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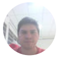







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# Development of Jojoba Oil (*Simmondsia chinensis* (Link) C.K. Schneid.) Based Nanoemulsions

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***SUMMARY*. Nanoemulsions are heterogeneous translucent systems, with intrinsic characteristics that make then in the spotlight of new product candidates for pharmacy, cosmetic and food industries. Surfactants have a main role in nanoemulsion stabilization, which is often associated to similarity among hydrophile-lipophile (HLB) values of oil phase and surfactants mixture. Jojoba oil is a natural raw material for pharmacy industry with economic significance and great potential for nanobiotechnology. The present study describes the development of jojoba oil based nanoemulsions. Measurement of mean droplet size confirmed nanoemulsion formation, in addition to a characteristic bluish reflection expected for this formulation type. Best formulations achieved were nanoemulsions with HLB value of 10 (235.0 nm) and 11 (240.0 nm). This data suggest that HLB value of jojoba oil may be in the zone of 10-11. To our knowledge, these are the first efforts for HLB characterization and nanoemulsion development of formulations with jojoba oil.**

*RESUMEN*. Las nanoemulsiones son sistemas heterogéneos translúcidos, con características intrínsecas que las colocan en el punto de mira de nuevos candidatos de productos para las industrias farmacéutica, cosmética y alimentaria. Los tensioactivos tienen un papel principal en la estabilización de la nanoemulsión, que a menudo se asocia a la similitud entre los valores de hidrofilia-lipofilia (HLB) de la mezcla de las fases de aceite y tensioactivos. El aceite de jojoba es una materia prima natural para la industria farmacéutica con importancia económica y gran potencial para la nanobiotecnología. El presente estudio describe el desarrollo de nanoemulsiones a base de aceite de jojoba. La medida del tamaño medio de las gotitas confirmó la formación de la nanoemulsión, además de un característico reflejo azuladoa esperable para este tipo de formulación. Las mejores formulaciones alcanzados fueron nanoemulsiones con valor de HLB de 10 (235,0 nm) y 11 (240,0 nm). Estos datos sugieren que el valor HLB de aceite de jojoba puede estar en la zona de 10-11. Hasta donde sabemos, estos son los primeros esfuerzos para caracterizar HLB y desarrollar una nanoemulsión de formulaciones con aceite de jojoba.

## INTRODUCTION

Nanoemulsions are heterogeneous systems constituted by two immiscible liquids. They are often presented as translucent kinetically stable systems, being sometimes referred as "approaching thermodynamic stability" [1,2]. Nanoemulsions have wide range of applications, such as oral, ocular, parenteral or transdermal administration and even as edible formulations [3,4]. Intrinsic characteristics of this special type of formulation make it in the spotlight of cosmetic, pharmacy and food industries [2,3,5]. Emulsification process, mainly dispersion of internal phase through external phase usually involves the

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presence of surfactants, which reduce interfacial tension, facilitate droplet disruption and cover droplets, allowing achievement of more stable products [6,7]. Hydrophile-lipophile balance (HLB) is also an important parameter that should be analyzed during formulation screening stage for development of nanoemulsions [8]. HLB is a semi-empirical scale for selecting surfactants [9] and it is possible to obtain stable formulations with smaller mean droplet size using blends of surfactants with HLB value close to the oil phase, making easier to achieve nanoemulsions [10]. Several nanoemulsions have been developed using plant origin oils with pharmaceutical and cosmetic significance, indicating the great potential of these natural products on a nanobiotechnology context [10-13].

*Simmondsia chinensis* (Link) Scheneider is commonly known as jojoba and is a native shrub of Mexico and USA [14]. The liquid extracted from its seed is commonly known as jojoba oil, being almost totally composed by monounsaturated esters of high molar mass alcohols and fatty acids [15,16], including oleic, eicosanoic, palmitoleic and behenic acids [17]. This raw material obtained from seeds has high stability and oxidation resistance. Due to these intrinsic chemical characteristics, it should be more appropriate to be referred as a liquid wax, since it is not a typical trygliceride composed oil [16,17]. Jojoba oil has been widely used in the cosmetic industry, hair care products, pharmaceuticals and also as low calories edible oil [18]. It is recognized by its ability to induce cutaneous regeneration [16], being used in many cosmetic formulations, such as lotions, moisturizers, massage oils and soothing creams [15].

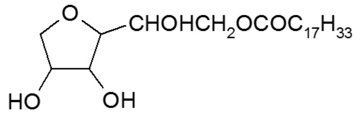
Despite the economic importance of products based on this natural product raw material, to our knowledge, studies regarding its nanobiotechnology potential remains almost unexplored. Thus, the aim of the present study was to investigate HLB value of jojoba oil using different blends of a pair of surfactants, in order to obtain nanoemulsions with this important vegetable oil.

