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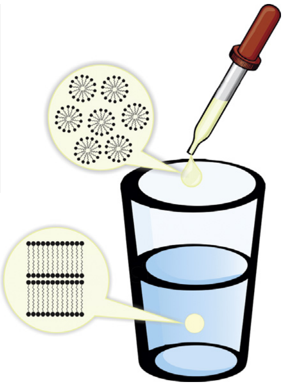
An environmentally safe larvicide against *Aedes aegypti* based on *in situ* gelling nanostructured surfactant systems containing an essential oil

S.G. Ferreira [a], V.S. Conceição [a], N.S. Gouveia [a], G.S. Santos [a], R.L.C. Santos [c], A.A.M. Lira [a], S.C.H. Cavalcanti [a], V.H.V. Sarmento [b], R.S. Nunes [a,*]

[a] *Department of Pharmacy, Federal University of Sergipe, 49100-000 São Cristóvão, SE, Brazil*
[b] *Department of Chemistry, Federal University of Sergipe, 49500-000 Itabaiana, SE, Brazil*
[c] *Department of Morphology, Federal University of Sergipe, 49100-000 São Cristóvão, SE, Brazil*

GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: Liquid crystalline precursors, which are *in situ* gelling nanostructured surfactant systems, can undergo phase transition in aqueous solution and become more structured aggregates, controlling release of larvicides and acting as biotechnology alternatives for dengue control. Such systems can contain bioactive substances as *Citrus sinensis* essential oil (CSEO) which exhibits biological activity against *Aedes aegypti (Ae. aegypti)* larvae.
Experiments: The formulations were composed by fixed concentration of CSEO stabilized by Polyoxypropylene (5) Polyoxyethylene (20) Cetyl Ether (PPG-5 CETETH-20): oleic acid (OA) 2:1, increasing water content. The phase diagram was established and systems structure was evaluated by polarized light microscopy (PLM), small angle X-ray scattering (SAXS) and rheology. Median lethal concentration was determined against *Ae. aegypti* larvae.
Findings: The phase diagram exhibited four regions: liquid crystal (LC), emulsion, microemulsion (ME) and phase separation. The PLM and SAXS distinguished microemulsions, lamellar and hexagonal LC

Abbreviations: CSEO, *Citrus sinensis* (L) Osbeck essential oil; *Ae. aegypti*, *Aedes aegypti*; PPG-5 CETETH-20, Polyoxypropylene (5) Polyoxyethylene (20) Cetyl Ether; OA, oleic acid; PLM, polarized light microscopy; SAXS, small angle X-ray scattering; LC, liquid crystal; EM, emulsion; ME, microemulsion; PS, phase separation; LC_{50}, Lethal concentration, 50%; $I(q)$, scattering intensity patterns; (q), scattering vector; Lα, lamellar liquid crystal; H_1, hexagonal liquid crystal; q_{max}, value of the scattering vector q at the maximum intensity; K, consistency index; N, flow behavior index; R^2, regression coefficient; G′, storage modulus; G″, loss modulus.

* Corresponding author at: Laboratório de Desenvolvimento Farmacotécnico, Departamento de Farmácia, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, Cidade Universitária, 49100-000 São Cristóvão, Sergipe, Brazil. Fax: +55 (79) 21056827.

E-mail address: rogeria.ufs@hotmail.com (R.S. Nunes).

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structures. Flow and oscillatory tests showed that increasing water content increases elasticity from Newtonian to non-newtonian behavior confirming the *in situ* gelation behavior. The larvicidal activity of formulations indicates that these nanostructured systems improved the oil solubility in aqueous medium and in addition are potential environmental larvicide against *Ae. aegypti* larvae.

1. Introduction

Citrus sinensis (L) Osbeck essential oil (CSEO) is a complex mixture of natural compounds, including hydrocarbon, terpenes and oxygenated compounds being its main component R-limonene (68–98%) [1,2]. CSEO is a viable alternative for dengue control since it is a potential bioactive agent against mosquito vectors, environmentally safe, low cost and biodegradable [3–6]. Dengue is a mosquito-borne infection caused by a RNA flavivirus and is mainly transmitted to humans via the *Aedes aegypti* female mosquito [7–9]. Control of the *Aedes* vectors to prevent dengue appears to be the only option to reduce dengue impact on the population [10].

