**Prediction of long-term stability of lyophilized antibody formulations under storage and accelerated conditions**

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Keywords: Monoclonal antibody, Protein aggregation, Lyophilized drug product, Modeling, Predictive stability, product shelf life

**Abstract**

The long-term stability of monoclonal antibodies is a critical consideration in the development of lyophilized biologic drug products. Despite concerns regarding the robustness of early predictions, the ability to accurately forecast long-term stability from accelerated stability studies remains of significant interest. In this study, we utilized a combination of accelerated stability studies (up to 6 months) and a stretched time kinetic model to predict the long-term stability under (up to 3 years) of various monoclonal antibody formulations. Specifically, we demonstrated the ability to reliably forecast protein stability at the intended storage condition (5 °C) using data collected over six months for multiple quality attributes at various temperatures, including standard storage (5 °C), accelerated (25 °C), and stress conditions (40 °C and 50 °C). Stability studies were conducted on several monoclonal antibodies, including IgG1, IgG4, and bispecific mAbs. Compared to traditional linear extrapolation methods, our approach demonstrated enhanced robustness and accuracy in predicting long-term stability.

**Keywords**

Monoclonal antibody, Protein aggregation, Lyophilized drug product, Modeling, Predictive stability, product shelf life

**Abbreviations**

# **Introduction**

Monoclonal antibody (mAb)-based biologics have emerged as a key therapeutic approach in recent years. As of December 2024, over 200 antibody-based therapies have been approved for market use, with nearly 1,400 investigational candidates currently in clinical trials for the treatment of various diseases. (1) However, the stability of these biologics remains a critical challenge due to their susceptibility to various degradation pathways, including covalent modifications, size alterations, and conformational changes, all of which can impact the biological activity of the product.

One of the most widely used approaches for predicting drug stability is the Arrhenius equation, which assesses the temperature dependence of degradation rates​ While this model is effective for small molecules, its application to biologics, particularly mAbs, is challenging due to the complexity of protein stability. The conventional Arrhenius model often fails to account for the multifaceted degradation pathways seen in biologics. Despite these challenges, a growing number of literatures demonstrates predictive stability for biologics, where the long-term stability of liquid drug product quality attributes is accurately predicted using temperature-dependent kinetics, including the application of the Arrhenius equation to liquid mAb drug products. (2-4)

In this study, we build upon previous work by applying a combination of stretched time kinetics and the Arrhenius relationship to predict the long-term stability under storage condition and accelerated condition of lyophilized mAb formulations. Our analysis focuses on the formation of high molecular weight (HMW) species, specifically dimers, as the primary degradation product under a range of temperature conditions (5 °C to 50 °C). By integrating stretched time kinetics into the Arrhenius model, we offer a robust method for predicting long-term stability under both storage and acerated conditions based on short-term stability data, providing an invaluable tool for early-stage product development.

# **2. Materials and Methods**

## **2.1 Reagents and chemicals**

Monoclonal antibodies were manufactured by Regeneron Pharmaceuticals Inc. (Tarrytown, NY). Sodium chloride, sodium phosphate monobasic, and dibasic were sourced from J.T. Baker (Phillipsburg, NJ, USA). Sodium perchlorate and urea were obtained from Sigma (St. Louis, MO, USA), while histidine and histidine hydrochloride monohydrate were supplied by Ajinomoto (Eddyville, IA). Sucrose was procured from Ferro Pfanstiehl (Waukegan, IL), and polysorbate 80 was purchased from Avantor (Phillipsburg, NJ). All reagents were of analytical grade or higher.

## **2.2 Stability studies**

The monoclonal antibody drug products, including IgG1, IgG4, and bispecific antibodies, were formulated at a concentration of 50 mg/mL in 10 mM histidine (pH 6.0), 5% sucrose, and 0.1% (w/v) polysorbate 80. The formulations were filled into glass vials and subjected to lyophilization under standard lyophilization cycles (details not shown), using an FTS LyoStar™ III lyophilizer. The lyophilized drug products exhibited less than 1% residual moisture and acceptable cake appearance.

Post-lyophilization, the vials were stored at the recommended long-term storage condition of 5 °C for up to 36 months. In addition to this, accelerated stability studies were conducted at 25 °C, while stress stability studies were performed at elevated temperatures of 40 °C, 45 °C, or 50 °C. Samples were analyzed at predefined intervals (1, 3, 6, 9, 12, 18, 24, and 36 months). Prior to analysis, the lyophilized drug products were reconstituted with sterile water for injection (WFI) to a concentration of 50 mg/mL.

In accordance with ICH guidelines, real-time storage conditions (5 °C), accelerated conditions (25 °C), and stress conditions (40 °C or/and 50 °C) were utilized for the stability studies. For the purposes of this study, 5 °C is referred to storage condition and 25 °C is referred to accelerated condition.

