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REPORT

Prediction of long-term stability of high-concentration formulations to support rapid development of antibodies against SARS-CoV-2

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ABSTRACT

Long-term stability of antibody therapeutics is required to ensure their safety and efficacy when administered to patients. However, obtaining shelf life supporting, long-term stability data are often a limiting factor for new drug candidates starting clinical trials. Predictive stability, which uses short-term accelerated stability data and kinetic modeling to forecast long-term storage stability, has the potential to provide justification to support establishing shelf life, although its application for biologics has only recently gained traction. We have developed empirical models for key stability-indicating quality attributes of high-concentration IgG1 liquid formulations. Using short-term accelerated stability data and Arrhenius-based approaches, including Arrhenius plotting and global fitting, we applied empirical kinetics to predict the long-term stability of seven anti-SARS-CoV-2 antibodies. Arrhenius plotting determines kinetics by plotting the reaction rate logarithm against inverse temperature, while global fitting simultaneously fits a model with data at multiple temperatures to comprehensively understand kinetics. These approaches were used to fit empirical kinetics to short-term data to predict long-term stability, leveraging stability data collected at shelf life storage conditions (5°C) and at least 1 month of accelerated stability data at three temperatures within 25–40°C. Model accuracy was demonstrated using long-term (up to 36 months) storage stability data at 5°C. The approach was applied successfully in anti-SARS-CoV-2 antibody drug development to enable rapid regulatory Investigational New Drug and Investigational Medicinal Product Dossier filings and support shelf life justification where limited shelf life stability data were available at the time of filing. Our results show that successful long-term stability predictions and shelf life estimation can be achieved with high accuracy using 1 month of accelerated stability data, which may be especially beneficial for rapid response programs with severely constrained development timelines. Thus, the described model demonstrates how predictive stability models can, in addition to enabling earlier decision-making in drug development, also be used to justify product shelf life in regulatory submissions, enabling faster patient access to life-saving drug products.

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Introduction

In the past 30 plus years, tremendous progress has been made in developing biological drug products to treat a wide range of diseases and conditions and thus improve and save patient lives. Monoclonal antibodies (mAbs) have made up a large part of the drug product landscape, as shown by the approval of over 100 antibody-based therapeutics by the US Food and Drug Administration (FDA).[1] The collective efforts made by industry and academia in developing these drug products have led to increased data and knowledge across all aspects of mAb drug development, which can be leveraged at multiple stages of drug product development to shorten the overall timeline and decision-making process. As biologics are sensitive to environmental conditions, establishing a suitable shelf life is essential to ensure they remain safe and maintain efficacy when administered to patients. Assessing the stability of antibody drug products is crucial for timely regulatory filings, but it is often a time-consuming component of drug development. This process requires real-time stability data to justify product shelf life, posing a significant bottleneck, especially for rapid development in response to a public health emergency. General stability requirements for pharmaceutical drug product development outlined in International Council for Harmonisation (ICH) guidelines Q1 and Q5C are widely adopted by regulatory agencies around the world, including the FDA and European Medicines Agency (EMA).[2] Briefly, shelf life setting is typically based on available long-term stability data, but recently, there has been a shift to allow shelf life assignment by predictive methods, such as the use of accelerated stability data or the extrapolation of storage stability data up to 12 months past the period covered by long-term stability data.[3–5] Risk-based predictive stability, which refers to stability predictions of pharmaceutical drug products through statistical, kinetic, or empirical approaches based on prior knowledge

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and/or short-term stability data, can play an important role in internal drug development decisions, but has only had limited impact on regulatory submissions thus far.[3,6]

Regulatory guidelines for risk-based predictive stability of biological products permit extrapolation up to the same duration as the existing experimental data, with a maximum limit of 12 months beyond the time span of the existing data.[4,5] Gathering real-time storage stability data over a period exceeding 6 months may not be practical for initial regulatory submissions. To expedite the early-stage development, implementing risk-based predictive stability methods by conducting short-term (1–3 months) stability studies at elevated temperatures and forecasting stability at the intended storage temperature can save valuable time and accelerate the path to clinical trials.