As most of the essential oils, CSEO is volatile and chemically unstable in the presence of air, light, humidity and high temperatures and water insoluble, invalidating its direct use [11]. Thus, surfactant systems can be used to circumvent those properties. Several studies have focused in the use of surfactant systems in order to enable the use of various substances and drugs [12–15]. The mixture of surfactant, oil and water can form aggregates with different structures as emulsions, micelles, microemulsions, liquid crystals (LC), among others [16–18]. The standard procedure used by several researchers for essential oils tests against *Ae. aegypti* larvae consists of preparing a coarse emulsion containing oil, water and surfactant [19]. The result is large droplets that promote the dispersion of the oil in water and larvae mortality. However, the LC_{50} (Lethal concentration, 50%) result still cannot defeat temephos, which has a diagnostic dosage of 0.012–1 ppm [20,21]. Therefore, decreasing the aggregates size to nanoscale would improve the dispersion of the oil in water, decreasing the LC_{50} value and thus being a viable candidate for *Ae. aegypti* larvicidal control.

The use of a cosurfactant is required for the formation of an optimal microemulsion region and reduces the droplet size [22]. Microemulsions (MEs) are characterized by ultra-low interfacial tension between the immiscible phases and offer the advantage of spontaneous formation, thermodynamic stability, simplicity of manufacture, solubilization capacity of lipophilic, hydrophilic and amphiphilic solutes, improved solubilization and bioavailability of hydrophobic drugs of active substances [23]. These systems can undergo into phase transition and structure modifications upon dilution with aqueous phase or evaporation of any volatile constituents [12,13,24,25]. The liquid formulation is easy to administrate, whereas post-application, it is transformed into a highly viscous rheologically structured system [12,26].

Liquid crystals (LC), also called as mesophases, presents the properties between a conventional liquid and solid crystal. They are structurally organized in lamellar, hexagonal or cubic phases which presents two or three dimensional nanometric-scale structures [27,28]. Lyotropic LCs are materials that are composed from at least two molecules: an amphiphilic molecule and its solvent. A hydrophilic solvent, such as water, hydrates the polar moieties of the amphiphiles via hydrogen bonding, while the flexible aliphatic tails of the amphiphiles aggregate into fused hydrophobic regions, based on van der Waals interactions [17,29]. Depending on each LC phase structure, the viscosity and self-assembly can be used to modulate the release of their content [30].

PPG-5-CETETH-20 or Procetyl® AWS, an alkoxylated cetyl alcohol surfactant, can be used as an *in situ* gelling agent for development of new controlled release formulations due to exceptional ability to self-assemble into lyotropic LC in the presence of water [12]. Oleic acid is a biocompatible fatty acid that consists of a long hydrocarbon chain and a carboxylate functional group [31]. In surfactant systems, it has been related with the use as oil phase [12,15,32], but it can also be used as cosurfactant, since it has the ability of reducing the surface tension.

Depending on the composition and characteristics of the formed systems, it is possible to establish lipophilic and hydrophilic sites in nanometric dimensions. The lipophilic sites can incorporate the CSEO, while structures arranged on a nanoscale in LC phases may be suitable to retain the CSEO components, establishing a possible control release. On the other hand, considering the composition of the aforementioned systems, it promotes a good level of the CSEO dispersion in an aqueous medium, allowing its use for the control of *Ae. aegypti* larvae.

Accordingly, the aim of this study was to develop and evaluate a promissory *in situ* gelling system based on CSEO that undergo into phase transition upon added in water being highly effective for *Ae. aegypti* larvicidal control. The systems were obtained by the mixture of PPG-5 CETETH-20, oleic acid (OA, cosurfactant), CSEO and water. The texture and nanostructure of the systems were evaluated by polarized light microscopy (PLM) and small angle X-ray scattering analysis (SAXS), respectively. The changes in the rheological properties were measured by steady shear rate and oscillatory tests. Finally, the median lethal concentration (LC_{50}) was assessed *in vivo* in *Ae. aegypti* larvae.

