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Short communication

Development and characterization of evening primrose (*Oenothera biennis*) oil nanoemulsions

Railane F. Rodrigues[a], Isabele C. Costa[a], Fernanda B. Almeida[a], Rodrigo A.S. Cruz[a], Adriana M. Ferreira[b], Jessica C.E. Vilhena[b], Alexandro C. Florentino[c], José C.T. Carvalho[b], Caio P. Fernandes[a,*]

[a] *Laboratório de Nanobiotecnologia Fitofarmacêutica, Curso de Farmácia, Universidade Federal do Amapá, Macapá, AP, Brazil*
[b] *Laboratório de Pesquisa em Fármacos, Curso de Farmácia, Universidade Federal do Amapá, Macapá, AP, Brazil*
[c] *Laboratório de Absorção Atômica e Bioprospecção, Curso de Farmácia, Universidade Federal do Amapá, Macapá, AP, Brazil*

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ABSTRACT

Evening primrose (*Oenothera biennis* L., Onagraceae) seeds oil has great economic importance due to its wide industrial application, mainly for medicines and nutraceutics. However, to our knowledge, it remains almost unexplored regarding development of innovative formulations, such as nanoemulsions. On the present study, required Hydroprophile–Lipophile Balance of evening primrose seeds oil was determined (HLB 12) and a stable nanoemulsion (Day 1: mean droplet size: 214.3 ± 0.69 nm, polydispersity index: 0.253 ± 0.012. Day 7: mean droplet size: 202.8 ± 0.23 nm, polydispersity index: 0.231 ± 0.008) was achieved. Moreover, pseudo-ternary diagram allowed delimitation of nanoemulsion region, contributing to nanobiotechnology of natural products.

Introduction

Oenothera biennis L. belongs to the family Onagraceae, being commonly known as evening primrose. Its seeds are used as raw material for production of oil extensively used in pharmaceutical industry, mainly due to its medicinal and nutritional properties. Evening primrose seeds oil (EPSO) has many folk uses, including treatment of eczema, asthma, rheumatoid arthritis, breast problem, premenstrual and menopausal syndrome (Montserrat-de la Paz et al., 2014). Moreover, EPSO may decrease the intensity of menopausal hot flashes (Farzaneh et al., 2013). It is recognized as a potential source of unsatured fatty acids (Granica et al., 2013; Yunusova et al., 2010). γ-Linoleic acid corresponds to (8–14%) of this oil (Ratz-Eyko et al., 2014), which is considered nutritionally more important than other herbal oils containing γ-linoleic acid (Wettasinghe et al., 2002). The non-saponifiable fraction of EPSO represents around 1.5–2% of the oil including phytosterols, which modulate proinflammatory mediators release (Montserrat-de la Paz et al., 2012).

Despite great economic importance of EPSO oil, studies regarding development of innovative nanoemulsions have not been reported. This special type of nanoformulation have mean droplet below 300 nm (Zhang et al., 2011), fine appearance, translucence or transparent aspect and bluish reflect (Forgiarini et al., 2000). It is often associated to kinetic stability (Solè et al., 2012) and also offer additional advantages, such as enhanced chemical stability of encapsulated substances, better water-solubilization of low polar substances, higher bioavailability, among others (Shen et al., 2011). Moreover, small droplets from nanoemulsions make possible to perform administration of this type of pharmaceutical through intravenous route (Bruxel et al., 2012), in addition to oral route.

Primrose nanoemulsion can be considered very promising as a phytopharmaceutical or even a nutraceutical, due to its pharmacological and nutritional properties. Moreover, aforementioned advantages of a nanodispersed system offer optimized conditions to enhance absorption, chemical stability of bioactive substances, physical stability during storage and wide range of administration routes. On this context, as part of our ongoing studies with vegetable oils, the aim of the present study was to develop evening primrose based nanoemulsions.

Material and methods

Oil from evening primrose (*Oenothera biennis* L., Onagraceae) seeds (Lot No. PRI 067/243) was obtained from Distriol (SP, Brazil).

* Corresponding author.
E-mail: caiofernandes@unifap.br (C.P. Fernandes).