## MATERIALS AND METHODS

### *Chemicals*

Jojoba oil was purchased from Florien (São Paulo, Brazil), sorbitan oleate (HLB: 4.3) and polysorbate 80 (HLB: 15) were purchased from La Belle ativos Ltda (Paraná, Brazil).

(1)



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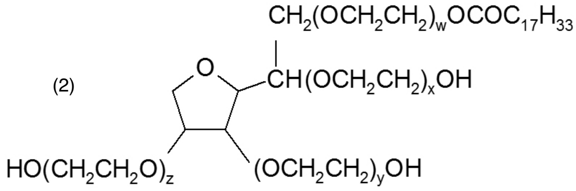

**Figure 1**. Chemical structures of sorbitan oleate (**1**) and polysorbate 80 (**2**).

### *Required HLB determination*

Each emulsion was prepared at a final mass of 25 g, containing 90% (w/w) of distilled water, 5% (w/w) of jojoba oil and 5% (w/w) of a mixture of emulsifiers [10]. Series of emulsions were prepared using sorbitan oleate as most hydrophobic emulsifier (1), and polysorbate 80 (2) as most hydrophilic emulsifier (Fig. 1). HLB values ranged from 4.3 (5% w/w of sorbitan oleate) to 15 (5% w/w of polysorbate 80) and were prepared by blending together the emulsifiers in different ratios. The HLB of each emulsifier mixture was calculated according to Eq. [**1**]:

$$HLBm = \frac{HLB_A \times A\% + HLB_B \times B\%}{100} \quad [1]$$

where $HLB_m$ = hydrophile/lipophile value of the mixture of surfactants; $HLB_A$ = hydrophile/lipophile value of the most hydrophobic surfactant; $HLB_B$ = hydrophile/lipophile value of the most hydrophilic surfactant; *A%* = percentage of most hydrophobic surfactant; *B%* = percentage of most hydrophilic surfactant; *A% + B% = 100.*

### *Emulsification method*

Emulsions were prepared by phase inversion method [19]. The required amounts of both emulsifiers were dissolved in the oil phase (jojoba oil) and heated at 65 ± 5 °C. The aqueous phase was separately heated at 65 ± 5 ºC and gently added and mixed with the oil phase, furnishing a primarily emulsion. Final homogenization was achieved using a T25 Ultra-Turrax homogenizer (Ika-Werke, Staufen, Germany) equipped with a 25 N-18 G disperser for 5 min (8000 rpm).

### *Macroscopical analysis*

Stability of all emulsions was evaluated immediately and after 1, 15, and 30 days of manipulation by macroscopic analysis, such as color, visual aspect, phase separation, creaming and sedimentation. During this period all emulsions were maintained under room temperature (25 ± 2 ºC) in screwcapped glass test tubes 10.

***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 and average droplet size was expressed as the mean diameter [20].

***Statistical analysis***

The significance of the results was analysed using One-way Anova with 95% of confidence interval) and Tukey´s test, using Software R (R Core Team, 2013). Differences among mean droplet diameters were considered significant when $p < 0.05$.

**RESULTS AND DISCUSSION**

The present study was performed in order to obtain jojoba oil based nanoemulsions. The mean droplet diameter of the emulsions measured after their production varied significantly ($p<0.001$). HLB values ranging from 4.3 to 15 were calculated according to Eq. [**1**] and presented particle size distribution ranging from 235.0 nm (HLB-10) to 5138 nm (HLB-15). Emulsions corresponding to HLB values of 4.3, 5, 6, 12, 13, 14, and 15 presented instable behavior, including critical macroscopical changes, such as creaming and phase separation, and therefore were discarded.

Nanoemulsions can be defined as emulsions with mean droplet diameter ranging from 30-300 nm [4]. This small mean droplet size allows obtainment of formulations with high stability to sedimentation, creaming, flocculation and coalescence, being also referred as "approaching thermodynamic stability" [2,21]. Despite emulsion with HLB value of 7 presented just afterwards manipulation a mean droplet size in accordance to this type of formulation (282.8 ± 24.25 nm), it presented some degree of creaming after 1 day and increment of droplet size (323.4 ± 0.9). This could be explained by coalescence of micelles, indicating loss of stability. Measurement of mean droplet size just afterwards manipulations indicated that formulations corresponding to HLB values of 8 (256.0 ± 1.82 nm), 10 (235.0 ± 3.69 nm) and 11 (240.0 ± 1.94 nm) could be characterized as nanoemulsions, while formulation with HLB value of 9 (302.5 ± 8.15) was characterized as a macroemulsion. It was observed no statistical difference between mean droplet size of emulsions with HLB of 8, 9, 10, and 11 just afterwards manipulation and after 1 day of manipulation ($p > 0.05$) (Fig. 2).