2. Experimental

2.1. Materials

PPG-5-CETETH-20 (Procetyl® AWS) was purchased from CRODA (Campinas, Brazil) and the OA from Synth (Diadema, Brazil). The high-purity water was prepared with a Millipore Milli-Q Plus purification system. CSEO was extracted by hydrodistillation in the laboratory.

2.2. Extraction of CSEO

CSEO was extracted by hydrodistillation with modified Clevenger apparatus. Sweet oranges were obtained in local market and their peels were dried and grinded before extraction. 200 g of the peels and 1.8 L of distilled water were used in the extraction. The essential oil was separated from hydrolate and was stored in amber glass container in refrigerator for later use.

2.3. Pseudoternary phase diagram construction and formulations preparation

The pseudoternary phase diagram construction was performed mixing different combinations of 2:1 PPG-5-CETETH-20:OA, CSEO and water at room temperature. The mixture of oil, surfactant and cosurfactant was diluted with water, under moderate magnetic stirring. The formulations were maintained during 24 h at room temperature to reach equilibrium. Transparent, clear systems were identified by PLM and SAXS, as described below. Based on the features of the formulations and PLM characterization, the ternary phase diagram was constructed using Sigma Plot Software v.11. Table1 shows compositions for each formulation.

2.4. Structural features of the formulations

The structural features of the formulations were analyzed by PLM, SAXS and Rheological measurements. *PLM*: The texture analysis of the systems at room temperature was observed using an Optical Olympus BX-51 Microscope equipped with a digital camera LC Color Evolution (PL-A662). Formulations were prepared by placing a drop of each one between a cover slip and a glass slide and then examined under polarized light. The isotropic or anisotropic behavior of the formulations was recorded. Pictures were taken at a magnification of 20.000×. *SAXS*: The nanometric phase structure was studied by SAXS measurements. The experiments were carried out on the D1B-SAXS1 beamline at the Brazilian Synchrotron Light Laboratory (LNLS, Campinas, Brazil). The X-ray beam was monochromatized (wavelength $\lambda = 1.499$ Å) by using a silicon-W/B4C toroidal multilayer mirror, collimated by a set of slits de fining a pinhole geometry, and detected on a Pilatus 300 k detector [33]. The sample-to-detector distance was 814 mm, covering a scattering vector q ($q = (4\pi/\lambda)\sin\theta$) ranging from 0.15 to 4.0 nm^{-1}, where 2θ = scattering angle. Measurements were performed at room temperature, and silver behenate powder (standard) was measured under the same conditions to calibrate the sample-to-detector distance, detector tilt, and direct beam position. Transmission, dark current and mica sheet corrections were performed. The parasitic scattering produced by slits was subtracted from the total scattering intensity. *Rheological measurements*: The formulations rheological analysis was carried out with a controlled-stress AR2000ex rheometer (TA Instruments). The geometry was plate-plate of 40 or 60 mm diameter (depending on the viscosity) with a 200 μm gap between the plates. Samples were carefully applied to the lower plate, ensuring that formulation shearing was minimized, and allowed to equilibrate for at least 3 min prior to analysis. All rheological determinations were carried out on all samples at 25 °C ± 0.2 °C. Steady shear rate sweep experiments (flow tests) were performed with shear rates ($\dot{\gamma}$) in the range of 0–200 s^{-1}. The shear rate region used was selected on the basis of the strength of resistance to the applied stresses. The rheological measurements were performed on both the up and down curves. The data from the shear cycle were fitted to a power-law model, using OriginPro v.8 Software. Dynamic frequency sweep experiments (oscillatory measurements) were performed within the linear viscoelastic region. The storage (G′) and loss (G″) moduli were evaluated using a frequency sweep between 0.1 and 100 Hz at 25 °C ± 0.2 °C and with constant oscillation stress at 0.5 Pa.

2.5. Median lethal concentration (LC_{50})

The larvae between third and fourth stages were used in the experiment. The formulations were used to prepare 100 mL solutions ranging CSEO concentration from 5 to 70 ppm. 20 Rockfeller larvae were placed in the solutions. Control solution was the inert formulation. After 24 h, it was observed the larvae mortality. LC_{50} and their confidence limits at 95% probability were calculated by probit analysis using Minitab v.16 Software.

