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HLB value, an important parameter for the development of essential oil phytopharmaceuticals

Caio P. Fernandes,^{1,2} Manuela P. Mascarenhas,¹ Fiorella M. Zibetti,¹ Barbara G. Lima,^{2,3} Rafael P. R. F. Oliveira,^{1,2} Leandro Rocha,^{2,3} Deborah Q. Falcão^{*,1,3}

¹Laboratório de Tecnologia Farmacêutica I, Faculdade de Farmácia, Universidade Federal Fluminense, Brazil;

²Laboratório de Tecnologia de Produtos Naturais, Faculdade de Farmácia, Universidade Federal Fluminense, Brazil;

³Programa de Pós-graduação em Ciências Aplicadas a Produtos para Saúde, Faculdade de Farmácia, Universidade Federal Fluminense, Brazil.

Abstract: Essential oils are used primarily as natural preservatives, flavourants and fragrances in cosmetic products. Several pharmacopeias possess monographs of plants which are good sources of essential oils, such as Brazilian Pharmacopeia, including *Illicium verum* Hook. f., Schisandraceae and *Rosmarinus officinalis*. Since determination of Hydrophile-Lipophile Balance (HLB) value of essential oils appears as a critical step for development of emulsions and other semi-solid formulations, evaluation of required HLB values for *I. verum* and *R. officinalis* essential oils is the aim of this study. They were obtained by hydrodistillation and several emulsions were prepared by changing emulsifiers. The couple sorbitan oleate/polysorbate 20 provided best emulsions and was used at different ratios, at a total blend concentration of 5% w/w. The lowest mean droplet diameters for *R. officinalis* and *I. verum* emulsions were obtained at HLB 16.5 (97.12 nm) and 16.7 (246.6 nm), respectively. Moreover, emulsions with *R. officinalis* were finer and presented some bluish reflection, characteristic of nanoemulsions. The lowest turbidity value for *R. officinalis* emulsion was also obtained at HLB 16.5 (0.33). Thus, the present study describes for the first time HLB values for *R. officinalis* (16.5) and *I. verum* (16.7) essential oils, contributing to their physicochemical characterization and technology development of phytopharmaceuticals.

Introduction

Essential oils (EO) are used primarily as natural preservatives, flavourants and fragrances in cosmetic products (Orafidiya & Oladimeji, 2002). In addition, studies related to biological properties of EO have been intensified, indicating ectoparasitic (Oladimeji et al., 2000), insect repellent (Oyedele et al., 2000), anticholinesterase (Lima et al., 2012), anti-diarrheal (Orafidiya & Oladimeji, 2002), antimicrobial and cytotoxic (Apel et al., 2006; Stefanello et al., 2011) activities.

Illicium verum Hook. f., Schisandraceae, commonly known as star-anise, is well known for its use as aromatic spice in culinary (Tuan & Ilangantileke, 1997). The EO from this species has (*E*)-anethole as main constituent, ranging from 70-94% of the relative composition (Wang et al., 2011) and this substance has fungicidal action against dermatophytes and insecticide

activity (Lima et al., 2008). It is described for the EO from *I. verum* some insecticidal (Zhao et al., 2012) and antiviral properties (Koch et al., 2008).

Rosmarinus officinalis L. belongs to the Lamiaceae family and is popularly known in Brazil as "alecrim". Despite its culinary use, various biological activities are also associated with this herb, which includes antimicrobial, antioxidant (Zaouali et al., 2010), antiproliferative (Tai et al., 2012), antidiabetic (Bakirel et al., 2008) and food preservation properties (Gachkar et al., 2007). This EO was also considered non-toxic in animal models (Lemônica et al., 1996).