It is interesting from a nanobiotechnology overview that jojoba oil derived nanoemulsions was easily obtained using high energy method of emulsification, despite the fact that high-speed homogenizers (*eg*. ultra-turrax) typically produces macroemulsions (2000-10000 nm) [6]. Nanoemulsions with HLB value of 10 and 11

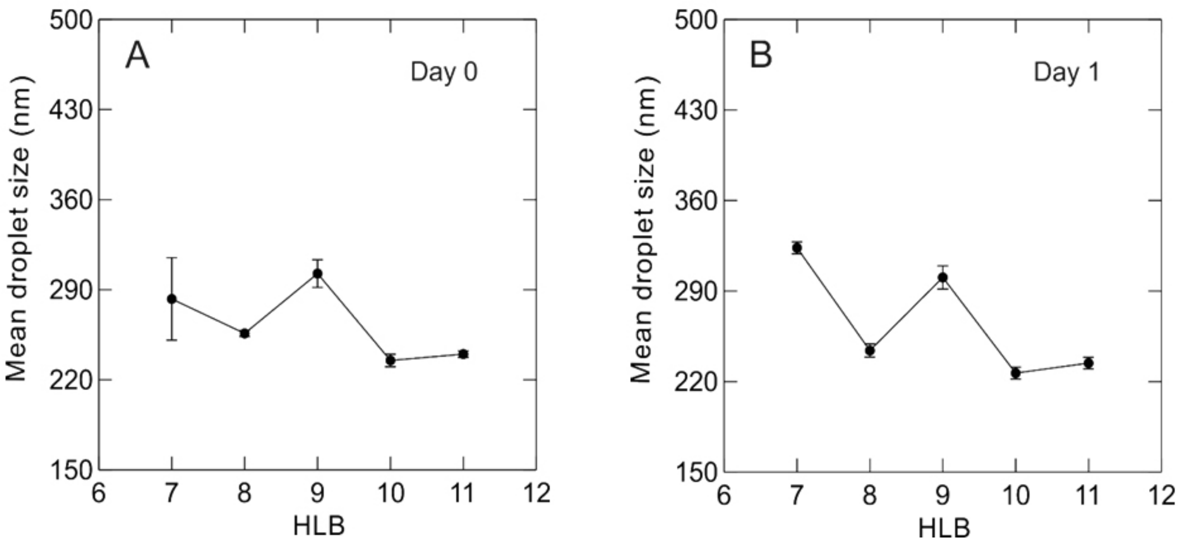


**Figure 2**. Mean droplet size variation of emulsion with jojoba oil in the HLB[a] range 7-11. **A**: Just afterwards manipulation. **B**: After 1 day of manipulation. [a] HLB values of surfactants blends were calculated according to Eq. [**1**].

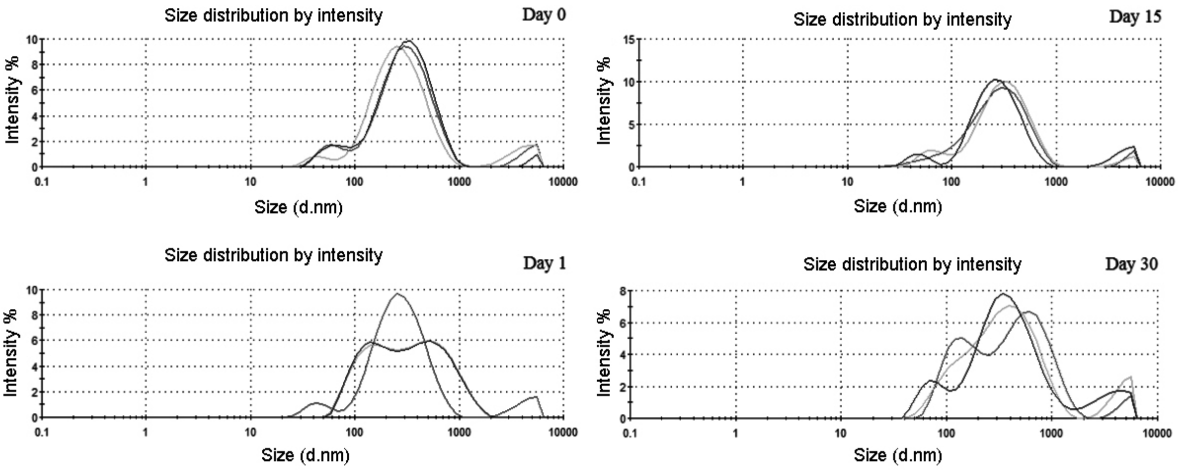


**Figure 3**. Particle size distribution for nanoemulsion with HLB value of 10. Mean droplet size: Day 0 (235.0 ± 3.69 nm); Day 1 (226.6 ± 0.86 nm); Day 15 (241.0 ± 2.37); Day 30 (278.1 ± 6.98 nm).