Predictive stability has been used for small molecule therapeutics to establish initial shelf life for regulatory submission, addressing the time constraints associated with collecting real-time stability data.[6] Prediction of small molecule stability focuses on the degradants produced from chemical reactions with well-understood reaction mechanisms, allowing for the implementation of Arrhenius kinetics, with some deviations or modifications as needed.[7–9]

While proteins and other biologics typically have more complex degradation mechanisms compared to small molecules, significant efforts in characterizing temperature-dependence of protein degradation have also been reported recently.[10] For chemical modifications, such as oxidation, where the reaction products are well-defined, reaction process can be tracked to determine if the kinetics has an Arrhenius temperature dependence. In contrast to these chemical degradation reactions, protein aggregation can be triggered through different pathways or a combination of pathways, and is thus often found to be non-Arrhenius due to the complexity of the aggregation process and temperature-dependent conformational stability of proteins.[10] However, Arrhenius behavior has been demonstrated for some therapeutic mAbs and recent studies have begun to outline the ability to predict long-term aggregation profiles from accelerated stability data,[11–13] which has the potential to enable shelf life estimation at an early development stage and is especially beneficial for high priority, rapid response programs.[14]

The use of Arrhenius kinetics to predict the shelf life stability of protein-based drug products has been gaining momentum to support earlier decision-making and faster speed to clinic during drug product development. Kuzman *et al.* previously applied a first-order model using Arrhenius kinetics to enable long-term predictions of up to 36 months using 3–6 months of accelerated stability data for multiple quality attributes, showing improvement in prediction accuracy compared to the limited linear extrapolation accepted in some regulatory filings.[12] First-order models were more recently applied to a suite of biotherapeutics consisting of mAbs, antibody-drug conjugates, and fusion proteins, with accurate long-term predictions reported for liquid and lyophilized drug presentations across a range of protein concentrations using 6 months of storage and accelerated stability data.[15] Both of these examples used first-order models to predict long-term stability, assuming a consistent kinetic mechanism across the accelerated and storage conditions. Bunc *et al.* reported on the importance of confirming a consistent kinetic mechanism when using accelerated data for the prediction of long-term stability, demonstrating a combination of thermodynamic and kinetic analyses.[11] By applying first-order models to predict protein aggregation of mAbs, distinct low- and high-temperature aggregation pathways were identified, with temperatures higher than 40°C leading to the increased prevalence of high-temperature aggregation pathways.[11] While the single-step first-order model cannot account for distinct mechanisms, Clenet introduced advanced kinetic modeling (AKM) which screens single- and two-step reactions to identify the model that best fits the data without over- or under-fitting.[16] This approach has been reported in the literature to accurately predict quality attribute trends during temperature excursions,[16] in-use periods,[13] and long-term storage[13,16] using 3–6 months of accelerated stability data.[17]

While AKM gives accurate kinetic predictions and there is a growing push for industry and regulatory agencies to adopt these good modeling practices,[15–17] it may not be amenable to rapid predictions. Literature published thus far has reported the use of between 3 and 6 months of accelerated stability data and has generally recommended the use of 20–30 data points to establish a reliable model for AKM.[17] However, the recent coronavirus disease (COVID-19) pandemic prompted rapid development of biologics to enable speed to clinics, which in turn requires early decision-making related to drug development, such as candidate selection or formulation development, and may limit real-time stability data collection. In cases like this, prior knowledge of biological drug stability behavior can be leveraged for rapid prediction of shelf life stability using data from as early as 1 month.

Herein, an empirical model was established based on an IgG1 mAb (mAb*) formulation with a well-known stability profile and applied on a platform basis to enable accurate prediction of shelf life stability and expedite the regulatory filing process for mAbs targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) developed during the pandemic. Specifically, we developed a risk-based empirical model using an Arrhenius approach for predicting long-term stability of key stability attributes with limited accelerated stability data (minimum 12 data points across 3 accelerated temperatures) and applied it to 7 anti-SARS-CoV-2 IgG1 mAbs (cov-mAbs) with similar high-concentration (≥100 mg/mL) formulations. Our results showed that long-term storage stability of up to 36 months of seven cov-mAbs can be predicted accurately using the 1 month accelerated stability data, and the results can be used for justifying product shelf-life in regulatory submissions, hence providing a framework for utilizing 1 month accelerated stability data to model long-term stability and facilitate rapid regulatory filing in accelerated drug product development.

