Sterile Filtration of a Parenteral Emulsion

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The Syntex adjuvant formulation (SAF) containing [thr[1]]-muramyldipeptide in an oil-in-water emulsion has proven to be an effective adjuvant eliciting both cell-mediated and humoral immune response. As a parenteral emulsion, sterility of the final product was a concern, and various methods of achieving sterility were considered. For emulsions, most conventional sterilization methods are not viable, requiring the more cumbersome technique of sterilizing individual components and assembling/manufacturing under sterile conditions. Emulsion vehicles were manufactured with various models in the Microfluidizer M110 series. All equipment examined was capable of reducing the average dispersed oil droplet size to approximately 160 nm, with varying size ranges. Operating at an internal equipment pressure of greater than 16,000 psi, with at least five cycles through the interaction chamber, the resulting emulsion had a narrow droplet size range distribution, with the largest droplets being small enough to enable sterile filtration. Under specific-manufacturing conditions, the adjuvant emulsion becomes easily filtered through a 0.22-μm cartridge filter, thus yielding a sterile end product. This is the first published example of emulsion sterilization being achieved by terminal filtration.

KEY WORDS: emulsion filtration; Microfluidizer; adjuvant; emulsion stability; emulsion processing; emulsion manufacture.

INTRODUCTION

The Syntex adjuvant formulation (SAF) was developed containing a muramyldipeptide analogue ([thr[1]]-MDP; referred to as temurtide) in an oil-in-water emulsion vehicle (1). This emulsion vehicle contains squalane (with a density of 0.811) as the internal oil phase, poloxamer 401 [Pluronic L121; with a hydrophile–lipophile balance (HLB) of 1.0], and polysorbate 80 (Tween 80, with an HLB of 15.0) as cosurfactants in a phosphate-buffered saline aqueous external phase. Pluronic L121 is a nonionic block copolymer of polyoxyethylene and polyoxypropylene which has been shown to possess adjuvant activity (2–4). Combined with [thr[1]]-MDP, this particular emulsion elicits both a cell-mediated and a humoral immune response, with a higher and more consistent response than other adjuvants. The Syntex adjuvant formulation has been included in many studies comparing various adjuvants with a variety of antigens; such studies include vaccines for cancer immunotherapy (5,6), hepatitis B (7); Epstein–Barr virus (8), and SIV (9). Because of its encouraging immune stimulating activity, SAF can improve vaccines and broaden their use in human populations.

Various manufacturing methods have been examined in an effort to develop the emulsion vehicle into a commercially stable product. Processes involving high-velocity fluid stream impaction combined with shear and cavitational forces produced very stable emulsions and resulted in manufacturing studies employing equipment necessary for this particular emulsification technique (10,11). Oil-in-water dispersions prepared in this manner exhibited an average particle size of approximately 160 nm. These emulsions proved to be more stable than the same formulation manufactured via mixing methods utilizing only one or two of the mechanical forces mentioned above. Centrifugation, utilized as a technique for accelerated physical stability testing, showed the emulsion to be particularly robust, demonstrating comparatively little creaming, with no coalescence of the oil phase (11). The ability to promote emulsion stabilization is likely due to a greater efficiency of dispersing the oil phase into small droplets, with a concomitant increased inclusion of poloxamer at the oil–water interface (10,11). These factors would contribute to emulsion stability by producing uniformity in droplet size and providing a mechanical barrier to coalescence.

Manufacture of a sterile emulsion can be quite tedious and costly. Conventional sterilization techniques such as temperature, radiation, and filtration are not necessarily viable methods for an emulsion because of thermal instability, production of free radicals promoting subsequent excipient degradation, and large droplets clogging pores of the filter membrane. The most practical alternative for ensuring sterility during emulsion manufacture is an aseptic process whereby each excipient is sterilized and the emulsion is assembled and mixed with sterile equipment under sterile conditions (12,13). This process, however, is least preferable for manufacture of a sterile product because the numerous steps involved provide a greater risk of possible contamination.

Operating pressures used during the manufacture of SAF emulsion played a key role in the product's particle size distribution. Although the average particle size was 160 nm, only those emulsions processed at greater than 16,000 psi could pass through a 0.22-μm (220-nm) sterilizing filter. At pressures below 16,000 psi, only minimal amounts of emulsion would pass through this type of filter. This observation suggests the presence of dispersed droplets larger than 220 nm, which rapidly clog the filter pores.

The purpose of this study is to examine factors involved in the manufacture of SAF emulsion which result in a distribution where all dispersed oil droplets are less than 220 nm, thereby facilitating sterile filtration of the parenteral emulsion.

MATERIALS AND METHODS

Reagents. All emulsion excipients were used without further processing or purification. Each excipient was received as follows: squalane, NF, Robeco Chemicals; poloxamer 401 (Pluronic L121), BASF Wyandotte Corporation; and polysorbate 80 (Tween 80), USP, Mazer Chemicals, or ICI Americas.

