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Nano-droplet formation in water/ethanol or isopropanol/mosquito repellent formulations

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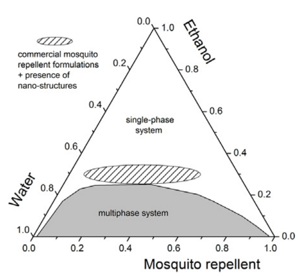
HIGHLIGHTS

- Nano-structures were found in water/alcohol/mosquito repellent systems.
- DLS/SLS confirmed findings for both natural and synthetic repellents.
- Nano-structures can be found in commercial formulations.
- Potential consequences for formulation efficacy.

GRAPHICAL ABSTRACT



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ABSTRACT

It was recently demonstrated that nano-structures were present in water/ethanol/oil systems, where the oil was either octanol or fragrance molecules. The goal of the present work is to check if such structures exist also in other, related systems and if a general concept can be deduced from these observations. To this purpose, natural and synthetic mosquito repellent molecules were investigated, which represent nearly all repellents used on the market. For the ternary water/alcohol (ethanol or isopropanol)/repellent systems ternary phase diagrams were established. The presence and the ordering of the nano-droplets were checked and characterized with dynamic and static light scattering and conductivity measurements. Based on these results it can be concluded that a nano-ordering with generally an organic continuum exists in hydro-alcoholic commercial mosquito repellents, and thus that these systems are not simply molecular solutions. This might have a consequence for diffusion processes in the skin.

1. Introduction

All around the world mosquitoes are the carriers of serious and lethal diseases, such as malaria, dengue, West Nile virus, yellow fever, and chikungunya [1,2]. According to the World Health Organization, 247 million malaria infections occurred in 2008 causing the death of 863,000 people, mainly children under the age of 5 [3]. Further, due to global warming, more and more species of mosquitoes carry such diseases [4]. Since the different pathogens are transmitted by injection of saliva into humans [5], the main protection is to prevent bites by minimizing contacts between the mosquitoes and their targets. In order to reach this goal, mosquito repellents are investigated and used worldwide, because they prevent mosquitoes from landing on humans and biting them.

Before the discovery of synthetic insecticides, natural products, for example nicotine from tobacco leaves, proved their efficiency against mosquitoes [6]. Nowadays, natural repellents are still used, such as citriodiol, *para*-menthane-3,8-diol (PMD) and 2-undecanone. Citriodiol is obtained by cyclisation of the citronellal from *Eucalyptus citriodora* oil (oil of lemon eucalyptus) in acid

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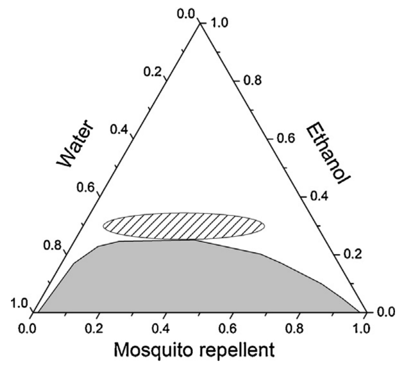

Fig. 1. Ternary phase diagram showing the solubilization area of mosquito repellents as it is commonly formulated (hatched area). The gray area is a multiphasic domain and the white area is a monophasic domain.

medium. This oil is obtained by hydrodistillation and can be then considered as an essential oil where citronellal is present at about 80% w/w. The tree was originally found in Australia and is now cultivated in warm places around the world, like Malawi by the firm *Citrefine International Ltd.*, which produces a melt of 60–70% (w/w) of *cis*- and *trans*-PMD, acetals from PMD and residual non-reactive terpene and terpenoid molecules [7].

Mosquito repellents can be formulated either by using directly the citriodiol or the pure PMD (cis and trans isomers) synthesized by the firm *Takasago International Corporation*.

2-Undecanone is obtained from a selective separation of rue (*ruta graveolens*) essential oil, its proportion being around 50% w/w [8]. Rue is a very common plant, which is native to the Balkan Peninsula and southeastern Europe.

Synthetic mosquito repellents, not found in nature, are also used, such as *N*,*N*-diethyl-*m*-methylbenzamide (DEET), 1-methylpropyl 2-(2-hydroxyethyl)piperidine-1-carboxylate (KBR 3023) and ethyl 3-[(acetyl)(butyl)amino]propanoate (IR 3535). DEET was first used in 1946 by the United State Army and entered civilian use in 1957. It is now the most widely used insect repellent. Its effectiveness was proven against a lot of insects, including mosquitoes, black flies, ticks and bedbugs [3].

