RESEARCH ARTICLE

AF03, An Alternative Squalene Emulsion-Based Vaccine Adjuvant Prepared by a Phase Inversion Temperature Method

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ABSTRACT: AF03 is a squalene-based emulsion adjuvant that is present in the adjuvanted pandemic influenza vaccine, Humenza™. In this report, we describe the design and development of this novel adjuvant formulation from the selection of the oil and surfactant system used in the adjuvant composition to the phase inversion temperature emulsification process that afforded AF03 as a long-term stable and well calibrated oil-in-water emulsion. The emulsion was characterized by its particle sizes, surface and interfacial tensions, viscosity, and long-term stability. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: vaccines; vaccine adjuvant; emulsion; squalene; surfactants; AF03; PIT; stability; Humenza®

INTRODUCTION

Vaccine adjuvants have become increasingly important to compensate for the reduced immunogenicity of purer vaccine preparations based on purified subunit, recombinant, or synthetic antigens.[1,2] A large variety of substances, which are very diverse in nature, structure, or composition, have been shown to have adjuvant activities in laboratory animals but many failed to progress in clinical development because of lack of efficacy or concerns about safety in humans.[3–5] Adjuvants that successfully passed clinical development and are now present in marketed prophylactic vaccines comprise aluminum oxyhydroxide and aluminum hydroxyphosphate, liposomes, a detoxified lipopolysaccharide, monophosphoryl lipid A, and squalene-in-water emulsions.[5,6]

The value of squalene emulsion adjuvants has been demonstrated through the development and European licensing of several pandemic and prepandemic influenza vaccines containing a squalene emulsion adjuvant. The licensing of these vaccines was supported by a number of clinical trials wherein the squalene-in-water emulsions outperformed aluminum salts at increasing vaccine immunogenicity, affording antigen dose sparing and influenza cross-strain neutralization without causing unacceptable adverse reactions.[7–9] Among them, Humenza™ (Sanofi Pasteur, Lyon, France) comprises a standard split virion vaccine, prepared from the pandemic influenza H1N1*v* strain, combined with a novel squalene-in-water emulsion, termed AF03. Although never marketed, Humenza™ (Sanofi Pasteur) has been injected into hundreds of human volunteers during its clinical development where it displayed remarkable efficacy owing to its AF03 adjuvant component.[10] Unlike MF59[11] and AS03,[12] the two microfluidized squalene-in-water emulsions found in marketed influenza vaccines from, respectively, Novartis Vaccines & Diagnostics (Cambridge, MA) and GSK Biologicals (Rixensart, Belgium), AF03 is manufactured up to industrial scale by using a phase inversion temperature (PIT) emulsification process.[13,14]

Here, we describe the selection and optimization of formulation and manufacturing process as to reproducibly obtain AF03 as a stable and well calibrated squalene-in-water emulsion, with a small particle size and a narrow size distribution through a simple and easy-to-scale PIT emulsification process. We also describe main physicochemical characteristics of the final AF03 emulsion adjuvant.

MATERIALS AND METHODS

Research and Good Manufacturing Practices (GMP)-grade squalene from shark liver oil (>98% purity) and

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Table 1. Surfactants used in PIT Emulsification Studies

Commercial Name	Chemical Name	HLB	Supplier
Eumulgin™ B1 (or Cetereth-12)	Polyoxyethylene[12] cetyl-stearylether ($C_{16–18}$)	13	Cognis
Eumulgin™ B2 (or Cetereth-20)	Polyoxyethylene[20] cetyl-stearylether ($C_{16–18}$)	15	Cognis
Eumulgin™ S10	Polyoxyethylene[10] stearylether (C_{18})	12.4	Cognis
Eumulgin™ S21 (or Steareth-21)	Polyoxyethylene[21] stearylether (C_{18})	15	Cognis
Simulsol™ 58 PHA	Polyoxyethylene cetyl-stearylether ($C_{16–18}$)	16	SEPPIC
Simulsol™ P4 PHA	Polyoxyethylene laurylether (C_{12})	10	SEPPIC
Montane™ 80 PPI	Sorbitan oleate	4.3	SEPPIC
Dehymuls™ SMO	Sorbitan oleate	4.3	Cognis