3. Results and discussion

3.1. CSEO extraction

The CSEO is a natural, sustainable resource extracted from sweet orange peels. Brazil is the world's leading producer of oranges with about 1/3 of world production. The 2012/2013 orange harvest resulted in nearly 16.36 million tons, and there is an expected growth of 8% for the 2013/2014 crop [34]. The CSEO extracted was obtained with nearly 10% of efficiency and the main component was R-limonene with about 96.30%. Similar results were found in the literature, Rossi and Palacios (2013) obtained 95.10% and Singh et al. (2010) obtained 90.66% [35,36].

3.2. Pseudoternary phase diagram construction and formulation preparation

Pseudoternary phase diagram was constructed to observe the existence of different phase regions and the balance between different phases [37]. Fig. 1 exhibit the pseudoternary phase diagram formed by PPG-5 CETETH-20: OA 2:1/CSEO/water. This diagram presents four different regions characterized by microemulsion (ME), LC, emulsion (EM) and phase separation (PS). The ME region was formed with low water content, until 20% of water, above 45% of PPG-5 CETETH-20: OA 2:1 and oil phase until 50%. The LC region was between 25 and 55% of aqueous phase, until 20% of oil phase and PPG-5 CETETH-20: AO 2:1 concentrations above 40%. The selected dilution line present the same CSEO concentration fixed at 5% and exhibits ME, LC and ME-LC transition systems.

3.3. Structural features

The nanometric structure of the phases was evaluated by SAXS and PLM. Fig. 2 shows scattering intensity patterns, $I(q)$ in function of scattering vector (q) for all the formulations and representative PLM (insert). We can observe that formulations A1, A2 and A3

Table 1
Composition, SAXS and Rheological parameters of the formulations formed by PPG-5 CETETH-20:OA/CSEO/water.

Formulation	Composition			SAXS parameters				Rheological parameters		
	W (%)	O (%)	S/Cos (%)	q_2/q_1	q_3/q_1	d (nm)	Structure	K (Pa s^n)	n	R^2
A1	5	5	90	–	–	–	ME	0.12	1.002	0.999
A2	10	5	85	–	–	–	ME	0.15	1.004	0.999
A3	15	5	80	–	–	–	ME	0.25	0.997	0.999
A4	20	5	75	2	–	–	ME + Lα	0.45	0.991	0.999
A5	25	5	70	2	–	–	Lα	3.90	0.470	0.994
A6	30	5	65	2	–	7.7	Lα	6.10	0.540	0.998
A7	35	5	60	2	3	8.4	Lα	–	–	–
A8	40	5	55	2	3	9.0	Lα	–	–	–
A9	45	5	50	2	3	10.0	Lα	–	–	–
A10	50	5	45	2	3	10.8	Lα	–	–	–

