

Characterization of Rosemary Essential Oil for biodegradable emulsions

S. Rodríguez-Rojo*, S. Varona, M. Núñez, M. J. Cocero

*Universidad de Valladolid. Escuela de Ingenierías Industriales – Sede Dr. Mergelina –
c/ Doctor Mergelina s/n 47011 Valladolid, España*

* Corresponding author: sorayarr@iq.uva.es

1 **Abstract**

2 The characterization of rosemary essential oil (EO) for its formulation in biodegradable
3 emulsions has been carried out. Firstly, the required HLB (hydrophile–lipophile balance) value
4 of the oil was determined to be 15 based on droplet size analysis and the stability of emulsions
5 with synthetic surfactants. Moreover the emulsion resulted to be stable after 50 days of
6 storage in ambient conditions. Secondly, four biodegradable and non-toxic surfactants derived
7 from starch were tested. The effect of these surfactants was analyzed by measuring interfacial
8 tension between the oil and the aqueous phase.

9 **Keywords:** Essential oil, emulsion, required HLB, surface tension, surfactant

10 **1. Introduction**

11 Essential oils (EOs) are commonly used as natural preservatives and fragrances in cosmetic
12 products. More recently, thanks mainly to their antimicrobial properties, new applications as
13 food preservatives (Burt, 2004), growth promoters in livestock (Calsamiglia et al., 2007),
14 natural pesticides in organic agriculture (El-Shafei et al., 2010) and insecticides (Phillips et al.,
15 2010) are emerging. EOs typically are volatile and they rapidly evaporate from surfaces. It is
16 thus desirable to formulate them, in its effective concentration, in a way that allows
17 minimizing the evaporation and protecting the oil, from high temperature, oxidation and UV
18 light, at the same time. Besides, such formulations should allow for a selective release and for
19 the increase of the shelf life of the oil. For this purpose, oil-in-water (O/W) emulsions are one
20 of the preferred formulations of EOs.

21 Stable emulsions are best formulated with emulsifiers or combination of emulsifiers having
22 HLB (hydrophile–lipophile balance) values close to that required for the oil phase (Aulton,
23 1995). The required HLB values of commonly used fixed and mineral oils have been
24 determined but those of EOs are yet to be studied.

25 The HLB of rosemary EO has been determined according to the method proposed and
26 validated by Orafidiya and Oladimeji (2002) based on the determination of droplet size and
27 size distribution of emulsions prepared with surfactants of a wide range of HLB values, being
28 the required HLB for the essential oil that of the surfactant leading to the most stable
29 emulsion, i.e. that of the minimum droplet size.

30 As previously mentioned, this required HLB value of the oil phase will be useful to select an
31 appropriate surfactant for the emulsion. These data is mainly available for synthetic
32 surfactants or it can be calculated from the chemical formula by group contribution methods
33 (Tadros, 2005; Wang et al., 2011). However, this information is not always available for
34 natural or natural derived surfactants, such as n-octenyl succinic anhydride (OSA)-modified
35 starches, whose application in emulsion formulation is increasing in the last years (Varona et
36 al., 2009; Wang et al., 2011).

37 In such cases, the measurement of the interfacial tension between the oil and a water solution
38 of the surfactant whose HLB value is unknown, provide reliable and fast information for the
39 selection of the appropriate surfactant and the minimum concentration needed to obtain a
40 stable emulsion (Huang et al., 2011) which is generally higher than the critical micelle
41 concentration (CMC) for the corresponding surfactant, provided that this information is
42 available. The smaller the interfacial tension between the oil phase and water, the more stable
43 will be the emulsion as it adsorbs onto the oil forming interfacial film, reducing the interactions
44 between both phases and the energy required to expand the interface of the phase to be
45 dispersed, in this case the oily one, for the formation of the emulsion (Tadros, 2005).

46 Hence, in the second part of this work the interfacial tension between the Rosemary EO and
47 the water for four biodegradable and non-toxic surfactants, n-octenyl succinic anhydride
48 (OSA)- modified starches, was measured and compared with the interfacial tension of the EO
49 and a synthetic surfactant with a value of HLB equal to that required HLB by the EO, in order to
50 select the most adequate OSA-starch and the minimum concentration required to obtain a
51 stable emulsion.