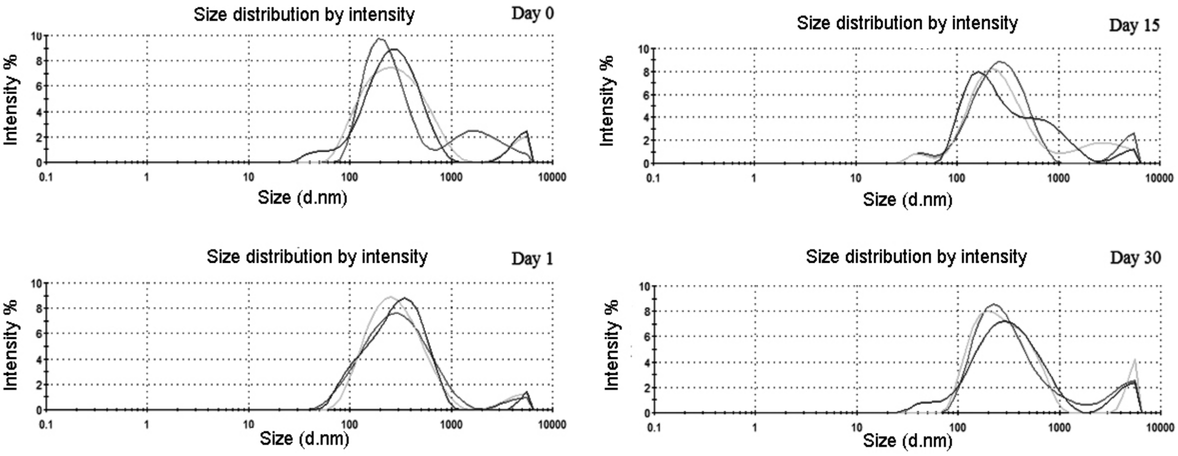


**Figure 4**. Particle size distribution for nanoemulsion with HLB value of 11. Mean droplet size: Day 0 (240.0 ± 1.94 nm); Day 1 (234.3 ± 1.82 nm); Day 15 (234.8 ± 4.13); Day 30 (267.6 ± 4.80 nm).

were the best formulations achieved, presenting smaller mean droplet size. They also showed a characteristic bluish reflection due to Tyndall effect, which is in characteristic for nanoemulsions [21]. Thus, their mean droplet size was monitored for 30 days (Figs. 3 and 4). During all storage period, macroscopical aspects were maintained and mean droplet size of both remained below 300 nm. These two formulations were also characterized as oil in water nanoemulsions. This characteristic is an advantage to development of cosmetic products easily removable by water, parenteral formulations and masking of unpleasant tastes for edible formulations [22], indicating the great potential of nanoemulsions obtained on the present study.

Emulsifiers have a crucial role in the emulsification [13] and utilization of emulsifiers mixtures usually enhances stabilization, when compared to the utilization of just one emulsifier [23]. Moreover, HLB value of an oil have also been recognized as an important parameter on emulsion development stages [8,24], since stable formulations are achieved with emulsifier system having HLB value close to that required for oil phase [20]. This concept allows prediction of best emulsifier system when required HLB value of oil phase is known, achieving nanoemulsions due to the fact that small droplets are obtained when HLB values of emulsifier system coincides with required HLB value of an oil [25]. Required HLB of many oils with wide range of applications in pharmacy and cosmetic industries have been determined, including marigold oil with value of 6.0 24, andiroba oil with value of 16.7 [26], rosemary oil with value of 15 [27], limonene with value of 6.4, orange oil with value of 8.7 [11], lavadin oil with value of 13.4 [13], eucalyptus with value of 9.8, lippia oil with value of 12.1, peppermint oil with value of 12.3 [20], olive oil with value of 12 [28] and canola oil with value of 7 [29]. On this context, information regarding HLB value of oils with pharmaceutical and cosmetic importance is crucial if development of nanoemulsions is desired. However, to our knowledge, HLB value of jojoba oil remained undetermined, despite its great economic importance. The present study indicates that smaller mean droplet size was observed for nanoemulsions with HLB value of 10 and 11, which were the most stable formulations. Since the smallest droplet size and most stable formulations occurs when the HLB value of a pair of surfactants coincides with required HLB value of an oil [30,31], our results suggest that required HLB value for jojoba oil may be close to the HLB range of 10-11.

## CONCLUSION

Jojoba oil is a natural raw material widely used by pharmaceutical industry. Despite its high economic potential, to our knowledge, jojoba oil remained unexplored regarding development of a nanotechnology based product. On the present study, it were obtained some stable nanoemulsions with great potential for commercial use. Moreover, we believe that the present study evaluates for the first time HLB of jojoba oil. We expect to contribute to pharmaceutical nanobiotechnology of natural products, providing good information for development of new products.

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