The only mosquito repellent more effective than DEET is KBR 3023, which was developed in the mid-1990s by Bayer and first used in Europe in 2001 with a 7% w/w solution or with a 20% (w/w) solution by the Australian Army [9].

IR 3535 was developed by the firm Merck KGaA and is classified by the Environmental Protection Agency as a biopesticide. It has been used in Europe for more than 20 years and was approved for use in the United States in 1999 [10].

Almost all mosquito repellent products available on the market are hydro-alcoholic formulations, incorporating water, ethanol and/or isopropanol and a repellent or a melt of them. As shown in Fig. 1, mosquito repellents contain generally between 10 and 50% (w/w) of repellent and the minimum quantity of ethanol or isopropanol necessary to solubilize this molecule.

Drapeau et al. [2] showed the possibility to obtain a surfactantless microemulsion in the water (42%, w/w)/isopropanol (38%, w/w)/PMD (20%, w/w) system, Klossek et al. [11] found the presence of nano-structures in the system water/ethanol/1-octanol by Dynamic Light Scattering (DLS), and Marcus et al. [12] had extended these studies and found these nano-structures in systems containing fragrance molecules. Therefore, the presence of similar nano-structures in the system water/ethanol/repellent appears very probable.

The aim of the present work is to check this point.

First, the influence of the mosquito repellent molecules on the domains of existence of the clear and single phase area of the water/ethanol or isopropanol/repellent systems is studied by establishing ternary phase diagrams. And second, dynamic (DLS) and static (SLS) light scattering experiments are performed in these single phase regions to investigate the presence of a nano-ordering.

2. Experimental

2.1. Materials

Citriodiol ((+)-cis-:(−)-trans isomers ratio equal to 66:34) was donated from Citrefine International Ltd. (Leeds, United Kingdom). PMD (purity ≥ 99%, (+)-cis-:(−)-trans isomers ratio equal to 62:38) was obtained from Takasago International Corporation (Paris, France). 2-Undecanone (purity ≥ 97%), DEET (purity ≥ 97%) and ethanol (purity ≥ 99.8%) were purchased from Sigma Aldrich Chemie GmbH (Steinheim, Germany). KBR 3023 (purity ≥ 97%) was donated by Lanxess (Langenfeld, Germany). Isopropanol (purity ≥ 99.8%) was purchased and IR 3535 (purity ≥ 98%) was donated by Merck KGaA (Darmstadt, Germany). LiCl (purity ≥ 99%) was obtained from Merck Schuchardt OHG (Hohenbrunn, Germany). All chemicals were used without further purification. All solutions were prepared using deionised water with a resistivity of 18.2 MΩ cm.

2.2. Methods and techniques

2.2.1. Ternary phase diagrams

The dynamic process described by Clausse et al. was used to determine the domains of existence of the monophasic regions for each system [13]. In screw top tubes, the repellent or water was mixed with ethanol or isopropanol at determined weight ratios to obtain a starting weight of 3 g. Water or the repellent was then added dropwise with Pasteur pipettes until the solution became turbid. The amount of water or repellent was recorded and the temperature was kept constant at 25 °C with a thermostatically controlled test tube rack.

2.2.2. Light scattering

Dynamic light scattering (DLS). The hydro-alcoholic solutions were prepared as described in Section 2.2.1. The samples were filtered with 0.2 μm PTFE membrane filters in order to remove dust and transferred into cylindrical light-scattering cells of 10 mm outer diameter. These cells were placed in a temperature controlled vat of toluene of a CGS-3 goniometer system from ALV (Langen, Germany) equipped with an ALV-7004/FAST Multiple Tau digital correlator and a vertical-polarized 22 mW HeNe laser (wavelength $\lambda = 632.8$ nm). All detection was performed at an angle of 90° and data were collected for 300 s at 25 °C. The software TableCurve 2D v5.01 was used to determine the size of the particles by fitting monomodal equations to the experimental correlation functions, which can be described with the following equation:

$$Y = a_0 + (a_1 \times e^{-a_z X})^2$$

where X is the delay time, a_0 is the constant baseline value, usually 1, a_1 refers to the dynamic part of the amplitude, and a_2 is the decay rate, linked to the diffusion coefficient D:

$$a_2 = Dq^2$$

and q is the scattering vector which is defined as:

$$q = \frac{4\pi n}{\lambda} \sin\frac{\theta}{2}$$