Results

Platform model development

Kinetics of IgG1 formulation key stability attributes

During the development of mAb*, stability studies were conducted at 5°C, 25°C, and 45°C; the 25°C and 45°C

conditions were included in order to accelerate changes in the quality attributes. At 45°C, the increase in high molecular weight species (HMW) over 2 months was somewhat linear ($R^2 = 0.97$) (Figure 1a). At 25°C, the increase in HMW over 6 months displayed a poor linear fit ($R^2 = 0.84$), starting with a fast initial increase followed by a slow but continued increase over time. HMW also increased significantly at 5°C over the studied time of 36 months (Figure 1a). The kinetics were also non-linear, similar to the observed trend at 25°C. Attempts were made to fit the kinetic data for HMW using several different empirical models to determine the rate constant (k). The square root of time kinetic model (Equation 1) established by Pikal *et al.*, which has previously been used to describe protein degradation in the glassy state for a lyophilized protein formulation and was adapted to describe HMW formation, best fit the stability data at 5°C and 25°C.[18] The increase in HMW for mAb* formulation fit well to the square root of time for 5°C and 25°C stability data, with R^2 of 0.99 at each temperature (Figure 1a). In addition, the 45°C data can also be fit to a square root of time ($R^2 = 0.97$), which was comparable to the linear fit ($R^2 = 0.97$).

$$HMW(t) = HMW_0 + k_{HMW} * t^{1/2} \quad (1)$$

$$LMW(t) = LMW_0 + k_{LMW} * t \quad (2)$$

$$Acidic(t) = Acidic_0 + k_{Acidic} * t \quad (3)$$

Low molecular weight species (LMW) significantly increased during storage at both 25°C and 45°C (Figure 1b). Unlike the HMW species, LMW species increased linearly at both 25°C and 45°C over time, as indicated by $R^2 \geq 0.98$ (Equation 2). At 5°C, very little change was observed for the LMW species for up to 36 months. Similarly, the acidic species increased significantly at 25°C and 45°C (Figure 1c), accompanied by concomitant decreases of the main peak. Results showed that acidic species increased linearly ($R^2 = 0.99$) over 6 months at 25°C and 2 months at 45°C (Equation 3). At 5°C, very little change in acidic species was observed over the course of 36 months.

Temperature dependence of key stability attributes

To characterize the effect of storage temperature on the kinetics of stability-indicating attributes, the stability of the mAb* formulation was studied at eight different temperatures using a separate lot of mAb* from the previous study. The aim of these studies was to establish a model at the defined temperature range that follows the same kinetics to permit the prediction of degradation rates at different temperatures. In addition to the 5°C, 25°C, and 45°C conditions studied previously, 20°C, 30°C, 37°C, 40°C, and 50°C conditions were included for this stability study to cover the full temperature range commonly used in stability studies of biological products. Figure 2a shows an example size exclusion-ultra-performance liquid chromatography (SE-UPLC) chromatogram and Figure 2b shows an imaged capillary isoelectric focusing (iCIEF) electropherogram of mAb* incubated at 5–50°C at 1 month.

For charge variants by iCIEF, the linear increase in acidic species over time was observed for all temperature conditions (Figure 3). The R^2 values of linear fittings (Table S1) were ≥0.94 for 25–50°C data. Lower R^2 values for 5°C and 20°C (0.32 and 0.45, respectively), are likely due to minimal changes observed at these conditions that are within method variability. Similar to acidic species, the LMW species by SE-UPLC also increased linearly over the storage time for all temperatures (Figure 3). The R^2 values of linear fits (Table S1) were ≥0.91 for 25–50°C data. Lower R^2 values for 5°C and 20°C (0.26 and 0.78, respectively), are likely due to the minimal change observed during the stability study. These observations indicate that LMW species formation and acidic species change over time retain the same kinetic behavior within the temperature range of 5–50°C. The apparent rate constants derived from empirical fitting of LMW species formation and acidic species increase were obtained from the linear fit for each storage temperature condition (Table S1).

