Advanced Kinetic Analysis as a Tool for Formulation Development and Prediction of Vaccine Stability

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ABSTRACT: We have used a protein-based vaccine, a live virus vaccine, and an experimental adjuvant to evaluate the utility of an advanced kinetic modeling approach for stability prediction. The modeling approach uses a systematic and simple procedure for the selection of the most appropriate kinetic equation to describe the degradation rate of compounds subjected to accelerated conditions. One-step and two-step reactions with unlimited combinations of kinetic models were screened for the three products under evaluation. The most appropriate mathematical model for a given product was chosen based on the values of residual sum of squares and the weight parameter *w*. A relatively simple *n*-th order kinetic model best fitted the degradation of an adjuvanted protein vaccine with a prediction error lower than 10%. A more complex two-step model was required to describe inactivation of a live virus vaccine under normal and elevated storage temperatures. Finally, an autocatalytic-type kinetic model best fitted the degradation of an oil-in-water adjuvant formulation. The modeling approach described here could be used for vaccine stability prediction, expiry date estimation, and formulation selection. To the best of our knowledge, this is the first report describing a global kinetic analysis of degradation of vaccine components with high prediction accuracy. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci
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INTRODUCTION

The stability of vaccines is a critical factor influencing their worldwide distribution and has a major impact on vaccine quality, potency, and storage conditions.[1,2] The thermal stability of vaccines is of great interest for the vaccine industry, government institutions, and philanthropic organizations attempting to increase the distribution of vaccines to people living in countries with poor infrastructure and unreliable transportation and storage facilities to preserve the vaccines that require refrigeration.[2–4]

The thermal stability of vaccines can be evaluated through numerous methods that look at physicochemical or biological changes in a vaccine upon exposure to elevated temperatures.[4–6] There are essentially two approaches to studying the thermal stability of vaccines: temperature ramping experiments and accelerated stability studies under isothermal conditions.

Temperature ramping experiments involve monitoring changes in the biophysical properties of a vaccine while temperature is increased at a given heating rate. In this case, thermal stability is evaluated via monitoring decomposition extent and/or some thermodynamic parameters such as the enthalpy and free energy.[7–9] Since thermal ramping experiments can be completed within a few hours, these studies are broadly used in vaccine formulation development and screening of stabilizing conditions.[10] Apart from thermodynamics, it is noteworthy to mention that kinetics also governs the proteins stability. High kinetic stability results in a low denaturation rate of the protein and thus long-term stability.[11] For kinetic analysis,[12,13] multi-heating rate experiments[9,14] and isoconversional analyses[15] are often used to determine the kinetic parameters for predicting the thermal stability of pharmaceutical products.[16,17]

Accelerated stability studies are designed to determine the rate of vaccine degradation over time as a result of exposure to temperatures greater than those recommended for vaccine storage.[18] Many different stability indicating assays can be used to monitor the rate of vaccine degradation as a function of time and these include viral titer, immunochemical assays, liquid chromatography, and gel electrophoresis, to mention a few.[2,4,19]

An accelerated stability program can serve for multiple purposes such as selection of stabilizing conditions, shelf life estimation, temperature excursion modeling, and to support manufacturing process changes that may be suspected to alter vaccine stability.[18] The most commonly used approaches to analyze data and predict long-term stability from accelerated stability studies consist of fitting data with a simple kinetic model (typically zero- or first-order kinetic models) to obtain the rate constant for two or more temperatures. This experimental procedure is followed by the calculation of the unknown rate constant at required temperature by extrapolation using an Arrhenius dependence of the reaction rate on the temperature.[19,20] However, stability predictions based on application of zero- or first-order kinetics are very often too simplified for description of the degradation of biological products, which frequently undergo complex and multistep degradation reactions.[21] As such, more sophisticated degradation kinetic models using Vogel–Fulcher–Tammann equation or Prout–Thompkins nucleation models may be useful to describe degradation in biological

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products.[22–25] Such more complicated approach in which a two-step kinetic model, including an n-th order and an autocatalytic component, was initially used to described the epoxy cure reaction[26] and was adapted more recently for protein aggregation kinetics.[27]

In this report, we use an advanced kinetic modeling approach in which the amount of reaction steps and kinetic models is unlimited and their use is validated by the statistical tools that allow choosing optimal number of the variable parameters used for fitting of experimental data.[28] This procedure was applied to evaluate the degradation kinetics of a protein- and virus-based vaccines, and an emulsion-based adjuvant formulation. Accelerated stability studies at three or more isothermal temperatures were conducted and the resulting data were fitted with one-step or two-step kinetic models. The most appropriate model was identified by statistical analysis and was afterward used to extrapolate the long-term degradation at any required storage temperature. It is important to mention that the selection of the kinetic models is purely based on statistical analysis and the goodness of fit without requiring that the chosen model is established as correctly describing the mechanism(s) of degradation of the complex systems under evaluation in this report. Pharmaceutical applications of this approach are discussed.

