**A simple, scalable, isothermal process for nanoemulsion formation**

**High Performance Emulsification using Adaptive Focused Acoustics**

**INTRODUCTION**

Nanoemulsions are a very effective vehicle for both the encapsulation of and the delivery of biologically active ingredients (1). However, current methods for the formation of nanoemulsions include extremely high heat, high shear force methods such as Microfluidizers, and complex chemistry, high temperature, low energy methods such as Self Assembling Nano Emulsions (SANE) are technically limiting (2,3). Findings show that the use of focused acoustic energy is very effective to form nanoemulsions across a wide range of sample volumes from under 0.50ml through continuous process flow. The ability to formulate monodisperse, nanoemulsions, with average particle sizes down to 20nm was previously demonstrated with a poly-dispersion index below 0.100. All results were achieved in very fast process time and with sample temperatures held below room temperature in all cases (e.g., <20 °C). Samples ranging from <1ml to continuous flow stream were prepared. Adaptive Focused Acoustics (AFA) has proven technical advantages in several biological based applications including DNA shearing, tissue homogenization, and cell lysis and disruption (2,4). The ability to apply a controlled mechanical, shear force energy (e.g., submicron jets) to the molecular solvent boundary layer benefits the formulation of nanoemulsions of biological, pharmaceutical, and any thermal sensitive (labile) materials. Characteristics of the technology include: fast and convenient operation, isothermal processing eliminating molecular damage due to overheating, high reproducibility, self contained, non contact processing, and the ability to scale from Batch Scale sample volumes through Continuous Flow Process.

**BATCH SCALE PROCESS**

AFA-based production of Oil/Water emulsions at batch scales showed that we can control the size by varying the PIP (FIGURE 2), effect of AFA retention (i.e., residence) time on the process (FIGURE 3) while maintaining isothermal processing temperatures (FIGURE 4) which can also influence the size of emulsions. The AFA technology enables lower volume evaluations during the developmental phase, which efficiently utilizes resources of expensive ingredients.

**CONTINUOUS FLOW PROCESS**

AFA-based, high volume production of Oil/Water emulsions have been previously reported. In addition, numerous formulations have been screened with this method. Understanding the role of physico-chemical parameters of AFA in the emulsification process will enable better control the process. In this study, the reproducibility, the scalability, and the control of the temperature of the AFA process is demonstrated. A continuous flow process system was configured to enable a real-time emulsion formation. Temperatures were measured at the inlet and outlet of the system. In all cases the maximum sample temperature was below 17 °C (FIGURE 5) and a reduction in power levels resulted in a direct reduction in sample temperature (FIGURE 6). The impact of flow rate was evaluated; more AFA residence time led to lower particle sizes and smaller tighter distributions (FIGURE 7). AFA as the potential to process high quality nanoemulsions from both a developmental scale to a continuous flow process, while maintaining low sample temperatures and computer controlled output quality (FIGURE 8).

**SUMMARY**

Focused-ultrasonic acoustics is both a very effective and beneficial technology to aid in the formulation of nanoemulsions. The ability to control a high power density, (delivered to the sample in a non contact manner), combined with the precise thermal control during the process, offers an unprecedented combination of benefits when compared to existing known technologies. AFA technology provides an innovative, robust, and an easily scalable process to thermally labile active ingredients.

**REFERENCES**


**AFA TECHNOLOGY OVERVIEW**

*The Covaris Adaptive Focused Acoustics™ (AFA) technology:*

- focuses high frequency (500 kHz) ultrasonic energy to a small region;
- Class A electronics precisely control the ultrasonic energy delivered to the targeted sample;
- is non-contact with optimized sample temperature control;
- is user friendly and provides highly repeatable sample processing;
- is widely used in biomedical fields, in low power applications such as acceleration of mass action events and in high power applications such as unbiased DNA fragmentation for next-generation sequencing.

**Pressure & Thermal Profile Comparison**

Covaris’ AFA technology has intrinsic advantages over traditional sonicators. Due to the highly focused ultrasonic wave, the power required to generate cavitation in water is magnitudes smaller than traditional sonicators. Higher efficient energy consumption of AFA avoids the excessive heat generation in the sample and reduces the potential thermal damages to the sample. The precisely controlled energy delivery improves the reproducibility of the sample processing for higher quality control.