Stable emulsions, especially where synthetic surfactants are used, are best formulated with emulsifiers or combination of emulsifiers having HLB (Hydrophile-Lipophile Balance) values close to that required of the oil phase (Orafidiya & Oladimeji, 2002). The HLB number is a semi-empirical scale for selecting surfactants (Griffin, 1949). On this context,

development of emulsions with blends of surfactants on a wide range of HLB values can provide a satisfactory determination of the required HLB of lipophilic phase, such as essential oils. Moreover, the HLB value of an essential oil appears as a critical step for development of emulsions and other semi-solid formulations. The number of experiments can be reduced early during the formulation screening stage by using these parameters (Schmidts et al., 2010) and can be used as an important parameter for quality control.

Several pharmacopeias possess monographs of plants which are good sources of essential oils. Since these complex mixtures are also used as raw material for different approaches, some of them have specific essential oils monographs with parameters of quality such as density, refractive index and optical rotation (Farmacopéia Brasileira, 2010). On this context, Brazilian Pharmacopeia was recently revised and the new version includes monographs of *Illicium verum* dried fruits and *Rosmarinus officinalis* essential oil from flowering aerial parts (Farmacopéia Brasileira, 2010). Thus, the present study aimed to investigate the evaluation of the required HLB values of the essential oils of *Illicium verum* and *Rosmarinus officinalis*.

Materials and Methods

Plant material

Dried fruits of *Illicium verum* (lot number 02/0908) were purchased from Santosflora Comércio de Ervas Ltda. (São Paulo, Brazil) and dried leaves of *Rosmarinus officinalis* (lot number 014) were purchased from Quimer Ervas e Especiarias (São Paulo, Brazil). They were pharmacognostically characterized by Prof. Cid Aimbiré M. Santos and samples were deposited at the "Herboteca Carlos Stelfled" from the Pharmacognosy Laboratory of the Departamento de Farmácia Universidade Federal do Paraná, with registry number 39-A and 13-A for *I. verum* and *R. officinalis*, respectively.

Chemicals

Sorbitan oleate (HLB=4.3) and Polysorbate 20 (HLB=16.7) were purchased from La Belle ativos Ltd. (Paraná, Brazil).

Extraction of the essential oils

I. verum (1.3 kg) and *R. officinalis* (1.8 kg) were individually turbolized with distilled water. Then, each material was placed in a 5 L round bottomed flask and submitted to hydrodistillation during 3 h using a Clevenger type-apparatus. At the end, the oils were

collected and stored at 4 °C for further analyses.

Gas chromatography/mass spectrometry analysis

Essential oils were analyzed by a GCMS-QP2010 (SHIMADZU) gas chromatograph equipped with a mass spectrophotometer using electron ionization. One microliter of each essential oil, dissolved in CH₂Cl₂ (1:100 mg μL⁻¹), was individually injected at RTX-5 column (i.d. = 0.25 mm, length 30 m, film thickness = 0.25 μm). The gas chromatographic (GC) conditions were as follows:

Rosmarinus officinalis: injector temperature, 200 °C; detector temperature, 240 °C; carrier gas (Helium), flow rate 1 mL min⁻¹ and split injection with split ratio 1:40. The oven temperature was programmed from 50 °C (isothermal for 10 min), with an increase of 2 °C min⁻¹, to 200 °C, ending with a 25 min isothermal at 200 °C.

Illicium verum: injector temperature, 220 °C; detector temperature, 250 °C; carrier gas (Helium), flow rate 1 mL min⁻¹ and split injection with split ratio 1:40. The oven temperature was programmed from 60 °C, with an increase of 3 °C min⁻¹, to 300 °C.

The mass spectrometry (MS) conditions were voltage 70 eV and scan rate 1 scan s⁻¹. The retention indices (RI) were calculated by interpolation to the retention times of a mixture of aliphatic hydrocarbons (C₉-C₃₀) analyzed in the same conditions (Van den Dool & Kratz, 1963). The identification of the substances was performed by comparison of their retention indices and mass spectra with those reported in literature (Adams, 2007). The MS fragmentation pattern of compounds was also checked with NIST (National Institute of Standards and Technology) mass spectra libraries. Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (GC/FID), under same conditions of GC/MS analysis and percentages obtained by FID peak-area normalization method.