MATERIALS AND METHODS

Materials

ALVAC poxvirus recombinant vector vCP2292 containing the melanoma gene inserts NY-ESO-1(M) and TRICOM was produced and purified as previously described.[29]

Pneumococcal histidine triad protein D (PhtD) was expressed as a recombinant protein in *Escherichia coli* and purified by serial column chromatography to purity greater than 95% as evaluated by reversed phase HPLC (RP-HPLC) and SDS-PAGE. Aluminum hydroxide (AH) adjuvant (Alhydrogel®) was obtained from Brenntag Biosector (Frederikssund, Denmark). Phosphate-treated aluminum hydroxide (PTAH) was prepared as previously reported.[30] All other chemicals were analytical grade reagents.

The oil-in-water emulsion was prepared by using the phase inversion temperature process. The concentrated oil-in-water emulsion [more than 30%, w/w, oil in phosphate-buffered saline (PBS)] was then diluted with PBS to its final concentration (~3%, w/w, oil in PBS).

Stability Monitoring

Purified ALVAC samples containing approximately 7.9 log CCID50 (50% of the cell culture infectious dose) in 10 mM Tris–HCl pH 7.4 were used for all stability studies. Under a laminar flow cabinet, two different ALVAC formulations (ALVAC-1 and ALVAC-2) were prepared by diluting ALVAC stock solution 1:2 with formulation buffer. All samples were dispensed in 3 mL glass vials (0.6 mL/vial) with septum closure and aluminum cap and were incubated under different temperatures for stability monitoring. Virus viability was subsequently tested by CCID50 at different time intervals.

PhtD formulations were prepared in PTAH adjuvant as previously described.[30] Formulations containing 100 μg/mL PhtD and 0.56 mg/mL aluminum were dispensed in 3 mL glass vials (0.6 mL/vial) with septum closure and incubated under different temperatures for stability monitoring. The stability of PhtD was evaluated as a function of time by RP-HPLC after desorption from the aluminum adjuvant as previously described.[30] By this technique, only the peak area of intact PhtD is integrated and used for the calculation of the concentration of intact protein. Therefore, this provided a direct measurement of protein degradation induced by elevated temperatures. Stability data are presented as the concentration of intact protein as a function of time.

The adjuvant formulation based on an oil-in-water emulsion was dispensed in 7 mL amber glass vials (4.6 mL/vial) with fluoropolymer stoppers and incubated under different temperatures for stability monitoring. Since acetone is one of the major degradation products accumulating in the formulation as a result of the oxidation of oil droplets in emulsion, in the stability study, its content was monitored by gas chromatography.

Advanced Kinetic Analyses

Data analysis was performed using AKTS-Thermokinetics software (version 3.6; AKTS Inc., Advanced Kinetics and Technology Solutions, Siders, Switzerland).[31] The tool considers a nonlimited amount of models using "one-step" and "two-step" kinetics. The rate of the reaction is commonly expressed by the Arrhenius equation that describes the empirical relationship between the reaction rate and the temperature.

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = k(T)f(\alpha) = A\exp\left(-\frac{E}{RT(t)}\right)f(\alpha) \tag{1}$$

with k, the rate constant, α, the reaction progress, and A, the pre-exponential factor. The units of A are identical to the unit of the rate constant k and will vary depending on the order of the reaction. E, the activation energy, R, the universal gas constant, T, the temperature in Kelvin, and $f(\alpha)$, represents a function describing the reaction kinetics. As proposed by Roduit et al.,[28] the truncated Šesták–Berggren[32] (SB) model was applied for $f(\alpha)$.

$$f(\alpha) = (1-\alpha)^n\alpha^m \tag{2}$$

where the exponents n and m are generally reported as noninteger. It follows that the general expression describing the rate of reactions which may proceed according to two subreactions has the form:

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = A_1\exp\left(-\frac{E_1}{RT}\right)(1-\alpha)^{n1} + A_2\exp\left(-\frac{E_2}{RT}\right)(1-\alpha)^{n2}\alpha^{m2} \tag{3}$$

For example, by taking n and m equal to 1, one can obtain the known Prout–Tompkins equation.[33,34] As a further example, the application of two SB models allows describing the autocatalytic reactions (with $n_1 = 1$, $m_1 = 0$, $n_2 = 1$, $m_2 = 1$) built up from two subreactions. The autocatalytic reactions are divided into two steps:

(1) Primary degradation resulting in the formation of a catalytic compound B.