52 2. Materials and Methods

53 2.1. Materials

54 The essential rosemary (*Rosmarinus officinalis L.*) oil was supplied by COCOPE (Peñafield, Spain).
55 It was obtained by steam distillation. The surfactants, Span[®] 20, Tween[®] 20 and Tween[®] 80,
56 were purchased from Sigma (Spain). The (OSA)-modified starches were kindly provided by
57 National Starch Food Innovation (Hamburg, Germany). The followings were tested: OSA-starch
58 derived from waxy maize (OSA-1), OSA-starch derived from waxy maize blend with dried
59 glucose syrup (OSA-2), OSA-dextrin derived from waxy maize (OSA-3) and OSA-dextrin derived
60 from tapioca (OSA-4).

61 The main components of the rosemary oil analyzed by Gas Chromatography are displayed in
62 Table 1.

63 2.2. Methods

64 2.2.1. Preparation of the emulsion

65 Oil-in-water emulsions were prepared by a two-step process. A surfactant solution (200 mL)
66 was initially prepared by dissolving the surfactant at a concentration of 1g/L in deionized water
67 (Milli-Q, Millipore) with the aid of a magnetic stirrer (IKA). Afterwards 50 mL essential oil was
68 gradually added to the suspension under continuous agitation for 5 min and a crude emulsion
69 was obtained. The resulting coarsely dispersed raw emulsion was then fed into the rotor-stator

70 machine (IKA® LABOR PILOT 2000/4) and processed during 2 min for fine emulsification at
71 70Hz, according to Varona et al, 2009.

72 2.2.2. Preparation of the mixture of surfactants

73 Emulsions were prepared using a combination of two surfactants, Span 20 whose HLB is 8.6
74 and Tween 20 whose HLB is 16.7. The proportion between these two surfactants was changed
75 in order to obtain HLB values in-between. The HLB of the mixture was calculated according to:

$$76 \text{ HLB} = x_A \cdot \text{HLB}_A + x_B \cdot \text{HLB}_B \quad (1)$$

77 where x_A and x_B are the weight fractions of each surfactant.

78 2.2.3. Droplet size analysis

79 Rosemary oil droplet size distribution of the emulsion was determined by laser diffraction
80 (Mastersizer 2000, Malvern Instruments) with a dual-wavelength detection system (Red light:
81 633 nm and Blue light: 436 nm). The sample was diluted with deionized water in the dispersion
82 unit (Hydro SM) to less than 1% (v/v) to prevent multiple scattering effects. The refractive
83 index of the dispersed phase, rosemary essential oil, was experimentally determined (Stanley
84 Abbey Refractometer) to be 1.468, in good agreement with literature values (Atti-Santos et al.,
85 2005). Particle size measurements are reported as the 10%, 50% and 90% quantiles of the
86 volume distribution, i.e. the 50% volume of droplets have a size smaller than the $d_{50,v}$ value;
87 The values are the average of 3 measurements. The maximum incertitude of the analysis was
88 5%, except for stored emulsions of HLB below 10.7 that reached values up to 20%.

89 In order to observe variation during the storage time, the measurements were made directly
90 after the production of the emulsion, and 21 and 50 days later following the same procedure.

91 2.2.4. Determination of the stability of emulsions

92 The emulsion stability, that means the ability of an emulsion to resist changes in its properties
93 with time, was analyzed using two different criteria: the amount of de-emulsified oil at two

94 time intervals (21 and 50 days) and the kinetics of sedimentation and creaming of the
95 emulsion in the following 60 minutes after preparation.

96 For both analysis, 7 mL of the emulsion were poured in a vertical glass tube with an inner
97 diameter of 13 mm (height of emulsion: 55 mm) and stored at ambient conditions.

98 The height of visible supernatant oil layer was recorded, and compared to maximum height of
99 oil (11 mm), i.e. the emulsion were completely de-emulsified; this ratio multiple by 100 was
100 the % of de-emulsinated oil.