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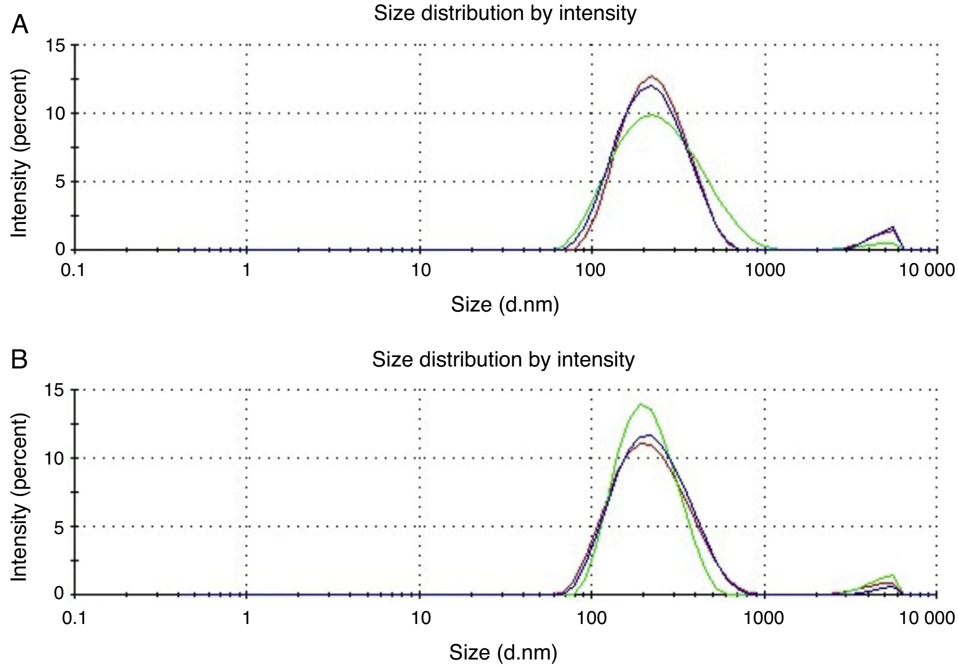

Fig. 1. Particle size distribution of nanoemulsions prepared with 5% (w/w) of evening primrose oil; 5% of surfactants (sorbitan monooleate:polysorbate 80; HLB – 12) and 90% (w/w) of water. (A) Day 1: mean droplet size: 214.3 ± 0.69 nm, polydispersity index: 0.253 ± 0.012. (B) Day 7: mean droplet size: 202.8 ± 0.23 nm, polydispersity index: 0.231 ± 0.008.

Table 1
Mean droplet size and polydispersity of nanoemulsions prepared during required HLB determination.

HLB	Day 1		Day 7	
	Mean droplet (nm)	Polydispersity index	Mean droplet (nm)	Polydispersity index
10	282.8 ± 1.2	0.655 ± 0.006	370.3 ± 6.5	0.715 ± 0.008
11	266.2 ± 11.5	0.493 ± 0.082	271.2 ± 7.1	0.575 ± 0.091
12	214.3 ± 0.7	0.253 ± 0.012	202.8 ± 0.2	0.231 ± 0.008
13	242.2 ± 3.9	0.479 ± 0.018	312.1 ± 16.4	0.685 ± 0.135