Emulsion Preparation. Each adjuvant vehicle was prepared with 10% squalane, 5% poloxamer 401, and 0.4% polysorbate 80 in an isotonic phosphate-buffered saline

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(PBS), pH 7.4 (0.736% sodium chloride, 0.0184% potassium chloride, 0.0184% potassium phosphate monobasic, 0.11% sodium phosphate dibasic, anhydrous). All components of the PBS were dissolved in sterile water for injection, and polysorbate 80 was then added and thoroughly mixed. Poloxamer 401 was added and allowed to hydrate in the aqueous environment; and finally, squalane was added. Initial mixing of the components was performed with agitation, either with a stir bar at high revolutions per minute or with a blade mixer. Final dispersions were prepared using a Microfluidizer M110T or M110Y (Microfluidics Corp., Newton, MA). These instruments differ in terms of their maximum achievable operating pressures; the M110T can achieve 13,000 psi, and the larger pump on the M110Y allows pressure up to 23,000 psi. For cleaning and sanitizing prior to emulsion manufacture, the Microfluidizers were thoroughly rinsed with 2–5 liters of ethanol followed by 5–15 liters of sterile water for injection. Emulsion was then cycled through the Microfluidizer, and aliquots were collected after each cycle for particle size analysis.

Because of the potential temperature increase during manufacture, the emulsification equipment was packed in ice, and processed emulsion was collected in a container surrounded by ice. Previous studies showed that uncontrolled heating during manufacture created a tendency for poloxamer aggregation and increased creaming of the oil droplets. Cooling this emulsion is critical for small, uniform droplet formation.

Filtration. Various filters were used dependent on the volume of emulsion to be filtered. For initial filtration feasibility, small emulsion aliquots were tested on Millex GV (Millipore, Bedford, MA) 0.22-μm membranes. Larger-scale filtration, for 2 to 8 liters of emulsion, employed a Millipak 60, Millipak 200, or Durapore CVGL01TP3, 0.22-μm cartridge filter (Millipore, Bedford, MA).

Sterility Testing. Sterility testing was performed by the QC Microbiology group of either Syntex or Wyeth-Ayerst. The direct method of sterility testing was performed according to USP guidelines. Test medium was composed of (i) fluid thioglycollate medium and (ii) soybean casein digest broth. Additionally, the medium contained 2% Tween 80 and 0.01% neutralizing buffer (Difco).

Particle Size Analysis. Droplet/particle size was determined by laser photon correlation spectroscopy. The instrument used for this analysis was a Nicomp laser particle sizer (Model 200) with a Nicomp computing autocorrelator (Model TC-100, Pacific Scientific, Silver Spring, MD). All samples were diluted in the emulsion's continuous (aqueous) phase prior to analysis.

Component Analysis. Excipient (squalane, poloxamer, and polysorbate) concentrations were analyzed pre- and postfiltration via GC and colorometric assay with cobalt thiocyanate active substance (CTAS).

RESULTS AND DISCUSSION

The Microfluidizer works by a combination of forces to disperse and reduce the droplet size of an emulsion's internal phase effectively. The interaction chamber of the Microfluidizer splits the incoming pressurized emulsion into two streams; at the center of the interaction chamber, the two streams collide at a high velocity, creating shear and impact forces; additionally, a large pressure drop occurs, creating cavitational force.

SAF emulsions were prepared comparing pressure differences between two Microfluidizer models. A working pressure of 8000–10,000 psi was used with the M110T, and a working pressure of 16,000–17,000 psi was used for the M110Y. After seven passes at the higher pressure, small batches of the resultant emulsion easily filtered through a Millex 0.22-μm membrane; evaluation of other aliquots collected showed that quantitative filtration did not occur at lower operating pressures or until the emulsion had completed five processing cycles at 16,000–17,000 psi. To assess the effect of manufacturing scale, a 2-liter batch of emulsion was prepared via six passes at 16,000–17,000 psi. The ability to filter this larger batch was tested with a Millipak 60 cartridge filter using positive nitrogen pressure. The entire emulsion volume easily filtered through the cartridge. Subsequent batches (5 liters) were prepared and successfully filtered through a Millipak 200 cartridge filter. All preparations were white fluid oil-in-water emulsions with a distinct bluish hue, indicating a submicron particle size. However, sterile filtration could be accomplished only when the emulsion was prepared at the higher working pressure. Physical properties of emulsions were not adversely affected by filtration, with no sign of phase separation after prolonged storage (12 months) at either 2–8°C or ambient room temperature. Furthermore, component analysis showed 95–100% recovery, indicating that filtration did not remove excipients from the emulsion.

