Research paper

Accurate prediction of vaccine stability under real storage conditions and during temperature excursions

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ABSTRACT

Due to their thermosensitivity, most vaccines must be kept refrigerated from production to use. To successfully carry out global immunization programs, ensuring the stability of vaccines is crucial. In this context, two important issues are critical, namely: (i) predicting vaccine stability and (ii) preventing product damage due to excessive temperature excursions outside of the recommended storage conditions (cold chain break). We applied a combination of advanced kinetics and statistical analyses on vaccine forced degradation data to accurately describe the loss of antigenicity for a multivalent freeze-dried inactivated virus vaccine containing three variants. The screening of large amounts of kinetic models combined with a statistical model selection approach resulted in the identification of two-step kinetic models. Predictions based on kinetic analysis and experimental stability data were in agreement, with approximately five percentage points difference from real values for long-term stability storage conditions, after excursions of temperature and during experimental shipments of freeze-dried products. Results showed that modeling a few months of forced degradation can be used to predict various time and temperature profiles endured by vaccines, i.e. long-term stability, short time excursions outside the labeled storage conditions or shipments at ambient temperature, with high accuracy. Pharmaceutical applications of the presented kinetics-based approach are discussed.

1. Introduction

The thermal sensitivity of formulated biologics, including vaccines, is a critical factor impacting product quality and potency, hence influencing their worldwide distribution [1,2]. The thermal stability of vaccines is of concern to the vaccine industry, health authorities, government institutions, and philanthropic organizations attempting to increase the distribution of vaccines to people living in countries with poor infrastructure, unreliable transportation and substandard storage facilities for the preservation of vaccines requiring refrigeration [3].

World Health Organization (WHO) sets guidelines for the stability evaluation of vaccines [4] and recommends conducting regular and accelerated stability studies. These guidelines aim to provide a framework for shelf life and storage conditions, monitor vaccine stability in the post licensure period and support manufacturing changes by demonstrating consistency of vaccine batches. Such guidelines can be used for the selection of optimal stabilizing conditions, shelf life estimation, temperature excursion modeling, and investigation of changes to manufacturing process that may potentially alter vaccine stability [5]. Many different stability indicating assays can be used, including viral titer, immunochemical assays, liquid chromatography and gel electrophoresis [6,7]. An often used parameter is shelf life, this refers to

the time period during which a drug product remains capable of acceptable performance. Several shelf life estimation methods exist, those described in International Conference on Harmonisation (ICH) guidelines [8] are generally based on linear or nonlinear regression and statistical modeling through poolability tests. Focusing on the fact that an agreement does not currently exist, an investigation of current statistical methods for estimating shelf life, based on stability data, was conducted by a Product Quality Research Institute (PQRI) working group [9]. Additionally, to improve ICH procedure, the application of quantile regression or mixed model tolerance interval methods and batch effects, was proposed [10,11].

Kinetics is typically used to estimate vaccine degradation rates through accelerated stability programs which expose products to temperatures greater than those recommended for vaccine storage (typically 5 °C, 25 °C, 37 °C). During kinetic analysis of the data, very often the simplified kinetics models, such as zero- or first order mechanisms, are considered. Such models fail to correctly describe the complicated course of decomposition of biological materials, which frequently show complex and multi-step degradation behavior [12–15]. The rate constant derived from simplified models are often of little value during kinetic workflow; for example the determination of the kinetic values of pre-exponential factor and activation energy from the Arrhenius plot

[7,16]. Superior kinetic description of the experimental decomposition data of biologicals [17,18] was obtained with kinetic parameters derived from two-step models, these better mimic the complicated decomposition of the investigated samples. Also of great use is the Šesták-Berggren approach [19], being a phenomenological kinetic model which allows kinetic analysis, even of complex reactions [14,20].

In this report, we applied a general procedure combining advanced kinetic and statistical analyses of vaccine forced degradation data to identify kinetic models which best describe loss of antigenicity for three variants of inactivated viruses in freeze-dried form. Kinetic analysis was applied to predict the long-term vaccine degradation of the freeze-dried products, either under isothermal or specific temperature excursions. Additionally, the antigenicity recoveries predicted by kinetic models were compared with the experimental data of tested samples. Pharmaceutical applications of the presented kinetics-based approach are discussed.

2. Material and methods

2.1. Materials

A multivalent freeze-dried inactivated vaccine by Sanofi Pasteur was used for this study; it is composed of three different viral variants referred as A, B, C. To allow the detection of the antigenicity recovery losses during temperature excursions outside of the refrigerated storage condition (cold chain break), a thermosensitive formulated product was selected. Final bulk products were prepared using an appropriate stabilizer containing a mixture of excipients (amino acids, sugar, surfactant and chelator) in a HEPES buffer to maintain pH at 7. All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). 0.5 mL of final bulk products were dispensed in 3 mL type I glass vials partially stoppered with septum closure and subsequently freeze-dried, then fully stoppered and crimped with aluminum cap. Dried products were investigated with low residual moisture content (0.7%) measured by coulometric titration (Karl Fisher).

