Journal of Pharmaceutical Sciences xxx (2016) 1-11

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

# Accelerating Vaccine Formulation Development Using Design of Experiment Stability Studies

Patrick L. Ahl [1, 2, *], Christopher Mensch [1, 2], Binghua Hu [1, 2], Heidi Pixley [1, 2], Lan Zhang [2, 3], Lance Dieter [1, 2], Ryann Russell [1, 2], William J. Smith [1, 2], Craig Przysiecki [2, 3], Mike Kosinski [1, 2], Jeffrey T. Blue [1, 2]

[1] *Vaccine Bioprocess Research and Development, Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania 19486*
[2] *Merck Research Laboratories, Merck & Co., Inc., Kenilworth, New Jersey 07033*
[3] *Infectious Diseases and Vaccines Discovery, Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania 19486*

ARTICLE INFO

*Article history:*
Received 12 April 2016
Revised 20 May 2016
Accepted 9 June 2016

*Keywords:*
analytical biochemistry
biotechnology
stability
vaccines
vaccine adjuvants
excipients

ABSTRACT

Vaccine drug product thermal stability often depends on formulation input factors and how they interact. Scientific understanding and professional experience typically allows vaccine formulators to accurately predict the thermal stability output based on formulation input factors such as pH, ionic strength, and excipients. Thermal stability predictions, however, are not enough for regulators. Stability claims must be supported by experimental data. The Quality by Design approach of Design of Experiment (DoE) is well suited to describe formulation outputs such as thermal stability in terms of formulation input factors. A DoE approach particularly at elevated temperatures that induce accelerated degradation can provide empirical understanding of how vaccine formulation input factors and interactions affect vaccine stability output performance. This is possible even when clear scientific understanding of particular formulation stability mechanisms are lacking. A DoE approach was used in an accelerated 37°C stability study of an aluminum adjuvant *Neisseria meningitidis* serogroup B vaccine. Formulation stability differences were identified after only 15 days into the study. We believe this study demonstrates the power of combining DoE methodology with accelerated stress stability studies to accelerate and improve vaccine formulation development programs particularly during the preformulation stage.


## Introduction

Successful vaccine development requires understanding of public health needs, disease pathology, and immunology. The overall benefits of vaccination are undeniable, but vaccine development is difficult, time consuming, and costly.[1] Vaccine developers are continuously searching for ways to accelerate vaccine development. Identification of appropriate antigens and adjuvants using animal studies is very time consuming and is often misleading.[2] Automated high-throughput procedures are now being used to rapidly screen the immune responses of antigen and adjuvant.[3] Acceleration of vaccine drug product formulation development is another urgent need within vaccine development. Numerous vaccine formulations must be screened to identify the formulation which provides the optimal efficacy, safety, and stability. Often, it is the formulation choices that are most important in determining vaccine drug product stability. Vaccine developers have automated several bioanalytical methods to monitor the stability of vaccine formulations.[4,5]

Successful optimization of vaccine formulation stability must typically overcome multiple challenges particularly in the early phases of development. These challenges often include (1) short formulation development timelines, (2) limited supplies of critical antigens and adjuvant, and (3) a wide range of available formulation conditions that should be examined. We have used a 2 formulation development concepts to overcome these 3 challenges. The short timeline challenge was overcome by conducting relatively short "stress testing" stability studies at a temperature

*Abbreviations used:* AAHS, amorphous aluminum hydroxyphosphate sulfate adjuvant; $AlPO_4$, aluminum hydroxyphosphate adjuvant; DoE, Design of Experiment; DSC, differential scanning calorimetry; ELISA, enzyme-linked immunosorbent assay; fHbpv1, factor H binding protein version 1; LOS, lipooligosaccharide; MenB, *N. meningitidis* serogroup B; 4-MUP, 4-methylumbelliferyl phosphate; NadA, *Neisseria* adhesion A; OMV, outer membrane vesicles; PBS, phosphate-buffered saline; $T_m$, thermal transition temperature midpoint; TRIS, Trizma base; WFI, sterile water for injection; WRAIR, Walter Reed Army Institute of Research.

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2016.06.014.

* *Correspondence to:* Patrick L. Ahl (Telephone: 215-652-5534; Fax: 215-652-5299).
*E-mail address:* patrick_ahl@merck.com (P.L. Ahl).

http://dx.doi.org/10.1016/j.xphs.2016.06.014
0022-3549/

slightly above the targeted storage conditions.[6] We found that a short-term stress stability study, that is, an accelerated stability study, can more quickly provide valuable formulation information. Challenges 2 and 3 can be overcome by the use of half-factorial or quarter-factorial Design of Experiment (DoE) designs with small sample quantities in early formulation development studies.[7,8] In this particular study, however, we used a full-factorial design. We found that a carefully planned DoE stability study can be used to build useful statistically significant mathematical models of formulation responses to key formulation input factors. A formulation screen of *N. meningitidis* serogroup B (MenB) outer membrane vesicles (OMVs) vaccines is used as an example in this report to demonstrate the advantage of the accelerated thermal stress DoE stability study approach for formulation development.

Bacterial meningitidis caused by *N. meningitidis* is a devastating disease that can kill patients within days of infection despite the use of modern antibiotics. Currently, 2 MenB vaccines are on the market. The GSK MenB vaccine Bexsero® is approved in Europe, Canada, Australia, and United States, whereas the Pfizer MenB vaccine Trumenba® is approved in the United States.[9,10] *N. meningitidis* is coated by a protein and lipooligosaccharide (LOS)-rich outer membrane which can bud off from the bacteria.[11] Several methods are available to extract and purify OMVs from the bacteria and OMV isolated from the bacteria are 100-200 nm in diameter vesicles.[12,13]

Endemic protection to *N. meningitidis* serogroup B invasive disease is possible by using 2 relatively conserved OMV membrane associated proteins: (1) Neisseria adhesion A (NadA) and (2) factor H binding protein version 1 (fHbpv1).[14]

NadA is a major OMV surface antigen that appears to be important for *N. meningitidis* binding to host epithelial cells.

