



Short communication

## Utilization of dynamic light scattering to evaluate *Pterodon emarginatus* oleoresin-based nanoemulsion formation by non-heating and solvent-free method


 Anna E.M.F.M. Oliveira<sup>a,b</sup>, Jonatas L. Duarte<sup>a,b</sup>, Rodrigo A.S. Cruz<sup>a,b</sup>, Edemilson C. da Conceição<sup>c</sup>, José C.T. Carvalho<sup>a,b</sup>, Caio P. Fernandes<sup>a,b,\*</sup>
<sup>a</sup> Laboratório de Pesquisa em Fármacos, Universidade Federal do Amapá, Macapá, AP, Brazil

<sup>b</sup> Laboratório de Nanobiotecnologia Fitofarmacêutica, Universidade Federal do Amapá, Macapá, AP, Brazil

<sup>c</sup> Laboratório de Pesquisa, Desenvolvimento e Inovação em Bioprodutos, Universidade Federal de Goiás, Goiânia, GO, Brazil

### ARTICLE INFO

#### Article history:

Received 30 June 2016

Accepted 22 November 2016

Available online 9 February 2017

#### Keywords:

Low energy method

Sucupira-branca

Colloidal system

Vouacapan diterpenes

Sesquiterpenes

Phytopharmaceutical nanobiotechnology

### ABSTRACT

*Pterodon emarginatus* Vogel, Fabaceae, is a great source of bioactive compounds. The most known and studied herbal derivative from this species is an ambar-colored oleoresin that contains vouacapan diterpenes and volatile terpenoids, such as  $\beta$ -caryophyllene. Some recent papers aimed to generate nanoemulsions using this oleoresin for biological applications. However, they used high-energy methods that elevate costs of the process or heating procedures, which offer the disadvantage of possible volatile substances loss. Thus, as part of our ongoing studies with nanobiotechnology of natural products, especially regarding preparation of nanoemulsions with promising plant-based oils by low cost and low energy methods, we decided to evaluate the ability of non-heating and solvent-free method to generate *P. emarginatus* oleoresin-based nanoemulsions. Two non-ionic surfactants were used to generate the nanoemulsions by a simple homogenization method with vortex stirrer. Low mean droplet size ( $<180$  nm) and low polydispersity index ( $<0.200$ ) were observed even after one day of preparation. The low coefficient of variation for the analyzed parameters of different batches and similar profile for droplet size distribution suggested reproducibility of the method. After 30 days, some degree of droplet growth was observed on nanoemulsion prepared with polyethyleneglycol 400 monooleate, while almost no alteration was observed for nanoemulsion prepared with polysorbate 85. Programmed temperature ramp analysis revealed that no major effects on droplet size and polydispersity index were observed, suggesting the robustness of formed nanoemulsions. Thus, the present study shows for the first time the formation of sucupira-based nanoemulsions by a simple, low cost and ecofriendly method. This study opens new perspectives for bioactive evaluation of this novel nano-product.

© 2017 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### Introduction

*Pterodon emarginatus* Vogel belongs to the family Fabaceae and it is widespread in Midwest region of Brazil, being also found in South-Eastern, Northeast and North regions (Lima and Lima, 2016). Its fruits, as well as those from other *Pterodon* species, are commonly known as sucupira-branca. This plant part is used for extraction of an oleoresin that has vouacapan diterpenes and sesquiterpenes. Sucupira-branca oleoresin has several biological properties, including analgesic, antiinflammatory, antinoceptive

and larvicidal activities (Hansen et al., 2010; Hoscheid and Cardoso, 2015).

Natural products from plant origin have been subjected to several studies aiming to generate novel nanotechnology-based bioactive systems. Regarding several types of nano-size formulations, herbal oils (including oleoresins) are very interesting for nanoemulsion preparation (Zorzi et al., 2015). Nanoemulsions are kinetic stable disperse systems constituted by two immiscible liquids, often stabilized by surfactant (s). The droplet diameter is on the nanometer range, often ranging from 20 nm to different upper limits (e.g. 500, 300, 200 and 100 nm), that varies according to author criteria (Solans and Solé, 2012). High-energy methods involved on nanoemulsion formation often make use of high energy devices that elevate costs of the process, such as high pressure homogenizers or ultrasonicators. Spontaneous energy methods

\* Corresponding author.

E-mail: [caiofernandes@unifap.br](mailto:caiofernandes@unifap.br) (C.P. Fernandes).

involve utilization of volatile organic solvents that may not be considered ecofriendly, considering their potential toxicity. Moreover, it is necessary to remove this component of the nanoemulsification process. Low-energy methods make use of intrinsic characteristics of the system, often involving phase inversion (Sugumar et al., 2016). Most common phase inversion methods are associated to phase transitions induced by change in temperature (phase inversion temperature – PIT) or composition (phase inversion composition – PIC) (Solans and Solé, 2012).

