TECHNICAL BRIEF (UST-009)

Neem oil nanoemulsion produced by Ultra Shear Technology: Finding Neem-O

Summary: The entomotoxic activity of neem oil rapidly deteriorates in water. This activity is preserved in neem oil nanodispersions produced by Ultra Shear Technology™ (UST™)

Background: Nearly half of the world's population lives in regions where malaria is endemic [1]. In 2019, the World Health Organization (WHO) reported 229 million cases of malaria worldwide [2], significantly higher than the total number of COVID-19 cases reported during the first 18 months of the pandemic [3]. Neem oil is widely used for its repellent and larvicidal activities on mosquito vectors [4]. Additionally, neem oil has antimicrobial properties and has been reported to have antiplasmodial properties similar to chloroquine [5].

The U.S. Environmental Protection Agency (EPA) has approved neem oil as a safe and natural pesticide which the U.S Department of Agriculture (USDA) has approved its use on organic crops. Azadirachtin, the primary insecticidal component of neem oil, is rapidly degraded by light, water, and soil and is therefore expected to have minimal environmental impact. Azadirachtin has a half-life of 36 to 48 h in water when exposed to natural sunlight [6]. However, the preservation of entomotoxic activity has been demonstrated when neem oil is formulated as a nanoemulsion [7, 8] and improved bioefficacy has been correlated with smaller oil droplet size [9].

Methods: Course emulsions of cold-pressed neem oil (Deepthi Organics, Greensboro, NC, USA) were prepared by rotor-stator homogenization. Course emulsions were 10% neem oil, 10% glycerol, and 5.4% anionic surfactant. Oil droplet size and polydispersity were determined by light microscopy and image analysis (ImageJ, Bethesda, MD, USA). Neem oil nanemulsions (Neem-O) were produced by passing the course emulsion through the UST NanoGap valve for 1-14 cycles at 45,000 psi. Nanoemulsions were analyzed after each UST cycle. Mean hydrodynamic diameter (Dh) and polydispersity of oil droplets was determined by dynamic light scattering (DLS). Nanoemulsions progressively clarified with each additional cycle. Clarification was quantified by UV/Vis spectroscopy. Entomotoxic activity of Neem-O in terms of lethal concentration and exposure time (LD_{50} and LT_{50}, respectively) was determined in *Daphnia pulex* (WM Tricker, Independence, OH, USA) as a representative copepod.

Results: Course emulsions were unstable suspensions prone to rapid phase separation. Large, highly polydisperse oil droplets ranging from 4,000 to 20,000 nm (8,580 ± 2,270 nm) were observed by microscopy. Polydispersity was 51.4%. The mean hydrodynamic diameter (Dh) of oil droplets was reduced to approximately 230 nm following a single pass through the UST NanoGap™ valve. Polydispersity was 23.0%. Oil droplet size progressively decreased over repeated UST cycles, reaching a minimum of 84 nm after 14 cycles (**Figure 1B**). Polydispersity was 23.8%.

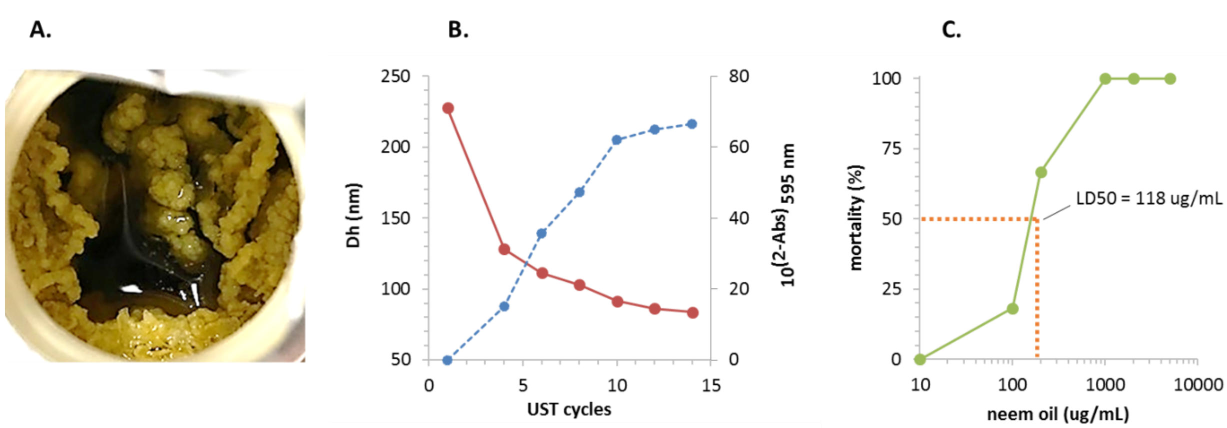

Figure 1. (A) 100% neem oil congealed at room temperature. (B) Progressive decrease in hydrodynamic diameter (Dh) of oil droplets produced over 1-14 UST cycles (red) corresponding to decreased light scattering and increased clarity (blue dashed line). LD_{50} in *D. pulex* measured over six hours for serial dilutions of Neem-O that was stored at room temperature for four months.

Entomotoxin activity was preserved in neem oil nanoemulsions for at least four months. An LD_{50} of 118 ug/mL was observed in *D. pulex* for Neem-O stored at room temperature for four months. A 1:100 dilution of Neem-O killed 50% of daphnids (LT_{50}) in less than two hours.

References:

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