Aliquots for particle size analysis were taken from emulsions prepared at 16,000–17,000 psi; small aliquots (about 70 ml) were collected after one, two, three, four, five, seven, and eight passes through the equipment. Emulsion particle sizes were determined via photon correlation spectroscopy. The results are listed in Table I. The average particle sizes for emulsions prepared at either of the two pressures were essentially equivalent. From the data in Table I, it appears that a small particle size is reached very quickly; after two passes at 16,000–17,000 psi, there was no further reduction of average particle size. To reach this end point at 8000–10,000 psi, four passes were required. Particle size data for

Table I. Prefiltration Particle Size Analysis Comparing SAF Emulsion Manufactured at Two Operating Pressures

	SAF emulsion manufactured at			
	16,000–17,000 psi		8000–10,000 psi	
No. of processing cycles	Mean size (nm)	Size range (nm)	Mean size (nm)	Size range (nm)
1	187	115–1000	228	110–1000
2	157	81–405	185	124–1000
3	—	—	164	78–728
4	164	118–315	157	84–756
5	164	90–308	150	90–450
7	165	81–270	—	—
7 filtered	163	37–189	—	—
10	—	—	151	77–390

emulsion produced after seven cycles, which had been sterile filtered, are also presented in Table I. This aliquot was easily and readily sterile filtered. The average particle size of the post-filtration emulsion remained equivalent to that of the prefiltration emulsion sample; the size distribution, however, showed decreased particle sizes at the high and low ends of the range.

The ability to sterile filter emulsions prepared at the higher operating pressure may be explained by comparison of droplet size ranges. Size range analysis shows that the population of larger particles was reduced by processing at 16,000–17,000 psi, with maximum particle size falling into the 0.2- to 0.3-μm range. This higher pressure, however, did not further reduce the average droplet size: 160 nm appeared to be the lower limit of average droplet size; this limit is likely due to formulation constraints. Droplet size ranges measured for emulsions prepared after several passes at 8000–10,000 psi indicated that large droplets were not completely dispersed even after 10 process cycles. These larger particles, even though they may be few in number, are very likely responsible for quickly clogging a 0.22-μm filter membrane, thus preventing sterile filtration. As these larger particles were reduced in size via the higher energy (higher pressure), sterile filtration became possible.

Bubble-point testing of the Millipore cartridge filters was performed as a check on filter integrity. Two methods of testing were performed. The first method was standard bubble-point testing with product. This method gave a reduced bubble point which is believed to be product specific (i.e., presence of squalane); validation testing of this method with *P. diminuta* is ongoing. The reduced bubble point seen with testing of this specific product leads to an alternate method as suggested by the manufacturer. After emulsion filtration, the filter cartridge was washed with 60% isopropanol/40% water; the bubble point was tested with this solution. The cartridge filters tested in this manner passed the bubble-point specification of ≥17 psi (14). Filtered batches of emulsion were tested for sterility as per FDA guidelines. All batches manufactured (seven total) showed no sign of microbial contamination and passed sterility testing as per FDA CFR:21. These results suggest that a sterile final product can be achieved for emulsions without the need for presterilization of formulation components and subsequent aseptic compounding.

Because of the high-energy mixing forces, heat transfer to the product resulted in a temperature rise and proved detrimental to emulsion integrity. An uncontrolled temperature rise during emulsion manufacture would allow the emulsion to achieve temperatures in excess of 60°C; at this temperature, squalane coalescence occurred. At temperatures above 40°C, SAF emulsion could not be filtered through a 0.22-μm membrane. Maintenance of an acceptable temperature range (<30°C) was achieved by surrounding the equipment's processing interaction chamber with ice and passing the emulsion through a cooling coil also surrounded with ice. For emulsions in general, though, increased temperature may enhance dispersion and reduction of particle size (15); the effect of temperature on the emulsion will be influenced by the excipients chosen for the specific formulation.

Because of the inherent thermodynamic instability of emulsions, the tendency to cream and coalesce is still present for SAF emulsion processed by high pressure emulsification. Although SAF emulsion droplets could be sufficiently reduced in size to allow sterile filtration, the effect could be only temporarily exploited. After 24 hr, the droplet size of SAF tended to increase, and sterile filtration was no longer possible due to filter clogging. This initial increase in droplet size distribution did not affect long-term stability; sterile-filtered SAF emulsion has been found to have at least 12 months of physical stability, with no sign of coalescence or phase separation.

Sterile filtration of an emulsion provides many advantages:

(1) the ability to manufacture with a less cumbersome procedure than the technique of sterilizing components separately with subsequent aseptic compounding and processing;
(2) a greater assurance of sterility by reducing the risk of contamination and removing microbes at the final step of the process;
(3) a greater applicability to GMP requirements (equipment which is sterilizable and a process which is readily validated); and
(4) the ability to validate the filtration step rather than the whole process.

In conclusion, droplet diameter and size distribution reductions achieved with a combination of mechanical forces (shear, impact, and cavitation) at high pressure provide the first published example of a macroemulsion which can be sterilized by terminal filtration. Such a capability may provide a major incentive toward the more widespread use of oil-in-water emulsions as vehicles for parenteral drug delivery.

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