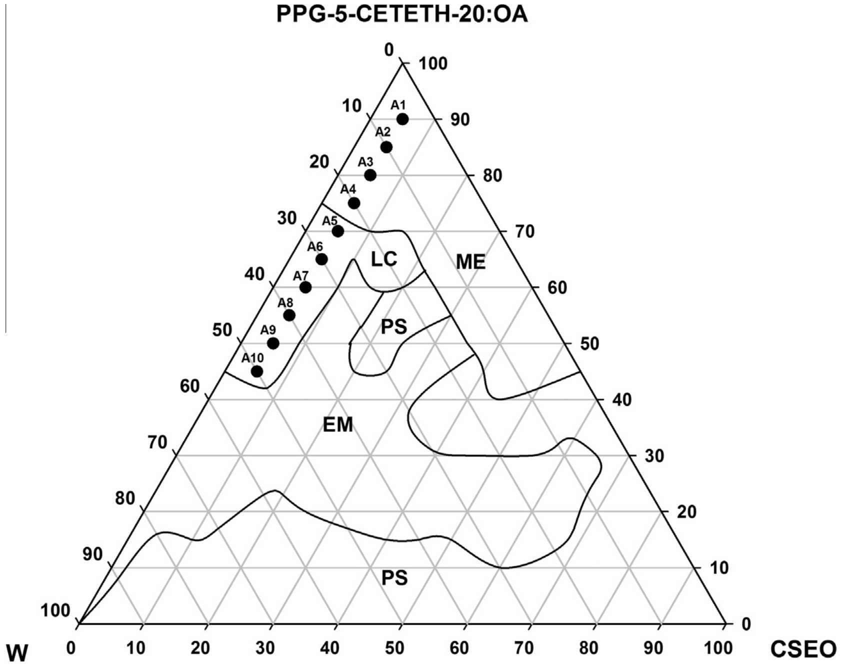

Fig. 1. Pseudoternary phase diagram formed by PPG-5-CETETH-20: oleic acid (P:OA) 2:1/*Citrus sinensis* essential oil (CSEO)/water (W) phase diagram. ME: microemulsion; LC: liquid crystal; EM: emulsion; PS: phase separation.

(Fig. 2a) present a broad and wide peak with the maximum of the peak shifted to smaller q-values (ranging from 1.4 to 0.92 nm^{-1}) as the aqueous phase content increased. These SAXS patterns are characteristic of ME systems [18] and are supported by PLM (insert of Fig. 2a superior), since ME are not birefringent and therefore, they generate a dark background when investigated under the polarizing microscope (characteristic of isotropic systems). Increasing aqueous phase content (formulation A4), there is an increase in the intensity and narrowing of peaks (Fig. 2a) suggesting a phase transition from less to more ordered structure possibly a LC [38]. These results are also in agreement with PLM (insert of Fig. 2a inferior) where we can observe a slight birefringence, but no characteristic texture, indicating that it is possibly a ME + LC phase transition system. Over 25% aqueous phase (A5–A10) narrow and well-defined peaks arise at high q values (Fig. 2b), suggesting a more structured system associated to LC phase. PLM (insert of Fig. 2b) corroborates with SAXS results due to presence of Maltese crosses characteristic of anisotropic lamellar LC [27].

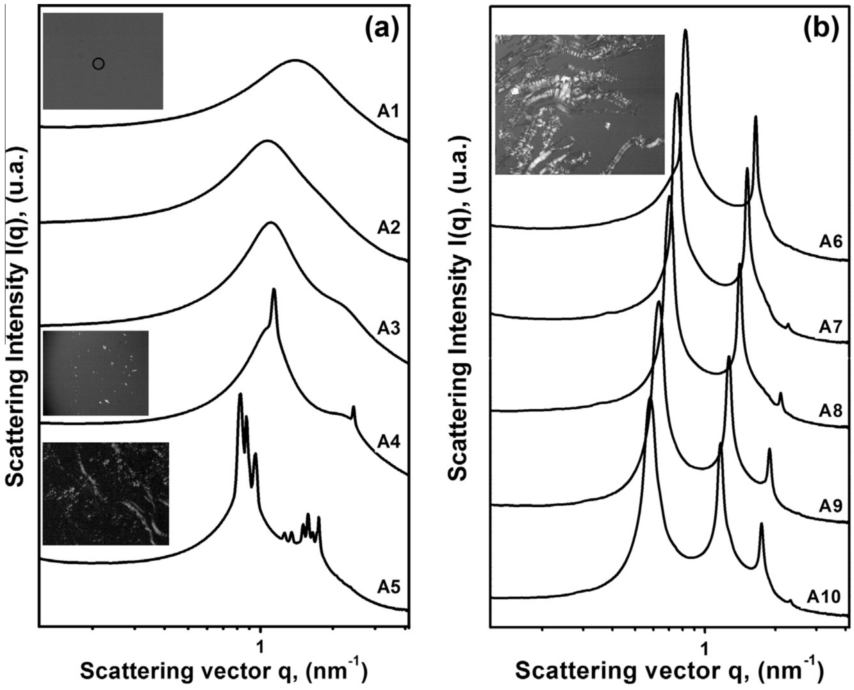

Fig. 2. Scattering intensity patterns $I(q)$ in function of scattering vector (q) for the formulations (a) from A1 to A5; (b) from A6 to A10 and representative photomicrographs (insert) obtained by polarized light microscopy (PLM).