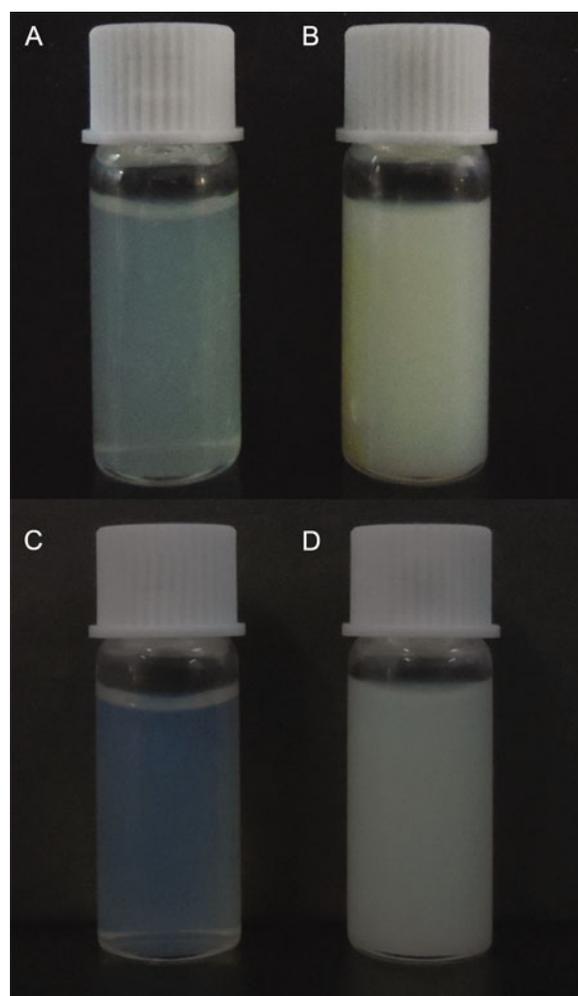
Growing interest is observed for nanobiotechnology studies with oleoresin from sucupira-branca (Hoscheid et al., 2015; Pascoa et al., 2015; Oliveira et al., 2016). The oleoresin obtained from the fruits of *P. emarginatus* that was used in the present study have been previously submitted to chemical characterization. Chromatographic analysis carried out using HPLC-DAD and comparison to authentic external standards revealed the presence of methyl 6 $\alpha$ ,7 $\beta$ -dihydroxyvouacapan-17- $\beta$ -oate, geranylgeraniol and  $\beta$ -caryophyllene, being in accordance with phytochemical profile of the genus (Oliveira et al., 2016). Thus, it is worth mentioning that utilization of a phase inversion temperature may lead some lost of volatiles, even if it is able to generate fine droplets. The present study aims to investigate the possibility of generating *P. emarginatus* oil in water nanoemulsions using a simple low energy, non-heating and solvent-free approach that can be considered very promising for development of viable, low cost and ecofriendly nanoproducts with this natural raw material.

## Material and methods

*Pterodon emarginatus* Vogel, Fabaceae, fruits were obtained at the Central Market of Goiânia, GO, Brazil, and were identified by Dr. José Realino de Paula. Voucher specimen was deposited at the Herbarium of Goiás Federal University (GO, Brazil) under the register number 41714. The oleoresin was previously obtained from fruits by cold pressing, being stored in amber glass flask and kept at  $-20^{\circ}\text{C}$  (Oliveira et al., 2016). Prior to utilization, the oleoresin was left under room temperature for two days and it was centrifuged to remove non-soluble compounds. Oil in water nanoemulsions were prepared by blending non-ionic surfactant (polysorbate 85, Sigma–Aldrich, St Louis, MO, or polyethyleneglycol 400 monooleate, Praid, SP, Brazil) and *P. emarginatus* oleoresin at a fixed surfactant to oil ratio (9:1). After complete homogenization of the oily phase (surfactant + oleoresin), distilled water was slowly added dropwise to this mixture under vigorous agitation using a vortex stirrer. Each emulsion presented final mass of 10 g and 90% (w/w) of water. Dynamic light scattering (DLS) analysis was carried out using a Zetasizer Nano ZS, Malvern, UK) equipped with a 10 mW “red” laser ( $\lambda = 632.8\text{ nm}$ ) and samples were measured at a  $90^{\circ}$  scattering detector angle for size measurements. The effects of dilution on mean droplet size, polydispersity index (pdi) and zeta potential were performed using different dilutions factors (1:10, 1:25, 1:50 and 1:100). Reproducibility of the nanoemulsion formation was carried out by preparing of three different batches of the nanoemulsions. Nanoemulsions were also subjected to analysis using a programmed linear ramp of temperature starting from  $25^{\circ}\text{C}$  to  $80^{\circ}\text{C}$  at  $5^{\circ}\text{C}$  intervals. Results are expressed as mean  $\pm$  standard deviation.

