Continuous-flow production of a pharmaceutical nanoemulsion by high-amplitude ultrasound: Process scale-up

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**A B S T R A C T**

High-pressure homogenization (HPH, including microfluidization) and high-amplitude ultrasonic processing are currently the leading two methods used to produce nanoemulsions of superior quality. Despite suffering from multiple important drawbacks, HPH is currently the technology of choice for the industrial manufacture of pharmaceutical nanoemulsions. The ultrasonic nanoemulsification technology is free from most of these drawbacks and frequently used in laboratory studies. The challenge for the ultrasonic method, however, has been bridging the gap between laboratory research and its industrial implementation. Due to limitations of conventional ultrasonic technology, scaling up has not been possible without a significant reduction in ultrasonic amplitudes, which compromises product quality. This limitation has been overcome by Barbell Horn Ultrasonic Technology (BHUT), which permits constructing bench and industrial-scale processors capable of operating at high ultrasonic amplitudes. In the present study, a high-quality MF59®-analog pharmaceutical nanoemulsion has been successfully manufactured using laboratory, bench and industrial-scale high-amplitude ultrasonic processors. The overall laboratory-to-industrial scale-up factor achieved by using BHUT was approximately 55. The ultrasonic amplitude and the resulting product quality were maintained identical at all three scales. To our knowledge, this work is the first reported instance of a successful and systematic industrial scale-up of any high-amplitude ultrasonic process.

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1. **Introduction**

Lipid nanoemulsions are complex, kinetically stable oil-in-water dispersions, homogenized with the aid of an emulsifier. In clinical practice, there are three major applications of nanoemulsions: (1) parenteral nutrition, (2) colloidal drug carriers and (3) vaccine preparations. Intravenous lipid nanoemulsions are an important source of fatty acids for pediatric and adult patients, used when oral nutrition is impossible or disadvantageous. Their size and stability are critically important [1]. Lipid nanoemulsions are also widely used as drug carriers because they easily incorporate lipophilic bioactive compounds, stabilize bioactive compounds that tend to undergo hydrolysis, and reduce side effects of potent drugs. Additionally, lipid nanoemulsions are biodegradable and can be produced on a large scale. Furthermore, nanoemulsions can be administered by almost all available routes including parenteral, ocular, nasal, oral, topical, and even aerosolization to the lungs [2]. There are approximately a dozen commercially available drugs encapsulated into nanoemulsions [2].

Recent years have seen major breakthroughs in how vaccines are formulated, with the approval of three new lipid-based adjuvants, each of which is formulated as a nanoemulsion. The lipid-based adjuvants approved for use in human vaccines include MF59® (Novartis), AS03 and AS04 (GlaxoSmithKline) [3]. MF59® and AS03 are squalene-based adjuvants, while AS04 combines monophosphoryl lipid A (MPL) with alum [3]. AS04 is approved for the use with hepatitis B virus and human papilloma virus in Fendrix and Cervarix vaccines (GlaxoSmithKline), respectively [4]. AS03 has been approved as a component of the pandemic flu vaccine Pre pandrix [5]. Squalene nanoemulsion most commonly used in vaccine formulations, MF59®, has already been licensed as a component of an influenza vaccine, Fluad® [6], and pandemic H1N1 vaccine [7]. MF59® has an established safety and efficacy profile: MF59®-based seasonal and pandemic flu vaccines have been distributed to approximately 80 million persons [7]. The global vaccine market is estimated at $32.05 billion in 2013 and is expected to reach $84.44 billion by 2022 [8].

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1.1. Overview of techniques used for the production of nanoemulsions

The industrial production of nanoemulsions requires significant energy deposition and intense shear forces [9]. Although low-energy emulsification methods do exist, they have the disadvantage of requiring high concentrations of surfactants, which must be removed before administration [10,11]. The shear forces necessary for the emulsification process can be provided by mechanical agitation, for example, stirring, high shear mixing, high-pressure homogenization (using a homogenizing valve-based device or a Microfluidizer®) or high-amplitude ultrasound. The latter two methods have been demonstrated to be superior to all others, being able to produce nanoemulsions with droplets much smaller than 500 nm in diameter and narrow size distributions [9,12,13].

High-pressure homogenizers require a coarse dispersion with droplets of about 1–10 μm in diameter to be prepared first, for example by a rotor-stator colloid mill [10], which is a significant limitation of this method. The premix is then pulled into a chamber and forced at an extremely high pressure (over 1000 bar) through a narrow valve (as in a valve homogenizer) or is split into two streams that go through separate micro-channels and subsequently collide with each other at very high velocities, producing droplet shear (as in Microfluidizer® devices). This process is very energy-intensive [10]. In addition, high-pressure homogenization utilizes expensive, large-footprint equipment that requires frequent and costly maintenance, is difficult to clean and service, and needs major redesign in order to enable aseptic processing [9,14].