surfactants from the Brij™ series (Brij56, Brij58, and Brij76) and Span™ series (Span65, Span80, Span83, and Span85) were purchased from SAFC (Lyon, France). Research-grade polyoxyethylene ether surfactants from vegetable origin (Table 1) were obtained from Cognis (Dusseldorf, Germany) and from SEPPIC (Castres, France). GMP-grade polyoxyethylene[12] cetyl-stearyl ether (Eumulgin™ B1-PH) was purchased from Cognis, and GMP-grade sorbitan oleate (Montane™ 80 PPI) from SEPPIC. Mannitol was purchased from Roquette Frères (Lestrem, France). Phosphate buffered saline (PBS; 7.5 mM Phosphate, 150 mM NaCl; pH 7.2), Tris buffered saline (TBS; 50 mM TRIS, 150 mM NaCl; pH 7.2), and citrate buffered saline (CBS; 10 mM Citrate, 150 mM NaCl; pH 6.5) were produced by Sanofi Pasteur.

EMULSION PREPARATION

Emulsions were prepared in two steps. In a first step, a crude oil-in-water (O/W) emulsion was prepared by mixing under stirring (1000 rpm for 10 min) at approximately 30°C an aqueous phase comprising a hydrophilic (high hydrophilic-lipophilic balance (HLB)) surfactant and optionally mannitol with an oil phase comprising a hydrophobic (low HLB) surfactant in squalene by using a laboratory reactor (IKA Eurostar; IKA, Staufen, Germany) equipped with a helix impeller, a thermometer, and a conductivity probe (Tacussel CD60; Radiometer Analytical, Villeurbanne, France). In a second step, the crude O/W emulsion was processed by the PIT method. First, it was heated under moderate stirring (~400 rpm) until conductivity dropped to zero, indicating that the O/W emulsion had turned into a water-in-oil (W/O) emulsion. Then, the heating was turned off, and the emulsion was cooled to room temperature. Upon cooling, the conductivity returned back to its maximal value, indicating that the W/O emulsion had inverted back into an O/W emulsion. For each of the surfactant and aqueous phase composition studied, the PIT, the final emulsion aspect, and particle sizes were carefully recorded.

MANUFACTURE OF AF03 AT PILOT SCALE

AF03 was prepared by using the PIT process as a concentrated O/W emulsion at a 2 kg scale and then diluted with PBS to its final concentration. In brief, the aqueous phase was prepared by dissolving 123.6 g of Eumulgin™ B1-PH (Cognis) into 1010 g of PBS containing 120 g of mannitol under stirring at 40°C. The oil phase was prepared by dissolving 96.4 g of sorbitan oleate into 650 g of squalene under stirring at room temperature. The two phases were then introduced into a stainless-steel-jacketed reactor equipped with a hydrofoil impeller (AGM-TC′LAB 50; Agitec, Montanay, France), a thermometer, and a conductivity probe (InPro 7100-25; Mettler Toledo, Viroflay, France) and emulsified by high-speed stirring at 1000 rpm (high shear) for approximately 4 min under nitrogen. Then, the emulsion was processed as described above by the PIT process with stirring at 200 rpm (low shear) to form a concentrated bulk emulsion. The process was monitored by in-line conductivity measurement on a CR7350 conductometer (Mettler Toledo). The concentrated bulk emulsion, containing 32.5% (w/w) of squalene, 11% total surfactant, 6% mannitol, and 50.5% PBS, was then diluted with PBS to a final squalene concentration of 5% or 3.3%, depending on the intended vaccine application. The diluted emulsion was sterile filtered through a 0.2 μm polyethersulfone membrane (Millipore express SHC; Merck Millipore, Molsheim, France) at a constant flow rate of 1 g/min/cm^2. Finally, the sterile emulsion was filled under nitrogen into amber glass vials for long-term storage.

PARTICLE SIZING

The emulsion particle sizing was performed by laser light diffraction (static light scattering) by using a Coulter LS230 (Beckman Coulter, Marseille, France) or a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). The equipments calculate sizes (20 nm–2 mm range) of particles in suspension from light scattering patterns obtained by recording the intensity of the light scattered by the particles at different angles (large, medium, and small angles) upon illumination of the sample by a laser beam. The Mie