Assays

Preparation of emulsion

Essential oil emulsions were prepared at a final volume of 20 mL, containing 90% w/w of water and 5% w/w of essential oils. The emulsifiers, Sorbitan oleate and Polysorbate 20, at total blend concentration of 5% w/w were used for the essential oil emulsions. The required amounts of both surfactants were dissolved in the oil phase. The aqueous phase was heated until 75±5 °C and the oil phase until 40±1 °C. Both phases were mixed by the inversion method with mechanical stirring (400 rpm) for 15 min (Aulton, 2005). Series of emulsions with HLB values ranging from 4.3 to 16.7

were prepared by blending together the emulsifiers in different ratios. A second set of emulsions was later prepared using smaller ratio intervals between the two most stable emulsions from the first series. The stability of all emulsions was evaluated 1, 30 and 60 days after manipulation by macroscopic analysis (color, visual aspect, phase separation, creaming and sedimentation) (Falcão, 2007). During this period all emulsions were maintained under room temperature (25±2 °C) in screw-capped glass test tubes.

Droplet size analysis

The droplet size and polydispersity were determined by photon correlation spectroscopy using a Zetasizer 5000 (Malvern Instruments, Malvern, UK). Each emulsion was diluted using ultra-pure Milli-Q water (1:25). Measures were performed in triplicate. An average droplet size was expressed as the mean diameter (Orafidiya & Oladimeji, 2002).

Turbidimetric method

Each sample (1 mL) was diluted with distilled water (25 mL) and the percentage transmission (%T) was measured at 600 nm (previously determined for distilled water used as the blank control) with a spectrophotometer. With the blank control set at 100% transmission, the turbidity of the diluted emulsion was calculated as:

$$\text{Turbidity} = 100 - \%T.$$

The results obtained were average of three determinations.

Results and Discussion

After the extraction, both essential oils presented a clear light yellow aspect. The essential oil from *Illicium verum* Hook. f., Schisandraceae, showed higher yield (2.4 %) when compared to the essential oil from *Rosmarinus officinalis* L., Lamiaceae (1.3 %).

The *I. verum* essential oil presented (*E*)-anethole (80.1%) and shisofuran (10.3%) as main constituents. This result is in accordance with literature data, which indicates that (*E*)-anethole is the major substance of essential oil from fruits of this species, ranging from 70-94 % (Wang et al., 2011). *R. officinalis* essential oil showed α -pinene (9.4%), camphene (3.3%), 1,8-cineole (44.0%) and camphor (16.1%). Moreover, these contents are higher than the minimum required on the monograph of *I. verum* dried fruits and *R. officinalis* essential oil (Brasil, 2010). The constituents and relative amounts of substances of essential oils from *I. verum* and *R. officinalis* are

indicated in Table 1.

Table 1. Relative abundance (%) of the essential oil constituents of *Illicium verum* and *Rosmarinus officinalis*.