## Results and discussion

*Pterodon emarginatus* oleoresin-based nanoemulsions prepared with polysorbate 85 or polyethyleneglycol 400 monooleate presented a fine aspect with bluish reflect (Fig. 1A and B) that is characteristic for this type of colloidal system. Based on our ongoing studies with natural oils and oleoresins, we have observed that



**Fig. 1.** Nanoemulsions prepared with oleoresin extracted from *Pterodon emarginatus* fruits and non-ionic surfactants at day 0: (A) polyethyleneglycol 400 monooleate or (B) polysorbate 85 and day 30: (C) polyethyleneglycol 400 monooleate or (D) polysorbate 85.

it is easier to form oil in water nanoemulsions using a system constituted by large amounts of water (90–95%). Moreover, it is well established that increasing surfactant to oil ratio often enhances kinetic stability by reducing the droplet size (Ostertag et al., 2012; Komaiko and McClements, 2015). Since we aimed to evaluate if it is possible to generate nanoemulsions using low energy, non-heating and solvent-free method, we opted to use this ratio as a background composition.

Several dilutions were performed just afterwards the preparation of *P. emarginatus* oleoresin-based nanoemulsions with polysorbate 85 or polyethyleneglycol 400 monooleate (Table 1). Higher mean droplet size and polydispersity index (pdi) were observed at the dilution ratio of 1:10, for nanoemulsions prepared with both surfactants, which also presented lower zeta potential (in module). On another hand, no major differences were observed for mean droplet size on the remaining dilution ratios (1:25, 1:50 and 1:100), which were around 160–170 nm for both nanoemulsions. Slightly lower polydispersity index was observed for nanoemulsion prepared with polysorbate 85, which was around 0.111–0.122, while pdi of droplets from nanoemulsion prepared with polyethyleneglycol 400 monooleate were around 0.149–1.152. Zeta potential of these nanoemulsions ranged from  $-21.9 \pm 0.5\text{ mV}$  (dilution ratio 1:25 for nanoemulsion prepared with polysorbate 85) to  $-28.7 \pm 0.7\text{ mV}$  (dilution ratio of 1:100 of nanoemulsion prepared with polyethyleneglycol 400 monooleate).

**Table 1**

Effect of dilution on droplet size, polydispersity index (pdi) and zeta potential of nanoemulsions prepared with oleoresin extracted from *Pterodon emarginatus* fruits and non-ionic surfactants (polyethyleneglycol 400 monooleate or polysorbate 85). Each measurement represents mean  $\pm$  standard deviation. Each analysis was performed in triplicate.

Dilution	Polysorbate 85			Polyethyleneglycol 400 monooleate		
	Size (nm)	Pdi	Zeta (mV)	Size (nm)	Pdi	Zeta (mV)
1:10	224.6 $\pm$ 0.6	0.265 $\pm$ 0.008	-16.1 $\pm$ 0.3	176.5 $\pm$ 1.8	0.188 $\pm$ 0.014	-20.1 $\pm$ 0.4
1:25	166.7 $\pm$ 1.8	0.113 $\pm$ 0.011	-21.9 $\pm$ 0.5	167.0 $\pm$ 1.9	0.149 $\pm$ 0.005	-25.2 $\pm$ 0.8
1:50	164.4 $\pm$ 0.5	0.122 $\pm$ 0.001	-24.9 $\pm$ 0.1	160.8 $\pm$ 0.2	0.152 $\pm$ 0.021	-23.3 $\pm$ 0.3
1:100	163.5 $\pm$ 0.2	0.111 $\pm$ 0.015	-26.2 $\pm$ 1.2	159.2 $\pm$ 0.9	0.150 $\pm$ 0.006	-28.7 $\pm$ 0.7

Dilution prior to droplet size distribution analysis is considered a critical parameter and optimized dilution factor should be determined, in order to avoid multiple scattering effects. Moreover, dilution of previously prepared nanoemulsions has been considered an important parameter that affects stability and droplet growth of nanoemulsions (Saber et al., 2013a,b, 2014). Considering that more concentrated stock nanoemulsion should be preferred for further dilutions in practical applicable products and since no major difference was observed for most of them, we decided to further investigate effect of storage or temperature using the nanoemulsion at 1:25 dilution.