Sorbitan oleate (HLB: 4.3) and Polysorbate 80 (HLB: 15) were purchased from Praid Produtos Químicos Ltda (São Paulo, Brazil). Surfactants and evening primrose seeds oil were pooled together and constituted oily phase, while aqueous phase was constituted by distilled water. After both phases reached the same temperature (65 ± 5 °C and), aqueous phase was gently added through the oily phase under magnetic stirring (750 rpm), affording a primarily emulsion. Final homogenization was achieved using a T25 Ultra-Turrax homogenizer (Ika-Werke, Staufen, Germany) equipped with a 25N-18G disperser for 5 min under 8000 rpm (Costa et al., 2014). Final mass was kept constant (25 g) for all formulations, constituted by 90% (w/w) of distilled water, 5% (w/w) of evening primrose seeds oil and 5% (w/w) of a mixture of surfactants. Firstly, several emulsions with different HLB values were achieved using different blends of sorbitan monooleate (HLB 4.3) and polysorbate 80 (HLB 15) in order to determine required HLB (rHLB) of the oil (Fernandes et al., 2013). Sorbitan monooleate:polysorbate 80 ratios were used as follows: 100:0 (HLB 4.3), 93.5:6.5 (HLB 5), 84.1:15.9 (HLB 6), 74.8:25.2 (HLB 7), 65.4:34.6 (HLB 8), 56.9:43.9 (HLB 9), 46.7:53.3 (HLB 10), 37.4: 62.6 (HLB 11), 28.0:72.0 (HLB 12), 18.7:81.3 (HLB 13), 9.3:90.7 (HLB 14), 0:100 (HLB 15). After rHLB determination, nanoemulsion region was determined using pseudo-ternary phase diagram and each corner corresponded to 100% of water, surfactant and evening primrose seeds oil. Composition (w/w) which allowed required HLB value determination was used as starting point (90% of distilled water, 5% of evening primrose oil and 5% of surfactants blend). Surfactants ratio (sorbitan monooleate/polysorbate 80, 1/2.1) was kept constant at required HLB value (Fernandes et al., 2014). All formulations were characterized after 1 and 7 days of manipulation. Macroscopical (visual aspect, translucence, opacity, bluish reflect, phase separation, creaming and sedimentation) aspects were analyzed. Polydispersity and mean droplet size 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 (Rodrigues et al., 2015) (Fig. 1).

Results and discussion

First set of formulations were prepared by ranging surfactant blends (sorbitan monooleate:polysorbate 80) at a wide range of HLB (4.3–15). Some of them presented unstable behavior (HLB 4.3, 5, 6, 7, 8, 9, 14 and 15), including presence of creaming and phase separation, in addition to large mean droplets and high polydispersity (data not shown). After one day of preparation, formulations at HLB 10, 11, 12 and 13 presented mean droplet size below 300 nm and therefore, were characterize as nanoemulsions (Zhang et al., 2011) (Table 1). After seven days, nanoemulsions at HLB 10, 11 and 13 had their droplet size and polydispersity index increased. These signals are associated to low homogeneity of particle size, which is associated to instable behavior of the system (Cheong et al., 2008). However, nanoemulsion at HLB 12 presented a slight decrease in both mean droplet size and polydispersity index, with a

Table 2
Composition, droplet size and polydispersity index of emulsions containing evening primrose seeds oil, sorbitan monooleate/polysorbate 80 (HLB 12) and water.