101 Particles migration phenomena (creaming or sedimentation) induce particle volume fraction
102 changes at the extremities of the sample. The creaming and sedimentation rates were
103 followed using a Turbiscan Classic MA2000 (Formulacion, France). The stability of emulsions is
104 followed measuring the variation of %backscattering of an 860 nm near infrared beam versus
105 the height of the sample, in steps of 40 μm , every 2 min for 60 min. The backscattering
106 detector receives the light scattered backward by the sample at an angle of 45° with respect to
107 the light source. The software of the equipment determines the calculation the slopes of the
108 %backscattering curves versus time in the lower and upper part of the emulsion (Figure 2,
109 inner box) in order to evaluate the kinetics of sedimentation and creaming, respectively. These
110 rates are then used to calculate the thickness of sedimentation and creaming after a defined
111 time (i.e. 60 minutes) for each emulsion in order to compare them.

112 2.2.5. Evaluation of modified starch surfactants

113 In order to evaluate the performance of the modified starch surfactant, the interfacial tension
114 between the EO and the aqueous phase was measured. The concentration of surfactant in the
115 aqueous phase ranged from 0 to 100 g/L. The interfacial tension was compared with that
116 achieved with a synthetic surfactant with a HLB equal to that required by the rosemary EO.
117 Measurements were made in triplicate using the Wilhelmy plate method with a tensiometer

118 EasyDyne (Krüss, Germany). The equilibrium time required for steady state interfacial tension
119 measurements was 2 min. The maximum standard deviation was 5%.

120 3. Experimental results and discussion

121 3.1. Required HLB

122 The required HLB value for the rosemary essential oil is between 12 and 15. According to the
123 analysis of mean drop size (Figure 1), expressed as the 50% quantile ($d_{50,v}$), a defined HLB
124 cannot be identified for a clear minimum value of the droplet size.

125 The de-emulsified oil after 50 days was neither a clear criteria, as only emulsions prepared
126 with a surfactant mixture HLB value smaller than 10.7 show a measurable (> 1 mm) layer of
127 supernatant oil. Besides, the emulsion prepared with a surfactant of HLB 14 displayed a thin
128 layer of oil with a height lower than 1mm. Only, the emulsions prepared with surfactants of
129 HLB 8.6 and 9.2 were completely de-emulsified after 50 days.

130 The analysis of the backscattered light across the emulsion allows determining the height of
131 emulsion sedimentation and creaming (e) for each emulsion, to evaluate its stability in the first
132 hour after their preparation (Figure 2). In a similar way to previous criteria of stability, all the
133 emulsions prepared with mixtures of surfactants with a HLB value higher than 10.7 have
134 similar values of "e".

135 In order to point out a specific value of required HLB in the range from 12 to 15, the width of
136 the droplet size distribution (DSD) based on the 10% and 90% quantile was analyzed. As
137 shown in Figure 3, fresh emulsions prepared with surfactant mixtures with HLB between 10.7
138 and 16.7 present a very narrow DSD. As the emulsions evolved with time, the width of the DSD
139 increases; after 21 days the initial HLB range is reduced to 13 to 15, and after 50 days, the
140 narrowest DSD is the one of the emulsion prepared with a surfactant mixture of HLB equal to
141 15. Hence, it can be concluded that the required HLB of the rosemary essential oil is 15.

142 This value is slightly higher than the values of other essential oils for aromatic herbs from the
143 order of Lamiales, such as lavandin oil with a value of 13.4 (Varona et al., 2009), lippia and
144 peppermint oils with values of 12.1 and 12.3, respectively (Orafidiya and Oladimeji, 2002).
145 Although, the HLB may be related with the hydrophilicity of the main component of the
146 essential oil, this is not always the case due to the great amount of components present in the
147 oil. In the lavandin oil, the main component is the linalool (ca. 45%), which is more hydrophilic
148 than the menthone (Phillips et al., 2010), the main component in the peppermint oil (ca.45%-
149 60%). However, the main component of the rosemary oil used in this work is the 1,8-cineole
150 (ca. 24%), which is more hydrophobic than the linalool, although HLB value of rosemary oil is
151 higher than that of lavandin oil. These can be due to the presence of other minor components
152 more hydrophilic, such as camphor (ca. 16%).

153 3.2. Evaluation of modified starch surfactants

154 As shown in Figure 4, the most adequate modified starches to be employed as surfacts with
155 rosemary essential oil, are OSA-4 and OSA-2, as they decrease the interfacial tension between
156 water and rosemary EO from 16.2 ± 0.6 mN/m to a lower value with respect to the other
157 surfactants tested. As reference, the interfacial values using a synthetic surfactant with the
158 HLB value required by the rosemary EO (Tween® 80, HLB =15) are also presented. The
159 minimum concentration of the surfactant that should be used to prepare stable emulsions of
160 rosemary EO is 25 g/L, which is 5 times higher than the critical micelle concentration of these
161 two surfactants, 4.5 g/L and 5.2 g/L for OSA-2 and OSA-4, respectively (Varona et al., 2009).