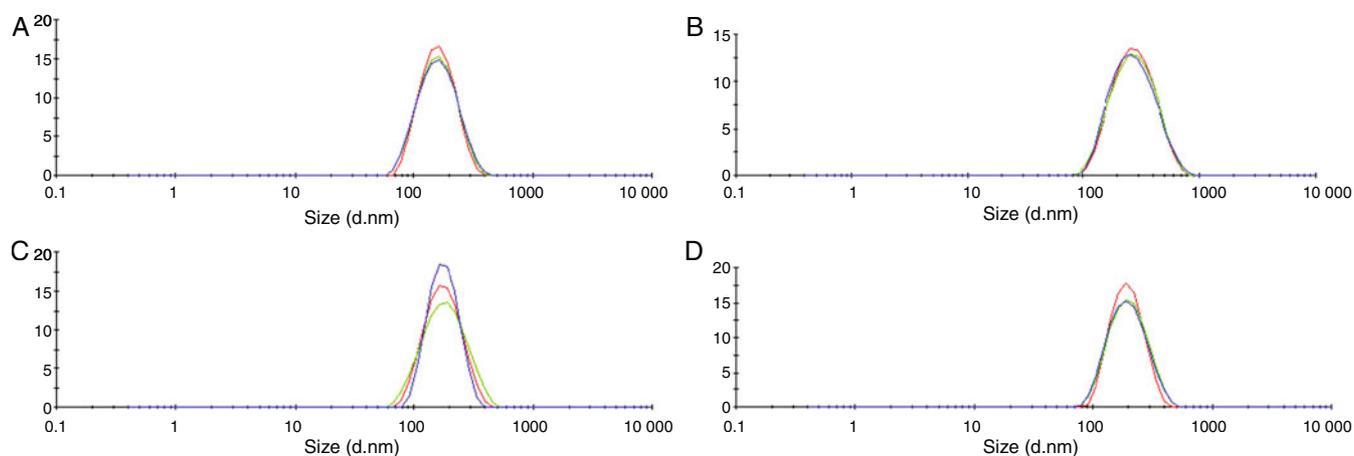
After 30 days of storage, the nanoemulsion prepared with polyethyleneglycol 400 monooleate (1:25 dilution factor) presented a slight creaming (Fig. 1C), while the nanoemulsion prepared with polysorbate 85 (1:25 dilution factor) maintained the visual appearance (Fig. 1D). It was observed that mean droplet size and polydispersity index of the nanoemulsion prepared with polyethyleneglycol 400 monooleate presented major increase (Fig. 2A and B). On another hand, lower increased on mean droplet size (181.5  $\pm$  0.9 nm) and polydispersity index (0.111  $\pm$  0.012) was observed for nanoemulsion prepared with polysorbate 85, showing a similar profile of droplet size distribution, when compared to day 0 (Fig. 2C and D). The surfactant type is a major parameter on nanoemulsion formation and stabilization. On this context, investigations regarding surfactant concentration are very important for development of optimal nanoemulsions (Guttoff et al., 2015). Considering that we opted on this study for a fixed surfactant to oil ratio, it is worth mentioning that differences between the surfactants may be responsible by differences on droplet size growth and nanoemulsion stabilization.

Fig. 3 shows the influence of temperature on droplet size distribution and zeta potential. Analysis of mean droplet size of nanoemulsion prepared with polysorbate 85 as function of temperature revealed that it remained almost constant from 25 to 35 °C (around 170 nm). Then, it rapidly decreased, being around