## **2.3 SE–UPLC**

Size-exclusion chromatography (SEC) was performed using a Waters Acquity Ultra-Performance Liquid Chromatography (UPLC) system equipped with UV detection at 280 nm. The chromatographic separation was achieved using an ACQUITY UPLC BEH200 SEC 1.7 μm 4.6 × 300 mm column (Waters cat. #186005226). The mobile phase consisted of 10 mM phosphate buffer with either 1 M sodium perchlorate or sodium chloride at pH 7.0. The flow rate was set at 0.4 mL/min with a total run time of 12 minutes. The high molecular weight (HMW) species, low molecular weight (LMW) species, and main peak were each integrated as distinct size variant groups.

**2.4 Data modeling and prediction**

Aggregation was monitored using the SE-UPLC method, with the total percentage of aggregates being calculated. Across all formulations and time points, dimers were identified as the predominant species among the aggregates. SE-UPLC overlay chromatograms indicated minimal fragmentation, regardless of temperature or IgG subclass types (*Figure S 1* and Figure S *2*). As a result, only the percentage of total HMW species was considered in kinetic modeling.

The stretched time kinetic model, as described by Pikal(1)., was employed to model the HMW formation in the lyophilized formulations. The relationship is described by the following equation:

Equation 1

Where,

%HMW0 = %HMW at T = 0

Kα = Time Coefficient (rate constant)

t = Time

*Arrhenius plotting*

The data obtained from SE-HPLC analysis were fitted using this equation. Subsequently, the Arrhenius equation (Equation 2) was applied to empirically determine the temperature dependence of the rate constant Kα ​, enabling the prediction of stability across a temperature range of 5 °C to 50 °C.

Equation 2

Equation 3

Where A is the pre-exponential factor and 𝐸𝑎 is the activation energy.

The activation energy Ea for each monoclonal antibody was calculated, allowing for the extrapolation of degradation rates at different temperatures. Short-term stability data (less than 6 months) from mAbs 8–10 were used to model the time coefficient, which was subsequently applied to the Arrhenius equation to predict the percentage of HMW species following long-term storage at 5 °C.

*Global fitting and generation of prediction intervals*

To evaluate long-term storage stability, a global fitting of Arrhenius kinetics was employed on accelerated stability data. Stability forecasts were derived from three months of data, considering time and temperature as independent variables that affect the variations in high molecular weight (HMW), low molecular weight (LMW), and acidic species concentrations.

A nonlinear regression analysis was conducted using a custom Python script to estimate the parameters associated with Equations 4, thereby determining activation energies and pre-exponential factors for each relevant attribute. The model's goodness of fit was assessed using the R² statistic. The resulting kinetic parameters were subsequently utilized to project long-term stability trajectories for HMW, LMW, and acidic species at 5°C or 25°C.

|  |  |
| --- | --- |
|  | (Equation 4) |

To enhance the precision of these predictions, a bootstrapping analysis was carried out to create 95% prediction intervals. For each forecast, a resampling methodology was applied to the experimental dataset—incorporating time, temperature, and the measured concentrations of HMW, LMW, and acidic species—resulting in the generation of 1,000 resampled datasets. Arrhenius kinetic modeling was executed on each resampled dataset, and the central 95% of the resulting forecasts were extracted to formulate confidence intervals. Prediction intervals were then established by accounting for both model fitting errors and anticipated data variability, adhering to established statistical methodologies.

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| --- | --- | --- | --- | --- |
| **mAb** | **Study Starting date** | **Data included in modelling** | **Data available** | **temperature** |
| 1 | REGN1500 | Y | 36m | 5, 25, 40,50 |
| 2 | REGN3767 | Y | 36m | 5, 25, 50 |
| 3 | REGN4336 | Y | 36m | 5, 25, 40 |
| 4 | REGN4461 | Y | 36m | 5, 25, 50 |
| 5 | REGN5069 | Y | 36m | 5, 25, 50 |
| 6 | REGN5668 | Y | 36m | 5, 25, 40 |
| 7 | REGN5678 | Y | 36m | 5, 25, 50 |
| 8 | REGN5713 | Y | 36m | 5, 25, 50 |
| 9 | REGN5714 | Y | 36m | 5, 25, 50 |
| 10 | REGN5715 | Y | 36m | 5, 25, 50 |
| 11 | REGN6194 | Y | 9m | 5, 25, 40 |
| 12 | REGN6569 | Y | 36m | 5, 25, 40,50 |
| 13 | REGN7075 | Y | 36m | 5, 25, 40 |
| 14 | REGN7257 | Y | 36m | 5, 25, 40,50 |
| 15 | REGN7544 | Y | 24m | 5, 25, 40 |
| 16 | REGN7945 | Y | 24m | 5, 25, 40 |
| 17 | REGN7999 | Y | 36m | 5, 25, 40,50 |
| 18 | REGN9035 | Y | 36m | 5, 25, 40 |
| 19 | REGN9533 | Y | 18m | 5, 25, 40 |
| 20 | REGN13335 | Y | 24m | 5, 25, 40 |