162 **4. Conclusions**

163 The characterization of rosemary EO regarding its formulation in emulsion has been carried
164 out. A required HLB value of 15 was determined according to the stability of emulsions in
165 terms of minimum droplet size and width of the drop size distribution over a period of 50 days
166 storage at ambient conditions. The performance of four biodegradable and non-toxic

167 surfactants derived from starch as stabilizers for rosemary essential oil emulsions in water, was
168 tested based on interfacial tension measurements. The best surfactants, OSA-2 and OSA-4,
169 reduced the interfacial tension between water and the essential oil in two thirds.

170 **Acknowledgments**

171 The authors thank the financial support of Junta de Castilla y Leon (Spain) through the project
172 GR11/2008. National Starch Group is acknowledged for supplying the OSAmodified starches
173 and giving permission to publish the results obtained with them. S.Rodríguez-Rojo thanks the
174 Spanish Ministry of Education for her postdoctoral grant. S. Varona thanks the University of
175 Valladolid through the FPI-UVa research program.

176 **References**

- 177 Atti-Santos, A. C., Rossato, M., Pauletti, G.F., Rota, L. D., Rech, J.C., Pansera, M.R., Agostini, F.,
178 Serafini, L. A., Moyna, P., 2005. Physico-chemical Evaluation of Rosmarinus officinalis L.
179 Essential Oils. Braz. arch. biol. technol. 48, 1035-1039
- 180 Aulton, M.E. (Ed.), 1995. Pharmaceutics: The Science of Dosage Form Design. Churchill
181 Livingstone, New York, pp. 290–292.
- 182 Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—
183 a review. Int. J. Food Microbiol. 94, 223– 253.
- 184 Calsamiglia, S, Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A., 2007. Essential oils as
185 modifiers of rumen microbial fermentation. J. Dairy Sci. 90, 2580-2595.
- 186 El-Shafei, G.M.S., El-Said, M.M., Attia, H.A.E., Mohammed, T.G.M., 2010. Environmentally
187 friendly pesticides: Essential oil-based w/o/w multiple emulsions for anti-fungal formulations.
188 Ind. Crop Prod. 31, 99-106.
- 189 [Huang, X., Kakuda, Y., Cui, W., 2011. Hydrocolloids in emulsions: particle size distribution and](#)
190 [interfacial activity. Food Hydrocolloid 15, 533-542.](#)

191 Orafidiya, L., Oladimeji, F.A., 2002. Determination of the required HLB values of some essential
192 oils. *Int. J. Pharm.* 237, 241-249.

193 Phillips, A. K., Appel, A. G., Sims, S. R., 2010. Topical Toxicity of Essential Oils to the German
194 Cockroach (Dictyoptera: Blattellidae), *J. Econ. Entomol.* 103, 448-459.

195 Tadros, T.F., 2005. *Applied Surfactants: Principles and Applications*. Wiley-VCH Verlag GmbH &
196 Co.KGaA. Weinheim (Germany).

197 Varona. S, Martín. A, Cocero. M.J., 2009. Formulation of a natural biocide based on lavandin
198 essential oil by emulsification using modified starches. *Chem. Eng. Process.* 48, 1121-1128.

199 [Wang, X., Li, X., Chen, L., Fengwei, X., Yu, L., Li, B., 2011. Preparation and characterisation of](#)
200 [octenyl succinate starch as a delivery carrier for bioactive food components. *Food Chem.* 126,](#)
201 [1218-1225](#)

Tables

Table 1. Main components of the rosemary essential oil analyzed by GC

Component	wt. %
1,8-cineole	23.8
α -pinene	15.6
camphor	15.6
camphene	9.2
Bornyl acetate	5.7
β -pinene	5.1
Borneol	4.4
caryophyllene	3.9
α -terpineol	1.5

