cipients and factors that need to be outlined to ensure an acceptable formulation is achieved. These include formulation screening as a function of pH, excipients, buffers, adjuvant, and their interactions, and therefore it is recommended that the formulation scientist should begin exploring ranges around them to build in robustness to the product. Utilization of DoEs allows multiple combinations of the formulation to be explored and optimized efficiently.

Design of experiments within vaccines is, at a minimum, broken down into two stages. The first stage will be a low-resolution study where a broad range of conditions for each input is explored (e.g., for pH a range from 4.0 to 8.0). Once the initial factors/interactions are identified that impact the product significantly, a more detailed exploration can be accomplished in stage 2 of DoE to establish the design space for the vaccine product. In addition, simulation and modeling efforts, where possible, can aid in reducing the number of experiments required in establishing the design space. Common factors explored within DoE for a vaccine formulation are outlined below (Table 3).

After a better understanding of the degradation mechanisms and the factors within the formulation that impact product stability is established, the formulation scientists can now focus on developing a stable product that will meet the necessary GTPP. It is however recognized that timeline/resource constraints might limit the detailed exploration of degradation mechanism and a business need might drive the product development forward with a suboptimal formulation. It is our recommendation that CQAs must be identified, investigated, and the formulation variables to ensure the stability is enhanced are explored. Here, the proper delivery method is explored, dose levels for the product through animal studies and early device development is determined. Once accomplished, a formulation is moved forward into safety assessment/toxicology studies and into phase I. The formulation may not be fully optimized, but there is a strong understanding of what will be required to meet the necessary GTPP and a formulation process, albeit not optimized for routine manufacture is in place so that clinical manufacturing at the pilot-scale can be achieved.

Table 3 Common factors explored in vaccine formulation development DoE

Common factors	Ranges investigated
Buffer species	Phosphate, histidine, succinate, citrate, acetate, tris
Buffer concentrations	5–50 mM
pH	4.0–8.0
Salt concentrations	0–300 mM
Sugar (sucrose, trehalose, lactose, etc.)	1–10%
Surfactant concentration (PS80, PS20, P188)	0–0.3%
Adjuvant	Aluminum phosphate, aluminum hydroxide, (proprietary adjuvants like MF59, AS03, AS04, etc.)
Antigen concentration or potency target	1–5 mcg/mL

DoE design of experiment