Presently, high-amplitude ultrasound-based techniques for producing pharmaceutical, food and cosmetics nanoemulsions are being actively developed as an alternative to high-pressure homogenization [9,14–17]. Intense shear forces necessary for nanoemulsification are generated by high-amplitude ultrasound through the associated effect of acoustic cavitation, which produces violently and asymmetrically imploping bubbles and causes micro-jets that impinge one liquid into the other in the form of nano-droplets. This effect has been extensively studied and reviewed [14,15,18–20], and demonstrated to be effective for small-scale preparations of pharmaceutical nanoemulsions [16,17].

It has been shown that high ultrasonic amplitudes, at least 80 μm peak-to-peak (μpp) [21–24], are required for efficient particle size reduction.

1.2. Scale-up limitations of conventional ultrasonic technology

Despite its potential, the high-amplitude ultrasonic method for producing nanoemulsions has been restricted to the laboratory scale. As explained below, when conventional ultrasonic liquid processors are scaled-up, their ultrasonic amplitudes are always reduced to levels insufficient for producing most types of pharmaceutical nanoemulsions [22,24]. Ultrasonic transducers, which convert the electrical energy supplied by an ultrasonic generator into mechanical vibrations, provide low displacement amplitudes: 20–25 μm peak-to-peak (μpp). In order to amplify the amplitudes and deliver the ultrasonic energy to the processed liquids, high-gain ultrasonic horns (sonotrodes) are used. Conventional ultrasonic horns (CH), however, do not allow independent design of amplitude amplification and output surface area. Only small-surface, large-amplification or large-surface, zero or negative-amplification horns are possible. The use of conventional high-amplitude ultrasonic processors is, therefore, limited to small-scale investigations, for which horn tip diameters of 10–20 mm are sufficient (Fig. 1a) [25,26]. Since industrial-scale ultrasonic processors require large-diameter horns, they are forced to run at low amplitudes and cannot produce high cavitation intensities.

High-power industrial-scale ultrasonic processors incorporating conventional sonotrodes (horns) are available [27]. However, these industrial processors cannot generate ultrasonic amplitudes above about 20 μpp. As a result, high power levels can only be attained by utilizing very large sonotrodes, which provide low power densities (due to low amplitudes), but high total powers (due to large radiating surfaces). It is important to point out that it is the amplitude of ultrasound, not the total power of the processor that determines its usefulness for many physical or chemical processes. For the production of high-quality nanoemulsions, the ultrasonic amplitude must be at least 80 μpp [21–24].

In order to successfully transfer a process from the laboratory to a production environment, one must maintain all processing parameters (amplitude, temperature, etc.) at the same level. Only if the amplitude can be maintained should the horn size (along with the corresponding power and productivity rate of the processor) be increased. The inability to scale up without sacrificing ultrasonic amplitudes has, therefore, been the most important limitation of conventional ultrasonic technology and the reason why it has not been able to compete with high-pressure homogenization in the industries requiring high-quality nanoemulsions.

1.3. High-amplitude processor scale-up with Barbell Horn Ultrasonic Technology

The scale-up limitation of conventional ultrasound has been successfully overcome by Barbell Horn Ultrasonic Technology (BHUT), which enables horn designs where amplification and output surface are independent variables, and permits constructing large-scale industrial processors that operate at high amplitudes [28–31]. BHUT-based processors have been used for the production of nanocrystals, liposomes and nanoemulsions, yielding high-quality products [22–24].

Fig. 1b illustrates a Half-wave Barbell Horn (HBH) having an output tip with the diameter of about 50 mm and two radiating surfaces that can generate ultrasonic amplitudes over 100 μpp. When a process is scaled-up by switching from a CH to a HBH-type horn, the processing capacity increases by a factor of about \(2(D_{\text{hbh}}/D_{\text{ch}})^3\), where \(D_{\text{ch}}\) and \(D_{\text{hbh}}\) are the respective output tip diameters of the two horns [22]. This relationship is based on the approximate
The ultrasonic horns corresponding to each processor were made from Ti6Al4V alloy and operated at the frequency of about 20 kHz. The ultrasonic amplitude in all studies was maintained at 90 μpp (except three laboratory experiments conducted at 20 μpp). The amplitude was confirmed by a high-speed photonic sensor (kd-300, MTI Instruments, Albany, NY). Power output levels during laboratory, bench and industrial scale experiments (at 90 μpp) were measured to be 79 W, 850 W and 2200 W, respectively.

For the preparation of squalene-based nanoemulsions, high-purity squalene oil, Tween 80 and Span 85 purchased from Sigma–Aldrich (St. Louis, MO) were used. The formulation was identical to that of the commercially produced MF59® nanoemulsion used for vaccine preparations: squalene oil (4.3%), Tween 80 (0.5%), Span 85 (0.5%), water (94.7%), 10 mM Na-citrate buffer. All components were combined and vigorously stirred together in the premix tank for 30 min, with no additional pre-processing. Particle size parameters such as the mean droplet size (MDS) and particle size distribution (PSD) were measured by dynamic light scattering (DLS) using Beckman Coulter N4 Plus and laser diffraction (LD) using Horiba LA-950, respectively. All measurements of physical parameters including MDS, PSD and metal content were performed at certified laboratories.