Figures and captions

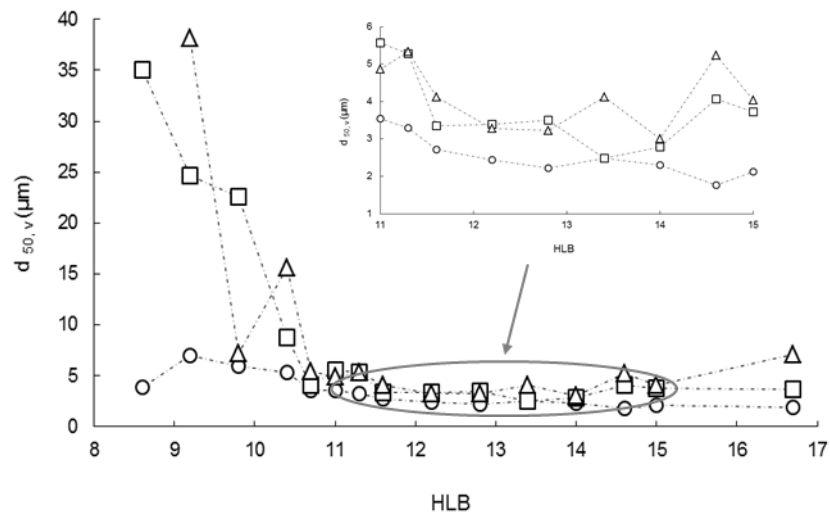


Figure 1. Drop emulsion diameter for different HLB values. Evolution with time: \circ - 0 days (preparation), \square - 21 days and Δ - 50 days.

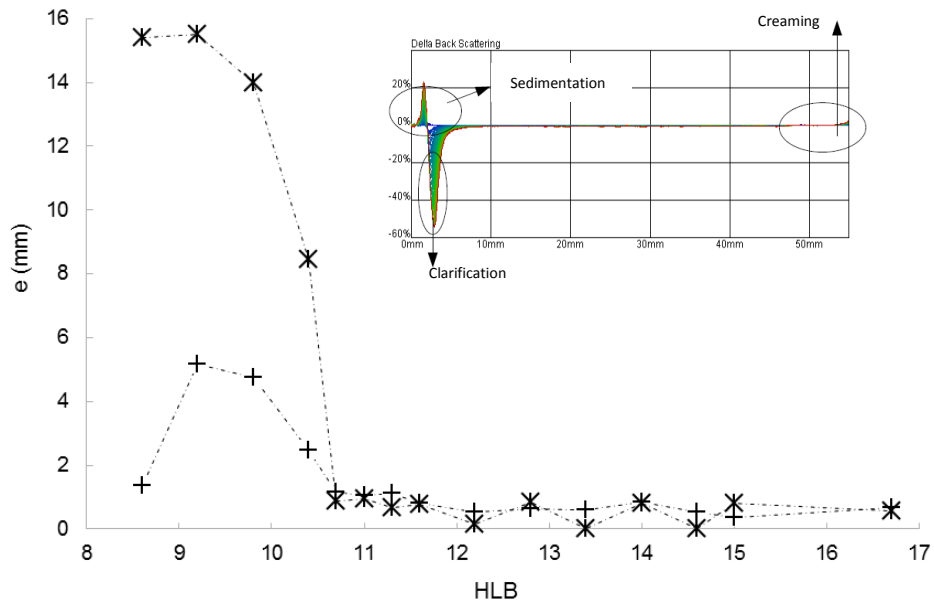


Figure 2. Thickness of the layer of sedimentation (+) and creaming (*) phenomena in the emulsion 60 minutes after preparation for different HLB values of the surfactant. Inner box: Backscattering profiles of the emulsion prepared with a HLB mixture value of 10.7, taking as reference the backscattering scan at time 0. Source data for the determination of the thickness of the referred layers.