Constituents	<i>Illicium verum</i>		<i>Rosmarinus officinalis</i>	
	RI	%	RI	%
α -pinene	934	0.1	930	9.4
camphene	-	-	943	3.3
β -myrcene	-	-	972	11.7
β -pinene	-	-	991	1.0
α -phellandrene	-	-	1001	0.2
γ -3-carene	1012	0.2	-	-
α -terpinene	-	-	1014	0.4
p-cymene	-	-	1022	2.4
limonene	1029	1.7	-	-
1,8-cineole	1032	0.1	1030	44.0
γ -terpinene	1058	0.1	1057	0.3
terpinolene	-	-	1086	0.2
terpineole	1089	0.1	-	-
linalol	1100	0.8	-	-
fenchol	-	-	1110	0.1
camphor	-	-	1141	16.1
isopulegol	-	-	1152	0.1
borneol	-	-	1162	3.5
terpinen-4-ol	1178	0.3	1174	1.2
verbenone	-	-	1188	4.1
α -terpineol	1192	0.2	-	-
shisofuran	1201	10.3	-	-
p-anisaldehyde	1250	1.8	-	-
<i>E</i> -anethole	1252	80.1	-	-
bornyl acetate	-	-	1283	0.5
thymol	-	-	1289	0.1
anisyl methyl ketone	1385	0.1	-	-
<i>E</i> -caryophyllene	-	-	1414	0.6
α -trans-bergamotene	1437	0.1	-	-
α -himachalene	-	-	1448	0.1
curcumene	-	-	1481	0.1
α -muurolol	1650	0.1	-	-
14-hidroxy-Z-caryophyllene	-	-	1668	0.1
foeniculin	1681	3.3	-	-
Total identified	99.2		99.5	

Several emulsions were prepared with both *R. officinalis* and *I. verum* essential oils. Optimized process parameters found in the preliminary study were applied as the heat temperature of the oil phase and the choice of the surfactant couple (data not shown). Different surfactants were previously tested in order to select the best couple. In this sense, the non-ionic emulsifiers

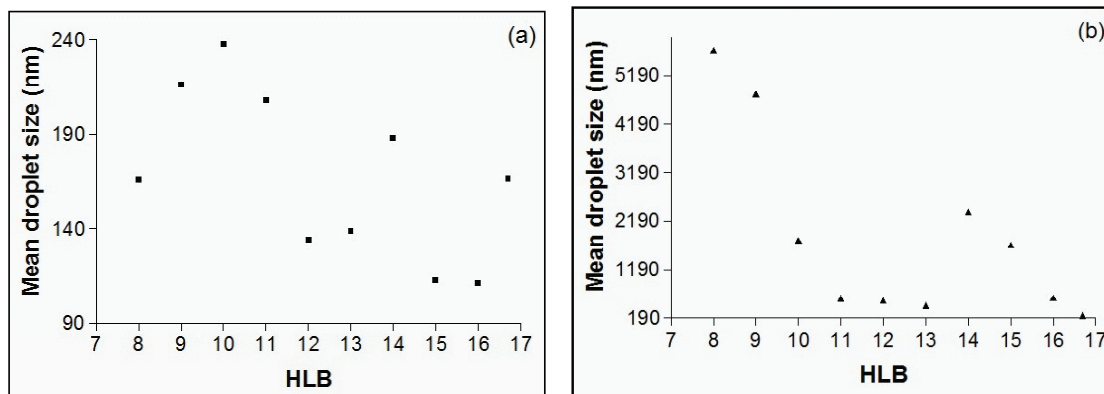


Figure 1. Mean droplet size of *Rosmarinus officinalis* (a) and *Illicium verum* (b) emulsions versus HLB.

from fatty acid esters, Sorbitan oleate and Polysorbate 20, showed best results and were used at total blend of 5% w/w at different ratios. According to the literature, this concentration is sufficient to obtain emulsions in a range which possibilities the establishment of HLB values, employing a single couple of surfactants (Orafidiya & Oladimeji, 2002).

The lowest mean droplet diameters for *R. officinalis* and *I. verum* emulsions were obtained at HLB 16.5 (97.12 nm) and 16.7 (246.6 nm), respectively (Figure 1). The size distribution for both emulsions is shown in Figure 2. The emulsions at the HLB values ranging from 4.3 to 7.0 cracked right after manipulation and it was not possible to evaluate the parameters droplet size and turbidity.