Antigenicity was tested by enzyme-linked immunosorbent assay (ELISA) at different time intervals.

2.2. Stability monitoring and antigen content by ELISA

Product stability was studied by storing freeze-dried formulations at 5 °C, 25 °C, 37 °C and 45 °C into temperature-controlled incubators for up to 6 months. This forced degradation plan was carefully designed to obtain experimental datasets able to be fitted by the software, with a minimum of 20 data points in total obtained at three or more storage temperatures, with significant degree of reaction reached for the more drastic condition. Hence, time intervals for the forced degradation plan were chosen, as follows: 0, 30, 90 and 180 days at 5 °C; 7, 14, 30, 60, 90 and 180 days at 25 °C; 1, 3, 7, 14, 30, 60 and 180 days at 37 °C, and 1, 3, 7, 14, 30 and 90 days at 45 °C.

During studies of the influence of temperature excursions, the two following temperature programs were applied to freeze-dried products before analysis by ELISA: (i) 1 month at 5 °C, followed by 9 months at 25 °C and then returned to 5 °C for 2 months; (ii) 1 month at 5 °C, followed by 6 months at 37 °C and then returned to 5 °C for 4.5 months. To evaluate the impact of experimental shipments under uncontrolled cold chain conditions, product samples kept for 534 days (~1.5 year) at 5 °C were shipped from Lyon (France) to Toronto (Canada) at ambient temperature. Another set of samples was also sent from Lyon (France) to Barcelona (Spain). Real temperature profiles were recorded with temperature monitoring devices (TempTale®4 USB Monitors, Sensitech Inc., Beverly, USA) placed into the box containing the vaccines. The temperature profiles recorded continuously during shipment were used for predictions. All samples came back to France and were kept at 5 °C before ELISA analyses.

The antigen titer of vaccine samples was determined by a variant-specific sandwich ELISA. Freeze-dried products were stored at 5 °C before analysis and reconstituted extemporaneously prior to testing. Three vials were analyzed for each kinetic time point and a mean of these independent dosage values was displayed. Results are systematically expressed as a percentage of the mean value at T = 0 and referred to as antigen recovery. To estimate prediction accuracy, percentage point was used as the arithmetic difference between antigenicity recoveries, experimentally determined and predicted.

2.3. Advanced kinetic analyses

A general stability modeling procedure using AKTS-Thermokinetics software [21] (version 4.1, AKTS AG, Advanced Kinetics and Technology Solutions, Siders, Switzerland) was already described in detail [20] and applied to predict stability for various products constituting vaccines [14]. Briefly, according to a least-squares regression analysis, the software uses 'one-step' and 'two-step' kinetic models based on the truncated Šesták-Berggren equation ('two-step' in Eq. (1) to determine the function(s) which best fits the experimental data. Hence, reaction rate was described as follows [20]:

$$\frac{d\alpha}{dt} = A_1 \exp\left(-\frac{E_1}{RT}\right)(1-\alpha)^{n1}\alpha^{m1} + A_2 \exp\left(-\frac{E_2}{RT}\right)(1-\alpha)^{n2}\alpha^{m2} \tag{1}$$

With α: the reaction progress, A: pre-exponential factor, E: activation energy, n: reaction order, m: a parameter introduced to take into account the possible autocatalytic behavior of reaction (1) and (2), for the first and second step, respectively. During evaluation one, two or more reaction stages may be considered, depending on the complexity of the reaction. For a two-step reaction, consecutive and competitive type models were screened. Competitive two-step models use one more adjustable parameter than consecutive two-step model, with the contribution of the reaction rate of the first and second stage to the overall reaction rate. The quality of regression fit is quantified according to *RSS* (Residual Sum of Squares) value. The combination of different kinetic models best describing the reaction course is identified with software according to the higher Akaikie Information Criteria and Baysian Information Criteria weighted scores, referred to as *wAIC* and *wBIC*, respectively. In kinetic analysis, when applying the different models describing the reaction course, an important issue is to find the best method for their discrimination [22]. The criteria for comparing models applied in this study were based on the information theory introduced by Akaike [23] and its Bayesian counterpart [24,25]. Both these criteria indicate not only which model is more likely to better fit the analyzed data, but also allow quantification of likelihood. An example of criteria application, using limited experimental points for the prediction of thermal stability of biological materials, is described in detail elsewhere [20]. Finally, by using the selected model, the software was employed to run bootstrap analysis (resampled 1000 times with replacement) and determine the confidence intervals (CI), in the form of the upper and lower 95 percentiles (predictive band at 95%), which were evaluated for each of the fitted parameters [20]. After determination of the best kinetic models the software was able to simulate the reaction progress during arbitrarily chosen aging time and under any temperature profiles. We have used this approach to continuously predict degradation progresses of variants when vaccines were kept under isothermal conditions (long-term stability), during temperature excursions and during exposures under real atmospheric temperature profiles (e.g. shipments at ambient temperature).

3. Results

3.1. Thermal degradation of virus-based vaccine described by two-step kinetic models

Lots of models, starting from the simplest ones (zero and first-order) and ending with more complex were screened to fit the six months