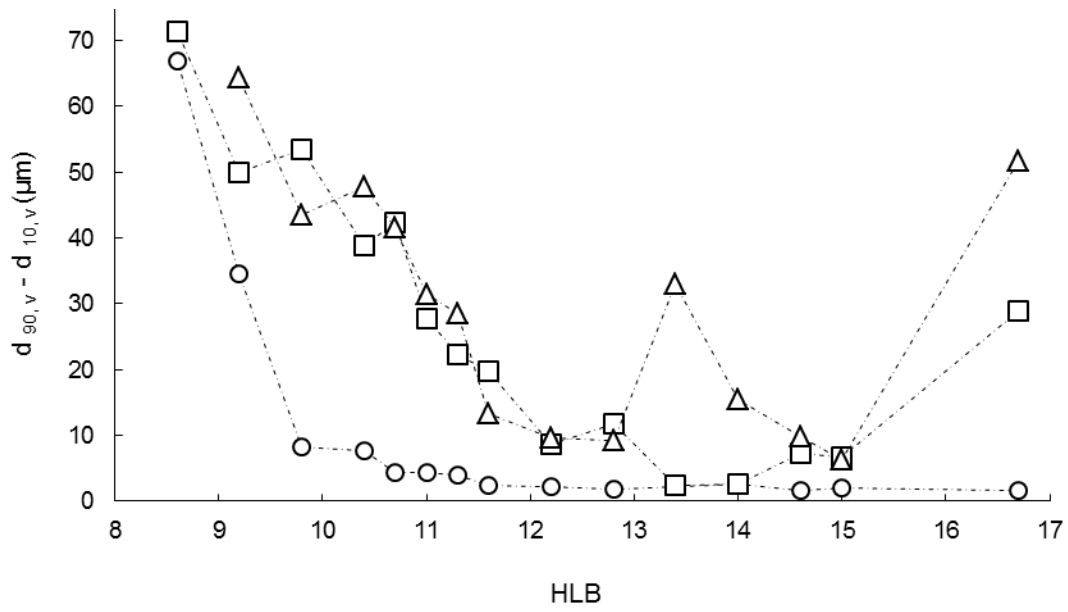


Figure 3. Width of particle size distribution ($d_{90,v} - d_{10,v}$) for different HLB values. Evolution with time: \circ - 0 days (preparation), \square - 21 days and Δ - 50 days.

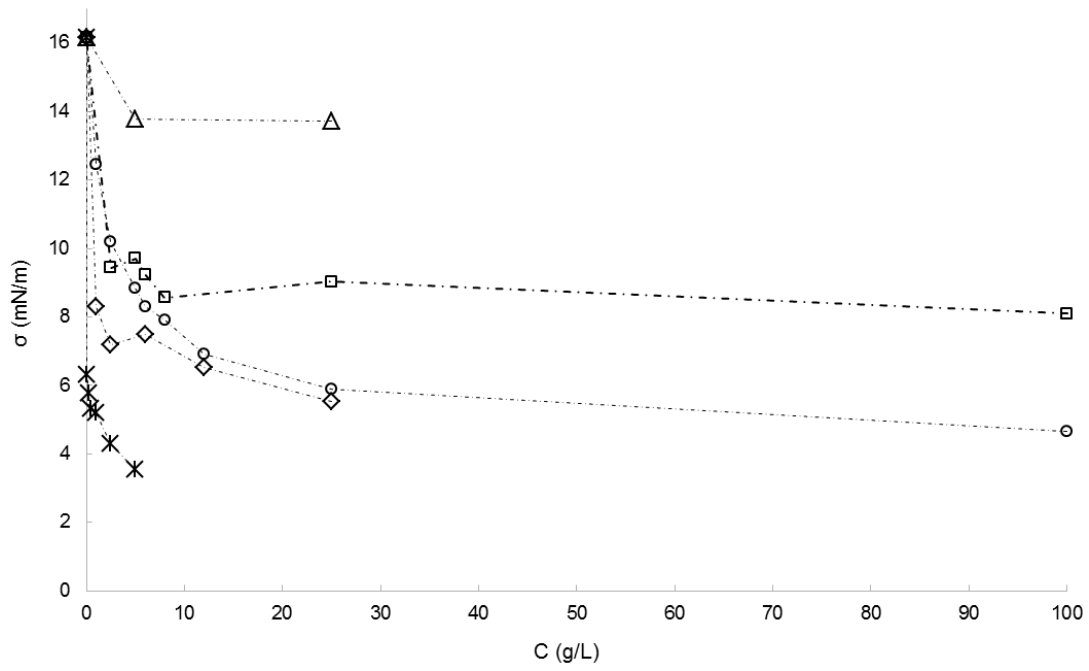


Figure 4. Reduction in interfacial tension water/ rosemary EO with the addition of surfactants:

OSA-1 (□), OSA-2 (◇), OSA-3 (Δ), OSA-4 (○) and Tween 80 (*).