	O (%)	S (%)	W (%)	Day 1		Day 7	
				Droplet size (nm)	Polydispersity index	Mean droplet size (nm)	Polydispersity index
1[a]	5	10	85	250.3 ± 12.9	0.370 ± 0.014	258.3 ± 4.0	0.375 ± 0.013
2[a]	5	15	80	201.6 ± 0.8	0.239 ± 0.005	210.2 ± 0.4726	0.278 ± 0.023
3[a]	5	20	75	246.1 ± 3.1	0.263 ± 0.008	242.5 ± 2.3	0.257 ± 0.013
4	5	25	70	269.5 ± 0.6	0.393 ± 0.006	314.0 ± 1.0	0.446 ± 0.027
5	5	30	65	289.5 ± 6.2	0.479 ± 0.003	342.5 ± 4.3	0.498 ± 0.011
6	10	5	85	728.4 ± 64.7	0.826 ± 0.123	719.5 ± 325.2	0.924 ± 0.095
7[a]	10	10	80	221.0 ± 2.1	0.219 ± 0.006	227.1 ± 3.7	0.235 ± 0.008
8[a]	10	20	70	271.9 ± 4.0	0.280 ± 0.013	263.6 ± 1.9	0.256 ± 0.006
9	15	5	80	6887.0 ± 8211.8	0.222 ± 0.144	6516 ± 607.3	0.335 ± 0.159
10	15	10	75	370.4 ± 5.1	0.355 ± 0.046	378.6 ± 12.4	0.381 ± 0.021
11[a]	10	15	75	222.4 ± 1.4	0.221 ± 0.004	220.2 ± 3.6	0.217 ± 0.010
12	10	25	65	423.1 ± 8.1	0.457 ± 0.016	391.8 ± 7.2	0.475 ± 0.019
13	10	30	60	321.7 ± 3.6	0.449 ± 0.002	465.2 ± 6.9	0.509 ± 0.028
14[a]	15	15	70	282.3 ± 4.6	0.257 ± 0.006	273.0 ± 3.8	0.246 ± 0.012
15	15	20	65	349.0 ± 8.5	0.468 ± 0.019	345.1 ± 8.3	0.336 ± 0.039
16	15	25	60	347.8 ± 1.7	0.359 ± 0.037	396.6 ± 4.4	0.450 ± 0.025
17	15	30	55	302.8 ± 2.9	0.290 ± 0.013	438.2 ± 8.0	0.396 ± 0.011
18	20	5	75	6382 ± 83.7	0.476 ± 0.063	7001 ± 1870	0.765 ± 0.408
19	20	10	70	487.9 ± 29.1	0.963 ± 0.042	848.4 ± 103.7	1.000 ± 0.000
20	20	15	65	491.7 ± 15.7	0.476 ± 0.034	429.9 ± 0.5	0.485 ± 0.025
21	20	20	60	523.1 ± 8.4	0.468 ± 0.019	544.3 ± 7.5	0.459 ± 0.022
22	25	5	70	5852 ± 451.9	1.000 ± 0.000	5250 ± 77.6	0.842 ± 0.274
23	25	10	65	1516 ± 151.5	0.999 ± 0.002	1607 ± 394.0	1.000 ± 0.000
24	25	15	60	539.1 ± 14.9	0.459 ± 0.045	537.1 ± 9.9	0.462 ± 0.006

[a] Nanoemulsions. O, evening primrose seeds oil content; S, surfactants content; W, water content.