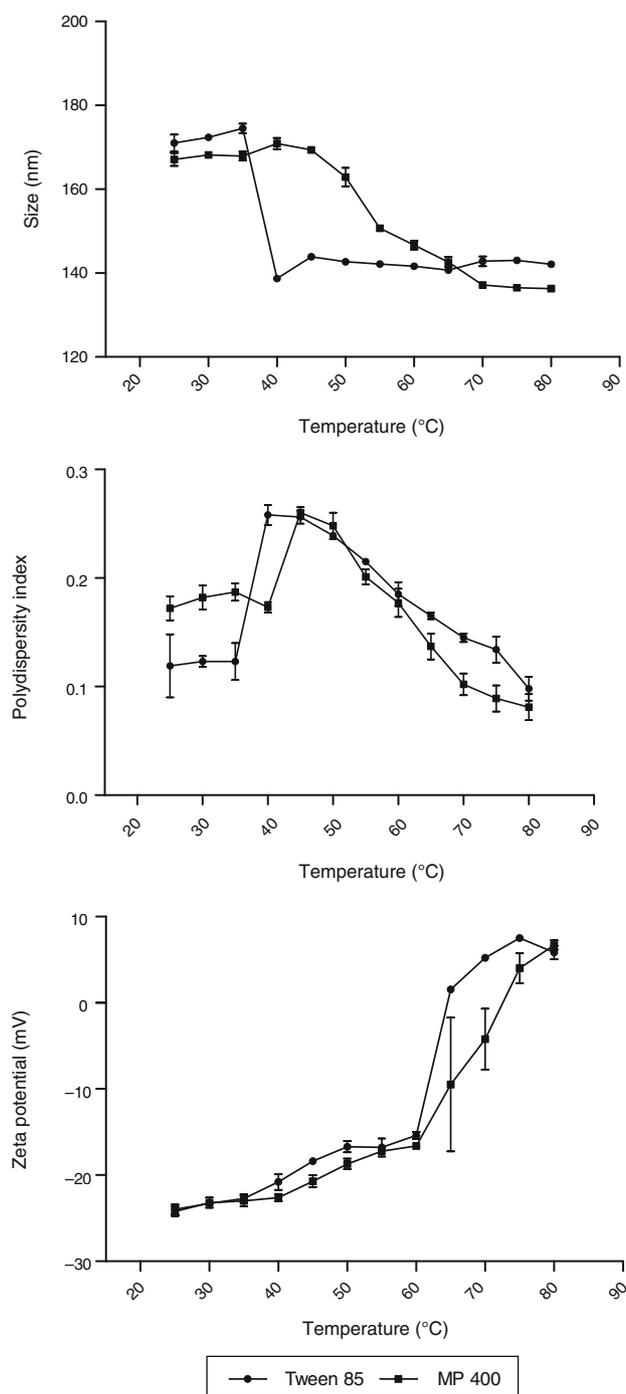
140 nm from 40 to 80 °C. On another hand, droplet diameter reduction which was observed for nanoemulsion prepared with polyethyleneglycol 400 monooleate occurred more gradually, also reaching low values at higher temperatures. Probably the coating film that this surfactant form through the droplets is more rigid due to the plasticizer character of polyethyleneglycol surfactants. Thus, rearrangement of the internal phase may not be so drastic as it was observed for polysorbate 85. Polydispersity index of both nanoemulsions started below 0.200, being the value associated to nanoemulsion prepared with polysorbate 85 slightly lower than the value observed for nanoemulsion prepared with polyethyleneglycol 400 monooleate. Then, they reached a maximum around 0.250 after 35 °C and decreased to minimum values below 0.100 at 80 °C. Zeta potential slight changed below 40 °C, however, overall gradual increase was observed as function of temperature.

Non-ionic surfactants stabilizes nanoemulsions by reducing interfacial tension and they also form a layer that promotes steric repulsion between droplets (Wang et al., 2009). The main mechanism for nanoemulsion destabilization is associated to Ostwald ripening, which induces formation of larger droplets (Solans and Solé, 2012). Interestingly, increased temperatures induced formation of smaller droplets while no increase on polydispersity index was observed above 0.300. Moreover, pdi values returned to basal values around 0.100. This parameter reflects homogeneity of droplet size distribution and gradual formation of larger droplets from a starting monomodal distribution would result on increased polydispersity index.

Migration of acid compounds from copaiba oleoresin was associated to negative zeta potential values on nanoemulsions prepared with this material (Dias et al., 2014). Vouacapan diterpenes, including some compounds with acid moiety, are characteristic of sucupira oleoresin and may explain these starting zeta potential values. Further increase of temperature may have induced growing partition of additional substances that do not easily dissociate, therefore reducing this zeta potential. Moreover, deposition



**Fig. 2.** Droplet size distribution of nanoemulsions prepared with oleoresin extracted from *Pterodon emarginatus* fruits and polyethyleneglycol 400 monooleate at (A) day 0/(B) day 30; and polysorbate 85 at (C) day 0/(D) day 30. Each analysis was performed in triplicate.



**Fig. 3.** Influence of temperature on droplet size, polydispersity index (pdi) and zeta potential of nanoemulsions prepared with oleoresin extracted from *Pterodon emarginatus* fruits and non-ionic surfactants (polyethyleneglycol 400 monooleate or polysorbate 85). Each measurement represents mean  $\pm$  standard deviation. Each analysis was performed in triplicate.

of some substances on the interface may form more compact films and therefore, enhancing the stability of the system (Wang et al., 2009).

