Journal of Colloid and Interface Science 456 (2015) 190–196

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

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

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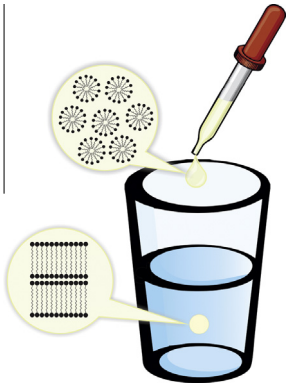
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 February 2015
Accepted 8 June 2015
Available online 15 June 2015

Keywords:
Liquid crystal
Microemulsion
In situ gelling
Citrus sinensis
Dengue
Aedes aegypti

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.

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http://dx.doi.org/10.1016/j.jcis.2015.06.012

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