The OMV antigen fHbpv1 is not embedded in the membrane bilayer. The molecule is anchored in the membrane by single fatty acid attached at the lipidated N-terminus.[15] Recently, researchers at the Walter Reed Army Institute of Research developed a vaccine that contained 3 MenB OMVs from antigenically diverse strains of the bacteria.[16,17] The LOS toxicity of the 3 OMVs was significantly reduced by disabling genes in all 3 OMV strains that were involved in the synthesis of LOS.[16] Vaccines prepared with these 3 particular OMV sources have been clinically evaluated.[18] Additional genetic modifications were performed at Merck with these 3 MenB strains to further increase the expression of NadA, fHbpv1, and other antigens to further optimize MenB strain protection.[19] The MenB vaccines containing these 3 distinct OMVs with enhanced expression of NadA and fHbpv1 have potential to be both safe and effective to provide broad protection against most MenB bacterial strains.

DoE is a key element of the Quality by Design approach recommended by the FDA to insure process understanding during pharmaceutical development.[20] DoE methods are routinely used in pharmaceutical manufacturing and during the pharmaceutical development of small molecules and devices. Currently, however, the DoE approach is not extensively used in vaccine formulation development with some exceptions.[21] The development of vaccine formulation is typically considered too complex for a simple DoE approach. In addition, vaccine stability studies done at 4°C often require months or years for formulation related stability differences to be statistically reliable. Fortunately, formulation parameters that control thermal stability can often be identified in days or weeks by higher temperature thermal stress studies at 37°C or higher.[22] DoE methodology combined with thermal stress stability studies below the antigen unfolding $T_m$ can often provide very predictive information about lower temperature formulation stability. Particularly, if the antigen degradation process basically follows Arrhenius kinetics, for example, simple protein unfolding. Thermal stress stability studies may not be as predictive for antigen degradation processes that are non-Arrhenius such as protein aggregation.[23] In any case, short-term high thermal stress DoE studies cannot unambiguously identify the optimally stable formulation for a vaccine. Longer stability studies at the targeted vaccine drug product storage temperatures, for example, 4°C, will be required for regulatory approval. However, thermal stress DoE stability studies can rapidly provide critical formulation information during early vaccine formulation development which can significantly reduce formulation development timelines.

This report will illustrate this approach to vaccine formulation development by showing how a 37°C DoE stress stability study of 17 aluminum adjuvant containing vaccine formulations for *N. meningitidis* serogroup B OMV was used to quickly determine which formulation input factors are most important for the MenB vaccine formulation thermal stability. Of course, other vaccine formulation stresses, for example, light, freezing, oxidation, and agitation, can also be examined by a DoE stability study approach. The temperature of 37°C was chosen because it is well below the differential scanning calorimetry (DSC) unfolding temperature of the antigens examined in the study. Although statistically significant stability effects were identified after only 15 days, the stability study was extended out to 77 days to confirm the 15-day stability trends. The following 4 formulation input factors were considered in this vaccine formulation DoE study: (1) pH, (2) aluminum adjuvant choice, (3) OMV to aluminum adjuvant (w/w) input ratio, and (4) added phosphate concentration. Two DoE formulation output responses were measured during the course of the 37°C study to access stability: (1) the normalized change in the NadA enzyme-linked immunosorbent assay (ELISA) response ($\Delta\%_{NadA}$) and (2) the normalized change in the fHbpv1 ELISA response ($\Delta\%_{fHbpv1}$). The ELISA response changes of these 2 major OMV protein antigens, NadA and fHbpv1, were particularly important because these assays correlated well with mouse *in vivo* NadA and fHbpv1 immunogenicity results from thermally stressed MenB OMV vaccines. Thus, we consider the NadA and fHbpv1 ELISA response assays as “stability indicating” for this particular vaccine. The MenB vaccine formulation DoE conclusions from the 37°C stress stability study were also found to be consistent with biophysical characterization of the antigens and results from a separate 11-month 4°C stability screen. This consistency demonstrates the value in the thermal stress DoE stability study approach for vaccine formulation development.

## Materials and Methods

### *Materials*

The *N. meningitidis* OMVs used in this study were supplied by the Merck Vaccine Bioprocess Department as described in the following. The amorphous aluminum hydroxyphosphate sulfate adjuvant (AAHS) was obtained from Merck Manufacturing, whereas the aluminum hydroxyphosphate adjuvant ($AlPO_4$) was supplied by the Merck Vaccine Formulation Department.[24] The monoclonal antibodies, streptavidin-conjugated alkaline phosphatase and 4-methylumbelliferyl phosphate (4-MUP) used in the NadA and fHbpv1 ELISA assays, were from the Merck Vaccine Analytical Department. Mouse serum measurements to determine anti-NadA, anti-fHbpv1, and anti-OMV serum antibodies were done coating ELISA plates with recombinant NadA, recombinant fHbpv1, and OMV, respectively. These reagents were obtained from Merck Vaccine Basic Research. The goat antimouse Ig AP—conjugated secondary antibody used in the mouse serum ELISA measurements were purchased from ThermoFisher Scientific. Physiological saline, phosphate-buffered saline (PBS), and sterile water for injection