In order to confirm the type of LC phase is important to check the position of the peaks produced from SAXS curves, which for structured systems such as LC, are referred to as Bragg peaks. For lamellar phase LC (Lα) the position of the peaks relative to the first and most intense peak should obeys the ratio 1:2:3, while hexagonal LC (H_1), $1{:}\sqrt{3}{:}2$ [27,28]. According this information, formulation A4 exhibits phase transition due to narrowing of the peak and an appearance of a second peak not well defined with ratio 1:2. In spite of that, the wide peak characteristic of the ME phase is still visible, demonstrating that an organized LC is not yet totally formed characterizing a ME + Lα transition. Formulation A5 shows a combination of lamellar structures probably due to a process of structural accommodation during the transition. Formulations A6–A10 exhibits three to four well-defined Bragg peaks (depending on water amount used in the formulation) with 1:2:3:4 peak ratio indicating that these systems are lamellar phase LC.

From the SAXS curves is possible to estimate the distance between lamellae in order to determine the lamellar periodicity using the equation $d = 2\pi/q_{\max}$, where d is correlation distance and $q_{\max}$ is the value of the scattering vector q at the maximum intensity $I(q_{\max})$. These values were calculated and are presented in Table 1. As we can see, d increases with the water amount (7.7–10.8 nm) suggesting that a process of swelling occurs. This process can be attributed to hydration of the surfactant molecule poly(ethylene oxide) (PEO) chains, which increases the repulsion between the head groups, increasing the distances between lamellae [38].

The significant structural changes exhibited by PPG-5-CETETH-20:OA/CSEO/water system from SAXS measurements and PLM results are important for their application in the various fields, especially for making formulations with controlled release of active ingredients. Since it is able to form lamellar phase LC when in contact with certain volume of the aqueous phase, our system is a promising *in situ* gelling system, which can enable the retention of larvicide for long periods and enhance their effectiveness. Rheological properties such as viscosity are an important parameter to be evaluated *in situ* gelling system.

Viscosity was determined by flow rheology measurements. Fig. 3 shows the shear stress as a function of shear rates of the formulations between 5% and 30% (A1–A6) of water content. For formulations > 30% (A7–A10), the viscosity values are above the limit of sensitivity of the equipment for this test and therefore, are not shown. As seen in Fig. 3, formulations A1–A4 exhibited Newtonian behavior (linear relationship between the shear stress and shear rate), while A5 and A6 presented non-linear relationship and consequently exhibited a non-Newtonian flow behavior [39]. Newtonian behavior is expected in ME systems due to their reduced droplet size and low interaction between them [40]. These results are in agreement with SAXS data that showed for formulations A1, A2 and A3 (Fig. 2a), a broad and wide peak characteristic of ME systems which is shifted toward lower q-values and that can be attributed to an increasing in the domain size. Despite of the SAXS profile does not present characteristics of ME, the formulation A4 exhibited Newtonian behavior. This somewhat different behavior shows that, although there is presence of intense and narrow peaks that indicates the existence of some interaction structural forces, it is not sufficiently strong to prevent an irreversible deformation under the action of shearing. It is important to emphasize that the A4 formulation lies in a region of phase transition and ME phase is still visible, demonstrating that the organized structures are not yet totally formed. A5 formulation, as seen in phase diagram (Fig. 1) is located in the beginning region of the lamellar phase LC and the presence of several narrow and intense peaks indicates the formation of structural interactions between lamellae that deform and flow instantaneously upon application of stress and display shear thinning behavior characteristic of structured fluids with pseudoplasticity. The same behavior can be explained to A6. Furthermore, both exhibited thixotropy showing that these formulations have a strong ability to recovery its structure when the stress is removed.

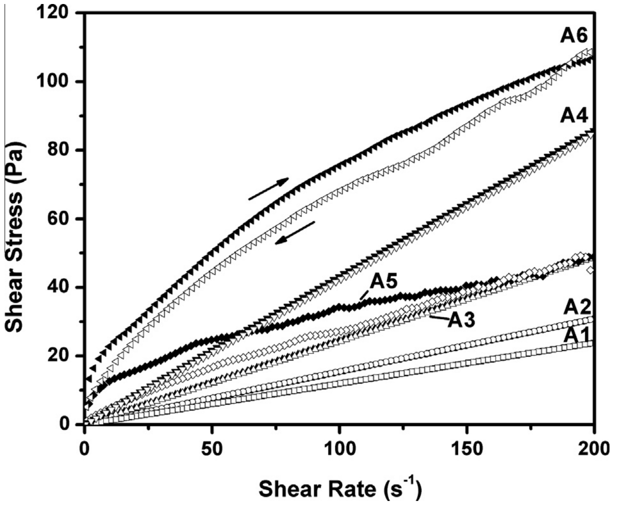

Fig. 3. Shear stress as a function of shear rates of the formulations between 5% and 30% (A1–A6) of water content.