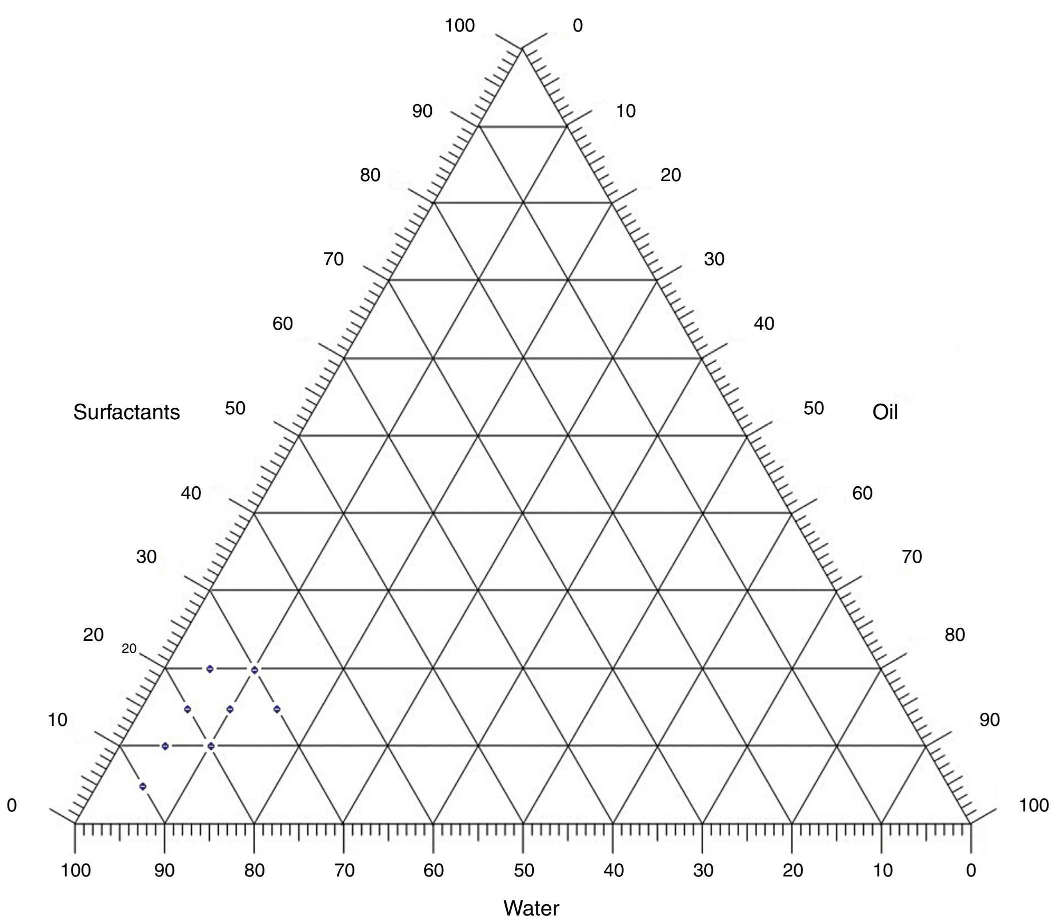

Fig. 2. Pseudo-ternary diagram constructed with evening primrose seeds oil/surfactants (sorbitan monooleate:polysorbate 80 – HLB 12) and water for nanoemulsion region determination.

great tendency to monomodal distribution. Micelles are in dynamic equilibrium with surfactant molecules, being continuously disintegrating and re-constructing until reaching kinetically stability (Patist et al., 2002).

Required HLB value of an oil can be determined by preparing a set of formulations at a wide range of HLB values, using a pair of surfactants (low and high HLB values). Considering the concept that optimal stability is achieved when HLB values of surfactant mixture and oil coincides, determination of formulation which presented smallest mean droplet size and narrow distribution can be used as a satisfactory criteria to determine HLB value of oils (Rodríguez-Rojo et al., 2012; Wang et al., 2009). On this context, evening primrose oil used in the present study was characterized regarding its required HLB value, being determined at HLB 12.

Pseudo-ternary diagram is a valuable tool to investigate influence of composition on nanoemulsion formation (Shen et al., 2011). It is usually constructed varying components of the formulation (oil-surfactants-water). Optimized surfactant mixture ratio at required HLB value is used and for this reason it is denominated a pseudo-ternary diagram instead of ternary diagram (Rodrigues et al., 2014). On the present study, 24 formulations were prepared. Mean droplet size ranged from 201.6 ± 0.8 nm (5% of oil, 15% of surfactants, 80% of water) to 6887.0 ± 8211.8 nm (15% of oil, 5% of surfactants, 80% of water) after 1 day of preparation (Table 2). Large droplets (>500 nm) were observed for some formulations with high oil content (10%, 15%, 20% and 25%). Moreover, high polydispersity levels (>0.500) suggested that some of them did not present stable behavior (Cheong et al., 2008). Further coalescence can explain appearance of new particle size populations and mean droplet increase after 7 days. This fact may be attributed to insufficient amount of surfactant, which may not be able to cover all droplets (Wang et al., 2009). Moreover, disruption of adsorbed layer (responsible by steric repulsion of droplets) and Ostwald ripening (major instability problem with nanoemulsions) (Tadros et al., 2004) may also be responsible by instability observed for some formulations. Nanoformulations which presented mean droplet size below 300 nm after 1 and 7 days of preparation were used to construct a nanoemulsion region (Fig. 2). This approach is very useful for nanoemulsion development, considering that any formulation prepared with oil, surfactants and water inside this region has great tendency to form nanoemulsions.

Several studies were carried out for vegetable oils concerning development of nanoemulsions. However, to our knowledge, evening primrose seeds oil remained unexplored regarding this area. On this context, nanoemulsions with good properties were successfully achieved in the present study, contributing to nanobiotechnology of natural products. Moreover, its required HLB value was obtained for the first time and pseudo-ternary diagram provided important information for further studies that aim to obtain nanoemulsions using evening primrose seeds oil, sorbitan monooleate:polysorbate 80 (HLB 12) and water.

Authors' contributions

RFR, ISC, FBA (undergraduate students), AMF, JCEV contributed running the laboratory work (HLB determination and pseudo-ternary diagram construction). RASC and ACF contributed to nanoemulsions characterization, analysis of the data and drafting the paper. JCTC and CPF designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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