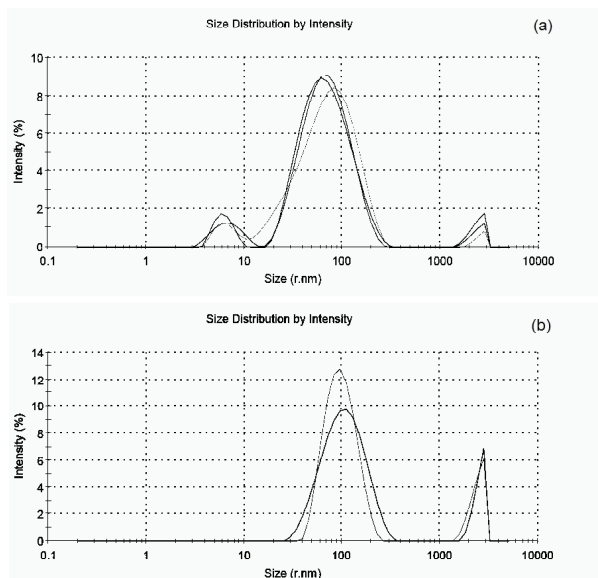


Figure 2. Particle size distribution for (a) *Rosmarinus officinalis* emulsion of HLB 16.5 (97.12 nm) and (b) *Illicium verum* emulsion of HLB 16.7 (246.6 nm).

The emulsions obtained with *R. officinalis* were finer and most of samples showed some bluish reflection, known as Tyndall effect, which is characteristic for nanoemulsions (Solans et al., 2005) (Figure 3). According to Solans et al. (2005) nanoemulsions are dispersions of droplet size typically in the range 20-200 nm. Due to their characteristic size, it possesses stability against sedimentation, creaming, flocculation or coalescence. This long term physical stability of nanoemulsions makes them unique, and they are sometimes referred to as “approaching thermodynamic stability” (Izquierdo et al., 2002; Solans et al., 2005). Moreover, nanoemulsions using essential oils can be especially interesting, since these types of formulations can be applied for delivery of fragrances, which may be incorporated in many personal care products. This could also be applied in perfumes, which are desirable to be formulated alcohol free (Tadros et al., 2004).



Figure 3. Picture of *Rosmarinus officinalis* emulsion (HLB 16.5) (left) and *Illicium verum* emulsion (HLB 16.7) (right) with droplet diameters of 91.12 nm and 246.6 nm, respectively.

For the *R. officinalis* essential oil it was possible to obtain emulsions with mean diameter between 97.12-237.8 nm and low polydispersity. On the other hand, the emulsions obtained with *I. verum* essential oil showed whiter appearance, which is characteristic for classical macroemulsions. The mean diameter droplet size of these samples ranged from 246.6-5704.0 nm. A light bluish reflection was observed only in the emulsion with higher HLB value (16.7), which also showed the lowest droplet size (246.6 nm). These emulsions are useful to the evaluation of required HLB of *I. verum* essential oil, which was the aim of this work. However, these results indicate that the couple of surfactants employed must be reviewed if the development of nanoemulsions based on this oil is desired.

The lowest turbidity value for *R. officinalis* emulsion was obtained at HLB 16.5 (0.33) and corroborates the required HLB found using the droplet size for this oil. The turbidity value obtained for different HLB of *I. verum* emulsions showed no significant difference (ANOVA, $p > 0.05$) and could not be used to identify the required HLB of this oil (Figure 4).

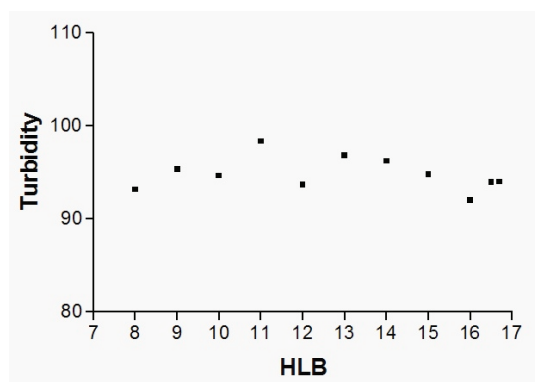


Figure 4. Turbidity values of *Illicium verum* emulsions versus HLB (ANOVA, $p > 0.05$).

