$ with CIPAC Standard Waters A and D [CIPAC
113 MT 18]. In the emulsion characteristics experiment, 5 ml of the formulation sample was
114 separately mixed with 95 ml standard water: (CIPAC A, 20 ppm hardness, pH 5.00 -
115 6.00, $\text{Ca}^{2+}:\text{Mg}^{2+}=1:1$ and CIPAC D, 342 ppm hardness, pH 6.00-7.00, $\text{Ca}^{2+}:\text{Mg}^{2+}=4:1$) in a
116 100 milliliter measuring cylinder to produce 100 milliliter of measuring cylinder to produce
117 100 milliliter of aqueous emulsion. The cylinder was then stoppered and inverted once then
118 it was noted if the emulsion was homogenous or not, after 30 sec it was inverted 10 times and
119 left to stand. The amount of free oil or cream that separated at the top or the bottom of the
120 emulsion was determined after the emulsion was allowed to stand undisturbed for various
121 time intervals (0.5, 2 and 24h and 24.5h :30 min after re-emulsification).

122 2.2.3.2.2. Storage Stability

123 Accelerated stability tests at elevated temperatures are designed to increase the rate of
124 chemical degradation or physical change of a product. Accelerated testing was performed at
125 elevated temperatures in an attempt to obtain information on the shelf life of a product in a
126 relatively short time. Accelerated testing involves extrapolations from higher to lower
127 temperatures and from shorter to longer storage periods.

128 Liquid formulations may be adversely affected by storage at low temperatures according to
129 the Food and Agriculture Organization (FAO) of Pesticides. The change in physical
130 appearance of samples was visually observed. that recommend testing of the relevant product
131 parameters before and after storage. Accelerated storage at elevated temperatures [CIPAC
132 MT 46.3] is performed by placing 50 ml of the product in a tightly capped bottle in an
133 incubator at $54^\circ\text{C} \pm 2^\circ\text{C}$ for 14 days. The volume of any separated material at the bottom of
134 the tube was then recorded. Storage at low temperatures may result in crystallization of active
135 constituent, significant changes in viscosity or separation of formulation. The liquid
136 formulations was also be tested at $0 \pm 2^\circ\text{C}$ or lower for 7 days [CIPAC MT 39.3]. A 100 ml of
137 the the sample was transferred to a glass tube and cooled in a refrigerator at $0 \pm 2^\circ\text{C}$ or lower
138 for 7 days. The tube was left to stand at room temperature for 3 hours. The volume of any
139 separated material at the bottom of the tube was recorded.

140 2.2.3.2.3. Centrifugation Test

141 Laboratory Centrifuge REMI Centrifuge Bombay-India- R32A.4000002 was used to
142 determine the stability of the emulsifiable concentrate formulations against gravity. Each

143 sample was centrifuged for 10 min at 5000 rpm to monitor whether phase separation occurred.
144 The formulation was centrifuged at 25°C.

145 **2.2.3.2.4. Persistent Foam**

146 Persistent foam is a measure the amount of foam likely to be present in a spray tank or
147 other application equipment following dilution of the product with water. Specified amount
148 of the prepared formulation is added to CIPAC standard waters (95 ml) in the measuring
149 cylinder and made up to the mark. The cylinder is stoppered and inverted 30 times. The
150 cylinder was left to stand undisturbed for the specified time. The volume of foam was noted
151 [CIPAC MT 47.2].

152 **2.2.3.2.5. Physical characterization**

153 **2.2.3.2.5.1. pH Measurement**

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

156 **2.2.3.2.5.2. Refractive index**

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

161 **2.2.3.2.5.3. Viscosity Measurement**

162 Viscosity of the prepared formulation was measured using "Brookfield DV II + PRO"
163 digital Viscometer (Brookfield, USA) UL rotational adaptor. The temperature was kept at
164 25°C during the measurement by water bath TC-502 USA and each reading was taken after
165 equilibrium of the sample. (ASTM, 2015).

166 **2.2.3.2.5.4. Surface Tension measurement**

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

171 **2.2.3.2.5.5. Density measurement.**

172 Density of the prepared formulation was measured using digital density meter model DDM
173 2910 with touch screen. Rudolph Research Analytical, USA. (ASTM, 2018).