In order to determine the viscosity of the formulations, a power-law model which expression is $\tau = K\dot{\gamma}^n$ was used, being K and n considered rheological parameters. K is the consistency index and n is the flow behavior index that represents a shear-thickening fluid if $n > 1$, a shear-thinning fluid if $n < 1$ and Newtonian fluid if $n = 1$. Table 1 shows the values of K, n, and R^2 (regression coefficient) for formulations A1–A6. As seen, all formulations show that R^2 values are around 0.999, indicating that the experimental data are well adjusted by the power law model. For n values obtained, except to A5 and A6, present close to unity confirming the Newtonian behavior for these formulations, which is expected for ME systems and ME-LC transitions [41]. For A5 and A6, the n values are less than unity, showing that these formulations present pseudoplastic shear-thinning behavior. Finally, the K values, as can be observed in Table 1, have a slight increase when the water amount increases from 5% (A1) to 20% (A4) and an abrupt increase for amounts over 20% (A5 and A6), showing the enhanced thickening property and consequently of the viscosity. All these results demonstrate the strong influence of the water on the LC phase formation and therefore, on rheological properties of the formulations.

The presence of the third peak, which intensity arises in the SAXS curves (Fig. 2a) for the formulations above 35% aqueous phase (A7–A10) indicate the formation of more organized structures, increasing interactions and consequently the viscosity. Therefore, the rheological properties of these formulations are not viable to be studied by flow tests and were submitted to oscillatory measurements, which provide wide range information about liquid crystalline structure; it is a non-destructive test and enables to measure simultaneously, both the viscous and elastic component of a formulation, characterizing the structural interactions in contact with the water.

Fig. 4 shows the dependence on frequency of the storage modulus (G′) and the loss modulus (G″) of the formulations consisting of 35%, 40%, 45% and 50% aqueous phase (A7, A8, A9 and A10, respectively). The examined formulations exhibit a viscoelastic gel-like behavior and are more elastic than viscous (G′ > G″) in the measured frequency range. Both G′ and G″ exhibit

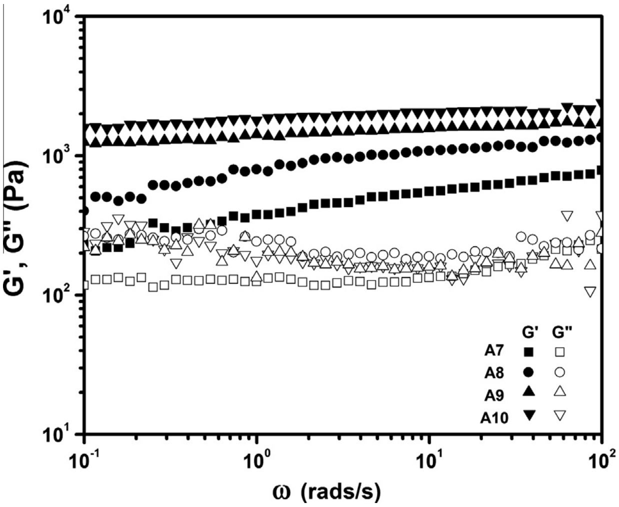

Fig. 4. Frequency-dependent behavior on the storage (G′) and the loss (G″) modulus of the formulations consisting of 35%, 40%, 45% and 50% aqueous phase (A7, A8, A9 and A10, respectively).

Table 2
LC_{50} against *Ae. aegypti* larvae after 24 h exposure of the formulations A3 and A4.