According to Orafidiya & Oladimeji (2002), both parameters droplet size and turbidity are useful to determine the required HLB of essential oils. For the *R. officinalis* emulsions, the turbidity values went through a minimum at the same HLB value that mean droplet diameter had a minimal. The correlation coefficient (Pearson r) between the turbidity values and the mean droplet size for this emulsion were 0.6468 ($r^2 = 0.4184$), showing some positive correlation ($p < 0.05$), as expected (Figure 5).

Since the formulation achieves the CMC (critical micelle concentration), it is known that a change of slope occurs in physical properties such as the intensity of light scattered by the dispersion. In the same way, as the droplet size decreases, the intensity of light scattered increases, leading to a reduction of

emulsion turbidity. The CMC is influenced by the surfactant structure and its polarity (Salager, 2000). It was observed greater droplet size for *I. verum* formulations and absence of variation in the turbidity values as function of HLB. These results suggest that the concentration of non-ionic surfactants derived from sorbitan esters should be higher than 5% if the development of *I. verum* essential oil emulsions is desired. The chemical composition of this oil is based on two majority substances, being the (*E*)-anetol the main compound (80.09%). This substance may play an important role in the physicochemical characteristic of this oil, including its required HLB value.

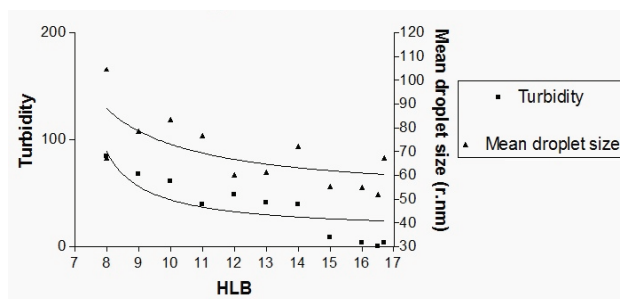


Figure 5. Curves of mean droplet diameter and turbidity of *Rosmarinus officinalis* emulsions versus HLB (ANOVA, $p < 0.05$).

According to the fact that minimum droplet diameter is related to the required HLB and emulsion stability, it is proposed that the most stable emulsion is the one which was formulated with the HLB of surfactants mixture nearest to required HLB of the oil phase (Prinderre et al., 1998; Salager, 2000). In this sense, we evaluated macroscopically all *R. officinalis* and *I. verum* emulsions until two months. After this period of storage, *R. officinalis* emulsion with HLB value 16.5 showed no macroscopic changes, maintaining its original fine appearance and bluish reflection. For *I. verum* emulsions, the one with HLB value 16.7 and smallest droplet size showed some degree of creaming, however, it was the more stable among those prepared with this essential oil. These results corroborate with the HLB values obtained using the other methods discussed earlier for both oils and it is especially important for *I. verum* essential oil, since the turbidity results could not be explored.

Conclusion

On the present work, it is described the required HLB values for the *R. officinalis* and *I. verum* essential oils, 16.5 and 16.7 respectively. Based on the semi-empirical scale proposed by Griffin these results indicates high HLB values, probably related to its chemical

composition based on mono and sesquiterpenes. Although the physicochemical characteristics of these oils are well established in the literature (Farmacopéia Brasileira, 2010) the required HLB values were for the first time evaluated. This parameter is an important tool for the technology development of phytopharmaceuticals and can be successfully employed in the formulation of natural products such as those based in essential oils. In addition, this information can also be used as parameters of quality for essential oils.

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***Correspondence**

Deborah Quintanilha Falcão
Laboratório de Tecnologia Farmacêutica I, Faculdade de Farmácia, Universidade Federal Fluminense Rua Dr. Mário Viana 523, Santa Rosa, 24241-000 Niterói-RJ, Brazil
deborah@vm.uff.br
Tel. 55 21 2629 9560
Fax: 55 21 2629 9578