3. Results and discussion

The results obtained for unfiltered MF59®-analog nanoemulsions are summarized in Fig. 3, showing the relationship between the processing rate and the MDS values. DLS was employed during process optimization steps since it is a fast and relatively economical method frequently chosen for this purpose. LD was used for final product evaluation and provided a significantly greater level of detail. The target MDS (determined by DLS) was chosen to be 250 nm. As shown below, this value corresponded to the D50 (median particle diameter for a volume distribution) of approximately 150 nm (determined by LD), which, according to our measurements and the literature data, is the D50 value of the commercial MF59® adjuvant [32] (see Table 1). Targeting this value permitted manufacturing the nanoemulsions at a relatively high rate, while ensuring that the product could be post-processed by filtration without significant losses in the dispersed phase [33].

As can be seen in Fig. 3, the target MDS was possible to obtain at all three scales, laboratory, bench and industrial. Plots of these data, presented in Fig. 3, have been used to approximate the
optimum processing rates corresponding to the target MDS at each scale (intersection with the vertical line at MDS = 250 nm). Direct scale-up of the nanoemulsion manufacture process has, therefore, been achieved, with the productivity increase by a factor of about 11 from laboratory to bench scale, and by another factor of about 5 from bench to industrial scale. The overall scale-up factor achieved by utilizing BHUT instead of conventional ultrasonic technology was, therefore, about 55. The ultrasonic amplitude and the resulting product quality were maintained identical at all three scales.

The nanoemulsion produced at 2.5 L/min using the ISP-3000 processor was passed through a 220 nm Cameo syringe filter (Sigma–Aldrich, St. Louis, MO), which did not affect the MDS. Dispersed material concentrations in the unfiltered and filtered samples were estimated by Static Light Scattering using a spectrophotometer (Perkin Elmer, LS-50) working in the light scattering mode (both excitation and emission wavelengths were 400 nm). The obtained results showed an approximate 8% loss of the dispersed phase due to filtration.

The effect of decreasing the ultrasonic amplitude was, further, evaluated by comparing the results obtained at 90 μpp and 20 μpp for equivalent processing rates. These experiments were carried out with the laboratory-scale processor, LSP-500. The results are presented in Fig. 4, showing a significant increase in the MDS values for the nanoemulsions obtained at the lower amplitude. The figure also demonstrates that even at dramatically reduced processing rates, the target MDS value cannot be achieved at this amplitude. Ultrasonic amplitudes, therefore, play a crucial role in the process of preparing high-quality nanoemulsions, and the ability of BHUT to scale up horn dimensions without having to reduce the amplitudes is essential for the industrial implementation of this process.

As with all high-shear techniques [34], one potential area of concern in our experiments was possible contamination of the nanoemulsion by metal particles during sonication due to cavitation erosion [35]. Metal contamination was evaluated by ICP-MS (Agilent 7500 ICP-MS analyzer), showing the total metal content of 37 ppm. Laser diffraction particle size distribution analysis, showed that the diameters of all metal particles were above 1 μm, centered at about 4.5 μm. Passing the product through a 220 nm filter reduced the metal contamination down to the background level.

Currently, the MF59® adjuvant is manufactured in 50 L batches using industrial Microfluidizer M7250 processors at the rate of 1.5 L/min per pass with a total 5 passes required [32], resulting in the final productivity of about 300 ml/min. The maximum productivity achieved with our industrial processor, ISP-3000, was approximately 2.5 L/min: about 8 times faster. In addition, the process was carried out in a continuous, single-pass mode, which is significantly more convenient than the multi-pass approach employed by the Microfluidizer.

Table 1 compares the performance of ISP-3000 and Microfluidizer M7250 systems by providing their MF-59®-analog nanoemulsion production rates and energy ratings. ISP-3000 is shown to be advantageous in terms of productivity and energy efficiency.

4. Conclusions

In this study we have shown the feasibility of using BHUT for the commercial-scale production of MF59®-analog nanoemulsion. To our knowledge, this work provides the first reported instance of a successful and systematic scale-up of any high-amplitude
ultrasonic process. BHUT-based ISP-3000 ultrasonic liquid processor provided the overall productivity scale-up factor of about 55 with respect to conventional high-amplitude ultrasonic technology and about 8 with respect to the industrial Microfluidizer M7250. Further optimization of the process and equipment is currently underway. Significant advantages of BHUT-based processors in terms of productivity, convenience, energy efficiency, sample preparation, footprint and equipment costs support their significant commercial potential in the field of pharmaceutical nanoemulsion production.

References