Sample	Structure	LC_{50} (95% CL) ppm
A3	ME	6.55 (6.33–6.77)
A4	ME + Lα	5.93 (5.70–6.16)
Control	–	0

LC_{50} = Lethal concentration (ppm) at which 50% of the larvae showed mortality.
CL = confidence limits at 95% probability.

frequency-independent behavior due to presence of completely cross-linked systems, typical of rigid and organized structures as LC lamellar phase. These results are consistent with SAXS measurements, indicating formation of a lamellar phase upon a water content increase [42]. All results of rheological analysis corroborate with those found by SAXS analysis, showing that the lamellar liquid crystalline systems have a well-organized structure. With aqueous phase increase, the systems structure becomes more ordered indicated by the increase in lamellae number (SAXS) and the increase in elasticity (rheology).

3.4. Median lethal concentration (LC_{50})

Formulations A3 and A4 were chosen for being systems in the boundary ME-LC, being A3 an ME and A4 an ME-LC phase transition system, therefore they are *in situ* gelling systems. Table 2 shows the LC_{50} values for these formulations. Both samples presented low LC_{50} values even though A3 presented a slightly higher LC_{50} value than A4, which means that they exhibited a great activity against *Ae. aegypti* larvae. Control was performed with the inert formulation and no mortality was observed.

Several authors published data on *Ae. aegypti* control using essential oils. Costa et al. (2005) identified the LC_{50} of 21.4, 19.5 and 18.5 ppm for three different essential oils, *S. aromaticum, L. sidoides* and *H. martiusii*, respectively [43]. Silva et al. (2008) identified 37 ppm for R-limone and 98 ppm for *L. gracilis* [19]. Temephos, an organophosphate commonly used by public health, has a diagnostic dosage of 0.012–1 ppm [20,21]. Bti (*Bacillus thuringiensis* israelensis), is used as an alternative to temephos, due to the resistance exhibited by *Ae. aegypti* [44]. However, Bti also showed resistance, and both of them present limited applied dosages in drinking-water as well as they exhibit persistence in the environment [20,45].

In previous studies, it was determined that the LC_{50} value for CSEO was 21.5 ppm (21.3–21.7) [46]. Since the CSEO is insoluble in water, the experiment requires the essential oil to be dispersed in water using a surfactant, tween80®. These data demonstrate that even though the two experiments use the surfactant for the essential oil dispersion, the proposed system exhibits a more potent activity.

In comparison with the LC_{50} values between other *Ae. aegypti* controls, the present system demonstrates the potent environmentally safe larvicide. These values are a result from the nanostructure formed in these formulations that has high solubilization capacity for bioactive substances as the CSEO. The formulations behave as LC precursors since they undergo into phase transition in aqueous medium, turning into systems that have a rigid matrix for CSEO release [12]. Thereby, sustainable resources as LC precursors containing CSEO are viable alternatives for *Ae. aegypti* control, since the CSEO is a natural product, with low toxicity and low cost.

4. Conclusion

In situ gelling nanostructured systems were obtained containing CSEO, PPG-5 CETETH-20, OA and water. These systems have several advantages such as low cost, easily prepared, environmentally safe and thermodynamically stable [4,12,16]. Since the obtained systems are liquid systems with low water content, they present long shelf life. The liquid preparation can be placed in water containers through dropper and the system would undergo into phase transition to a more ordered structure.

The increase of aqueous phase content from 5% to 50% enhanced a phase transition from less ordered structure as ME to a more ordered structure as lyotropic LC. The result of the structural features exhibited the transition of an initially liquid isotropic system, with low viscosity (characteristic of ME systems) to a structured, viscoelastic and anisotropic system (characteristic of LC) with the increase of the water content. The obtained LC precursors are environmentally safe, containing a sustainable resource as CSEO and showed LC_{50} values of 6.55 and 5.93 ppm against *Ae. aegypti* larvae.

Only a few articles relate LC precursors and there is limited data regarding this system. It has a nanoscale structure in which can be incorporated lipophilic and hydrophilic substances and in contact with water, the system is transformed to a lyotropic LC establishing a possible control release of the substance. Similar studies of LC precursors obtained positive outcomes for their purpose and discovered that the LC can present a rapid or slow release [12,13].

It is hypothesized that the *in situ* gelling system has the potential to be used as a delivery vehicle for CSEO for *Ae. aegypti* larvae control. *In situ* gelling system has not been used for such purpose, and therefore it is a major development in the field since the researchers are still seeking new alternatives to control dengue and the vaccine is still under development.

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