We decided to investigate the reproducibility of the method on nanoemulsion formation and low coefficient of variation values of three individual batches were observed for droplet size ( $CV_{MP400} = 0.00499$ ;  $CV_{T85} = 0.0113$ ), pdi ( $CV_{MP400} = 0.0534$ ;  $CV_{T85} = 0.0732$ ) and zeta potential ( $CV_{MP400} = 0.0416$ ;  $CV_{T85} = 0.0741$ ). Fig. 4 reflects the technical feasibility of the method to induce formation of nanoemulsions with low droplet

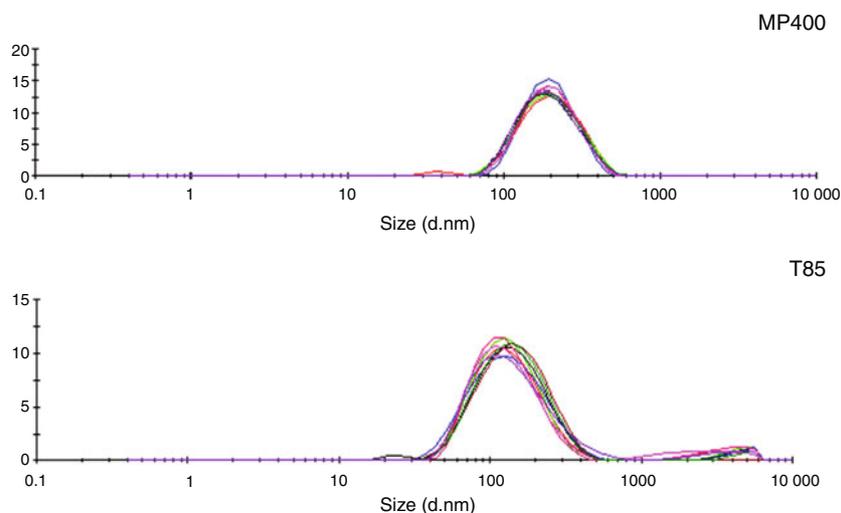
size and low polydispersity index ( $<0.300$ ), being in accordance with satisfactory results for size distribution of aqueous nanoemulsions (Tan et al., 2016). A study aiming to optimize the preparation of nanoemulsions with copaiba oleoresin from *Copaifera multijuga*, which belongs to the same botanical family (Fabaceae) of *P. emarginatus*, performed a triplicate of center point from a factorial design experiment and also observed low coefficient of variation for analyzed parameters (Dias et al., 2014). Thus, overall our results suggest the reproducibility of the method for the preparation of nanoemulsions using *P. emarginatus* oleoresin with a high prevalence of droplets below 200 nm.

Droplet size distribution of nanoemulsions from the three different batches also reveal a less abundant droplet population below 100 nm, for those prepared with both surfactants. Moreover, a droplet population with size above 1000 nm (microdroplets) was observed on nanoemulsion prepared with polysorbate 85. Since the first studies with *P. emarginatus* oleoresin, it was observed that diterpenes gradually precipitate on this viscous oily raw material (Mahajan and Monteiro, 1973). Considering the high viscosity of the *P. emarginatus* oleoresin, some microparticles (or microcrystals) of the diterpenes on suspension may remain on the supernatant that was used for nanoemulsification, even after a centrifuging step, being responsible by this micro-size population. Further studies aiming to better investigate this hypothesis or concomitant formation of another systems (e.g. multiphase region, microdroplets/coarse droplets) are required to solve these questions.

A larvicidal nanoemulsion prepared with the same oleoresin that was used in the present study, a mixture of polysorbate 80/sorbitan monooleate (HLBmixture = 11) and water presented mean droplet size around 130 nm, polydispersity index around 0.200 and zeta potential around  $-30$  mV. The nanoemulsification method involved homogenization of oily phase (*P. emarginatus* oleoresin and surfactants) at  $80^\circ\text{C}$  for 30 min (Oliveira et al., 2016). A procedure that also involved heating process was performed aiming to prepare *P. emarginatus* based nanoemulsions due to the anti-inflammatory potential (Pascoa et al., 2015). It is worth mentioning that volatile substances from the oleoresin may be lost during heat of the oily phase. For this reason, utilization of effective nanoemulsification methods that do not involve a heating step should be considered an advantage, considering that they would protect the undesired loss of volatiles, including some terpenoids.

*Rosmarinus officinalis* essential oil was formerly used for development of an oil in water nanoemulsion using phase inversion temperature (PIT) method and droplets with mean diameter around 100 nm were obtained (Fernandes et al., 2013). Latter, nanoemulsion phase inversion method using a titration process was performed for achievement of bioactive rosemary-based nanoemulsions using the same natural raw material. After seven days of storage, mean droplet size around 115.0 nm and low polydispersity index ( $<0.300$ ) were observed (Duarte et al., 2015).

The nanoemulsions prepared with oleoresin from *Copaifera duckei* were prepared by using phase inversion temperature method. Low mean droplet size (145.2 nm) and relatively broad droplet size distribution (pdi = 0.378) were observed for this nanoparticle after one day of preparation (Rodrigues et al., 2014). The oleoresin from another copaiba species (*C. multijuga*) was nanoemulsified using a high-pressure homogenizer and droplets size around 120–140 nm and zeta potential around  $-20$  mV were obtained (Dias et al., 2012). On another study, nanoemulsions prepared with *C. multijuga* oleoresin were prepared using high-pressure homogenization method or spontaneous emulsification using organic solvents. Most of nanoemulsions prepared by high energy method presented mean droplet size around 160–200 nm and monomodal distribution, being considered more efficient than solvent-based method in this study (Dias et al., 2014).