174 **2.2.3.2.5.6. Flash point.**

175 Measurement of flash point of the prepared formulations was carried out by tag open cup
176 method by Koehler instrument company, INC,USA. The flash point was recorded as the
177 temperature at the thermometer when a flash appeared [CIPAC MT12].

178 **3. Results and Discussion.**179 **3.1 Active Ingredient Content.**

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

185 **Table.2. Effect of different storage conditions on active ingredient content in 25 %**
186 **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		% 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

187

188 **3.2. Stability Study of Emulsion.**

189 The emulsion stability and re-emulsification test states that the formulation when diluted at
 190 30°C±2 with CIPAC standard waters A and D shall comply with the specification of
 191 emulsification of emulsifiable concentrate formulation (JMPS FAO/WHO pesticides
 192 specifications, 2010). The maximum level of cream and precipitate layer didn't exceed 2 ml
 193 after 0.5, 2 and 24.5 hrs from dilution. Results in Tables (3-5) showed that all formulations
 194 passed successfully through emulsification stability and re-emulsification test before and after
 195 storage when diluted with standard CIPAC waters A and D except for formulation number 5
 196 with HLB value 13.5 in the different storage conditions though in CIPAC water D at 0°C for
 197 7 days it was within the acceptance limit.

198 **3.3.Physical Parameters.**

199 The physical properties of the emulsifiable concentrate formulations of difenconazole are
 200 illustrated in Tables (6-8). Performance in accelerated testing and kinetics of pH profiles are
 201 key in a formulation's chemical stability (Issa et al., 2000). The pH values of the prepared
 202 formulations were in range (5.45-6.61), which indicated that the formulations had acidic
 203 character. The prepared formulations had a surface tension range (32.58-33.98 mN/m).
 204 Lower surface tension is a desirable characteristic for most agricultural sprays because it
 205 facilitates the spreading of droplets upon impact on leaves or other target surfaces, to increase
 206 the surface active area and improve penetration and uptake of the product into the plant
 207 (Giardino et al., 2006). The variation of density was (0.98-1.0042 g/cm³). The flash point is a
 208 measure of the tendency of a sample to form flammable mixtures with air in controlled
 209 laboratory conditions and is a parameter for storage and handling when considering as
 210 flammable materials (Encinar et al., 2005). The prepared formulations in all the storage
 211 conditions showed high flash points values (54-56°C). The variation of refractive index was
 212 (1.5044-1.5122) and the viscosity of the prepared formulations ranged from (3.81 to 4.96
 213 mPas)  hally, the volume of foam from the samples was low and within the acceptable
 214 limits for foam.

215 **Table.3. Emulsion stability and re-emulsification of emulsifiable concentrate**
 216 **formulations of difenconazole as function of (HLB) of blend mixed surfactants at**
 217 **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

218

*CL : Creamy layer (ml)

219 **Table.4. Emulsion stability and re-emulsification of emulsifiable concentrate**
 220 **formulations of difenconazole as function of (HLB) of blend mixed surfactants at**
 221 **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

222

*CL : Creamy layer (ml)

223 **Table.5. Emulsion stability and re-emulsification of emulsifiable concentrate**
 224 **formulation of difenconazole as function of (HLB) of blend mixed surfactants at**
 225 **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

226

*CL : Creamy layer (ml)



228

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 (°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

229

230

231

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

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 (°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

232

233 **Table.8.** Physical properties of emulsifiable concentrate formulations of difenconazole
 234 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

235

236 4. Conclusion.

237 Emulsifiable concentrate formulations prepared by blend mixed surfactants with different
 238 HLB values were characterized according to their active ingredient content, pH, refractive
 239 index, surface tension, viscosity, flash point, effect of centrifugation, persistent foam and
 240 emulsion stability. Difenconazole could be formulated as a stable emulsifiable concentrate
 241 showing good physical characteristics at different HLB values except at an HLB value of
 242 13.5. Further studies could be performed to evaluate the antifungal ctivity of the prepared
 243 formulations and also compare the activity with commerical formulation available in the
 244 market.

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