**Fig. 4.** Droplet size distribution of *Pterodon emarginatus* oleoresin-based nanoemulsions prepared with polyethyleneglycol 400 monooleate (MP400) or polysorbate 85 (T85). Each measurement (in triplicate) represents three different batches of nanoemulsions.

The oleoresin from *P. pubescens* mixed with phospholipon 90G was added through aqueous dispersions of several surfactants and then nanoemulsified using high-speed shear homogenizer. Mean diameter of droplets ranged from 199 to 860 nm and lowest size were observed for nanoemulsions prepared with polyethylene glycol (PEG-40) castor oil/sorbitan oleate and PEG-40 hydrogenated castor oil/sorbitan oleate (PEG-40H) (Hoscheid et al., 2015). The andiroba (*Carapa guianensis*) oil was subjected to nanoemulsion preparation by a self-nanoemulsifying method using acetone as organic phase constituent. On this same study, an aroeira (*Schinus molle*)-based nanoemulsion was prepared using a high-pressure homogenizer (Baldissera et al., 2013).

## Conclusion

The present study shows that it is possible to generate *P. emarginatus* nanoemulsions by a non-heating and solvent free approach, without using high energy equipment. This ecofriendly concept has major advantages due to possible reducing costs and even less impairment to environment and health. It is worth mentioning that further studies should be necessary to develop optimal *P. emarginatus* nanoemulsions using this method. However, we believe that it opens great perspectives for fast and simple preparation of natural product-based nanoemulsions for biological evaluation, being comparable or even potentially more effective than most used methods. It also provides great information for studies aiming to develop this type of colloidal system, since this information are still scarce for complex Brazilian natural oils.

## Authors' contributions

AEMFMO (PhD student), RASC (PhD student) and JLD (undergraduate student) contributed running the laboratory work, analysis of the data and drafted the paper. ECC contributed to oleoresin extraction, characterization and critical reading of the manuscript. 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.

## Acknowledgments

The authors thank CNPq (Rede Amazônica de Pesquisa em Biofármacos-407768/2013-0), Fapeap (Prodetec Araguari – Process n° 250.203.035/2013) and PROPESPG (PROPUBLIC and PAPESQ) for financial support.

## References

- Baldissera, M.D., da Silva, A.S., Oliveira, C.B., Zimmermann, C.E.P., Vaucher, R.A., Santos, R.C.V., Rech, V.C., Tonin, A.A., Giongo, J.L., Mattos, C.B., Koester, L., Santurio, J.M., Monteiro, S.G., 2013. Trypanocidal activity of the essential oils in their conventional and nanoemulsion forms: *in vitro* tests. *Exp. Parasitol.* 134, 356–361.
- Dias, D.O., Colombo, M., Kelmann, R.G., Souza, T.P., Bassani, V.L., Teixeira, H.F., Veiga Jr., V.F., Limberger, R.P., Koester, L.S., 2012. Optimization of headspace solid-phase microextraction for analysis of  $\beta$ -caryophyllene in a nanoemulsion dosage form prepared with copaiba (*Copaifera multijuga* Hayne) oil. *Anal. Chim. Acta* 721, 79–84.
- Dias, D.O., Colombo, M., Kelmann, R.G., Kaiser, S., Lucca, L.G., Teixeira, H.F., Limberger, R.P., Veiga Jr., V.F., Koester, L.S., 2014. Optimization of Copaiba oil-based nanoemulsions obtained by different preparation methods. *Ind. Crop. Prod.* 59, 154–162.
- Duarte, J.L., Amado, J.R.R., Oliveira, A.E.M.F.M., Cruz, R.A.S., Ferreira, A.M., Souto, R.N.P., Falcão, D.Q., Carvalho, J.C.T., Fernandes, C.P., 2015. Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. *Rev. Bras. Farmacogn.* 25, 189–192.
- Fernandes, C.P., Mascarenhas, M.P., Zibetti, F.M., Lima, B.G., Oliveira, R.P.R.F., Rocha, L., Falcão, D.Q., 2013. HLB value, an important parameter for the development of essential oil phytopharmaceuticals. *Rev. Bras. Farmacogn.* 23, 108–114.
- Guttoff, M., Saberi, A.H., McClements, D.J., 2015. Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: factors affecting particle size and stability. *Food Chem.* 171, 117–122.
- Hansen, D., Haraguchi, M., Alonso, A., 2010. Pharmaceutical properties of “sucupira” (*Pterodon* spp.). *Braz. J. Pharm. Sci.* 46, 607–616.
- Hoscheid, J., Cardoso, M.L.C., 2015. Sucupira as a potential plant for arthritis treatment and other diseases. *Arthritis*. <http://dx.doi.org/10.1155/2015/379459>.
- Hoscheid, J., Outuki, P.P., Kleinubing, S.A., Silva, M.F., Bruschi, M.L., Cardoso, M.L.C., 2015. Development and characterization of *Pterodon pubescens* oil nanoemulsions as a possible delivery system for the treatment of rheumatoid arthritis. *Colloids Surf. A: Physicochem. Eng. Asp.* 484, 19–27.
- Komaiko, J., McClements, D.J., 2015. Low-energy formation of edible nanoemulsions by spontaneous emulsification: factors influencing particle size. *J. Food Eng.* 146, 122–128.
- Lima, H.C. de, Lima, I.B. *Pterodon* in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB29842> (accessed 28.06.16).
- Mahajan, J.R., Monteiro, M.B., 1973. New diterpenoids from *Pterodon emarginatus* Vog. *J. Chem. Soc.* 1, 520–525.
- Oliveira, A.E.M.F.M., Duarte, J.L., Amado, J.R.R., Cruz, R.A.S., Rocha, C.F., Souto, R.N.P., Ferreira, R.M.A., Santos, K., Conceição, E.C., Oliveira, L.A.R., Kelecom, A., Fernandes, C.P., Carvalho, J.C.T., 2016. Development of a larvicidal nanoemulsion with *Pterodon emarginatus* Vogel Oil. *PLOS ONE* 11, e0145835.

- Ostertag, F., Weiss, J., McClements, D.J., 2012. Low-energy formation of edible nanoemulsions: factors influencing droplet size produced by emulsion phase inversion. *J. Colloid Interface Sci.* 388, 95–102.
- Pascoa, H., Diniz, D.G.A., Florentino, I.F., Costa, E.A., Bara, M.T.F., 2015. Microemulsion based on *Pterodon emarginatus* oil and its anti-inflammatory potential. *Braz. J. Pharm. Sci.* 51, 117–126.
- Rodrigues, E.C.R., Ferreira, A.M., Vilhena, J.C.E., Almeida, F.B., Cruz, R.A.S., Florentino, A.C., Souto, R.N.P., Carvalho, J.C.T., Fernandes, C.P., 2014. Development of a larvicidal nanoemulsion with Copaiba (*Copaifera duckei*) oleoresin. *Rev. Bras. Farmacogn.* 24, 699–705.
- Saberi, A.H., Fang, Y., McClements, D.J., 2013a. Effect of glycerol on formation, stability, and properties of vitamin-E enriched nanoemulsions produced using spontaneous emulsification. *J. Colloid Interface Sci.* 411, 105–113.
- Saberi, A.H., Fang, Y., McClements, D.J., 2013b. Fabrication of vitamin E-enriched nanoemulsions by spontaneous emulsification: effect of propylene glycol and ethanol on formation, stability, and properties. *Food Res. Int.* 54, 812–820.
- Saberi, A.H., Fang, Y., McClements, D.J., 2014. Stabilization of vitamin E-enriched mini-emulsions: influence of organic and aqueous phase compositions. *Colloids Surf. A: Physicochem. Eng. Asp.* 449, 65–73.
- Solans, C., Solé, I., 2012. Nano-emulsions: formation by low-energy methods. *Curr. Opin. Colloid Interface Sci.* 17, 246–254.
- Sugumar, S., Ghosh, V., Mukherjee, A., Chandrasekaran, N., 2016. Essential oil-based nanoemulsion formation by low- and high-energy methods and their application in food preservation against food spoilage microorganisms. In: Preedy, V.R. (org.) *Essential Oils in Food Preservation, Flavor and Safety*. Academic Press, pp. 93–100.
- Tan, T.B., Yussof, N.S., Abas, F., Mirhosseini, H., Nehdi, I.A., Tan, C.P., 2016. Forming a lutein nanodispersion via solvent displacement method: the effects of processing parameters and emulsifiers with different stabilizing mechanisms. *Food Chem.* 194, 416–423.
- Wang, L., Dong, J., Chen, J., Eastoe, J., Li, X., 2009. Design and optimization of a new self-nanoemulsifying drug delivery system. *J. Colloid Interface Sci.* 330, 443–448.
- Zorzi, G.K., Carvalho, E.L.S., von Poser, G.L., Teixeira, H.F., 2015. On the use of nanotechnology-based strategies for association of complex matrices from plant extracts. *Rev. Bras. Farmacogn.* 25, 426–436.