For HMW species by SE-UPLC, the increase over time at temperatures in the range of 5–50°C is best fit using square root of time kinetics (Figure 3), with a lowest R^2 of 0.98 at 5°C (Table S1). Inspection of the HMW species peaks observed by SE-UPLC revealed that dimer formation is the main HMW species formed at temperatures from 5°C to 40°C, while an additional increase of HMW peak representing higher-order

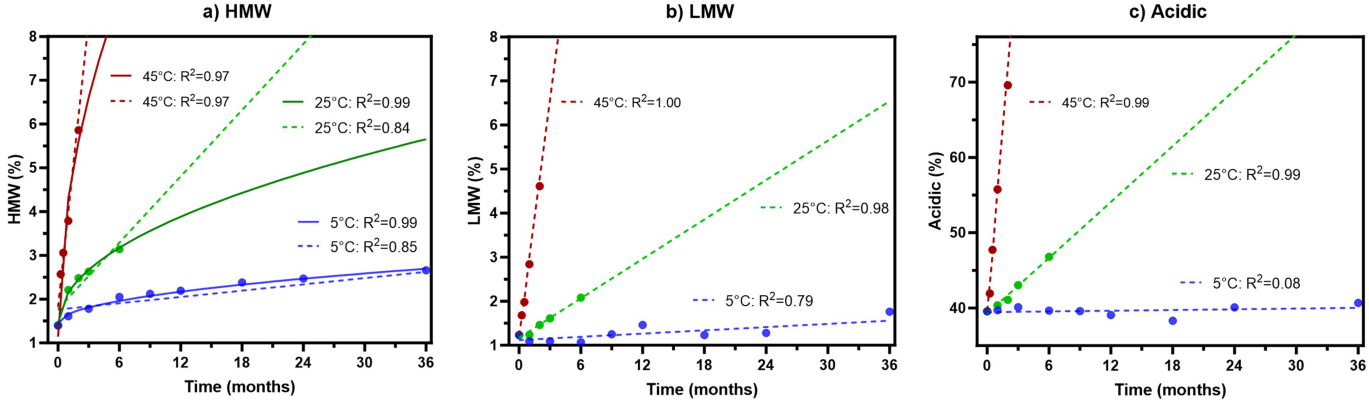

Figure 1. Kinetic modeling of mAb* at 5°C, 25°C, and 45°C for a) HMW species, b) LMW species, and c) acidic species. Data (filled circles) from 5°C, 25°C, and 45°C were used to assess the kinetic models. Linear (dashed line) and square root of time (solid line) fits are displayed.

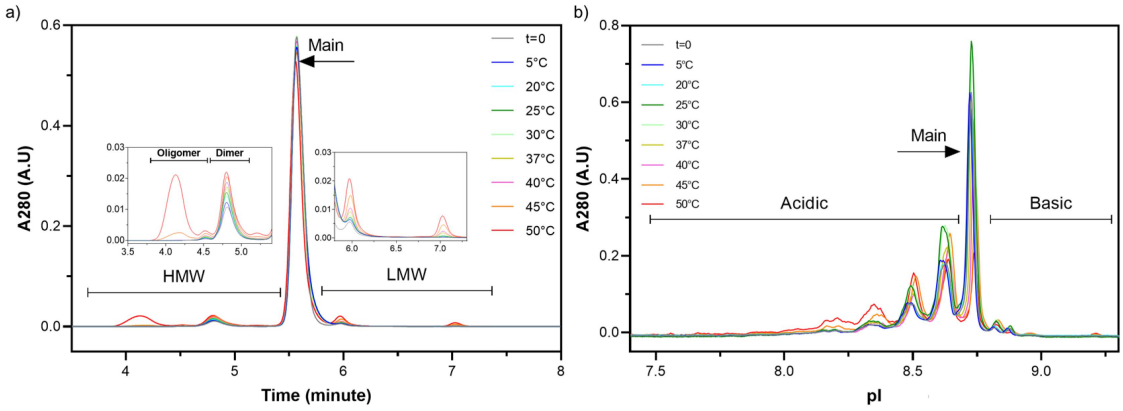

Figure 2. Illustration of a) SE-UPLC chromatograms and b) iCIEF electropherograms of mAb* incubated at 5–50°C for 1 month. At 5–40°C the HMW species formed are dimers, while higher-order oligomers are also observed at 45°C and 50°C.

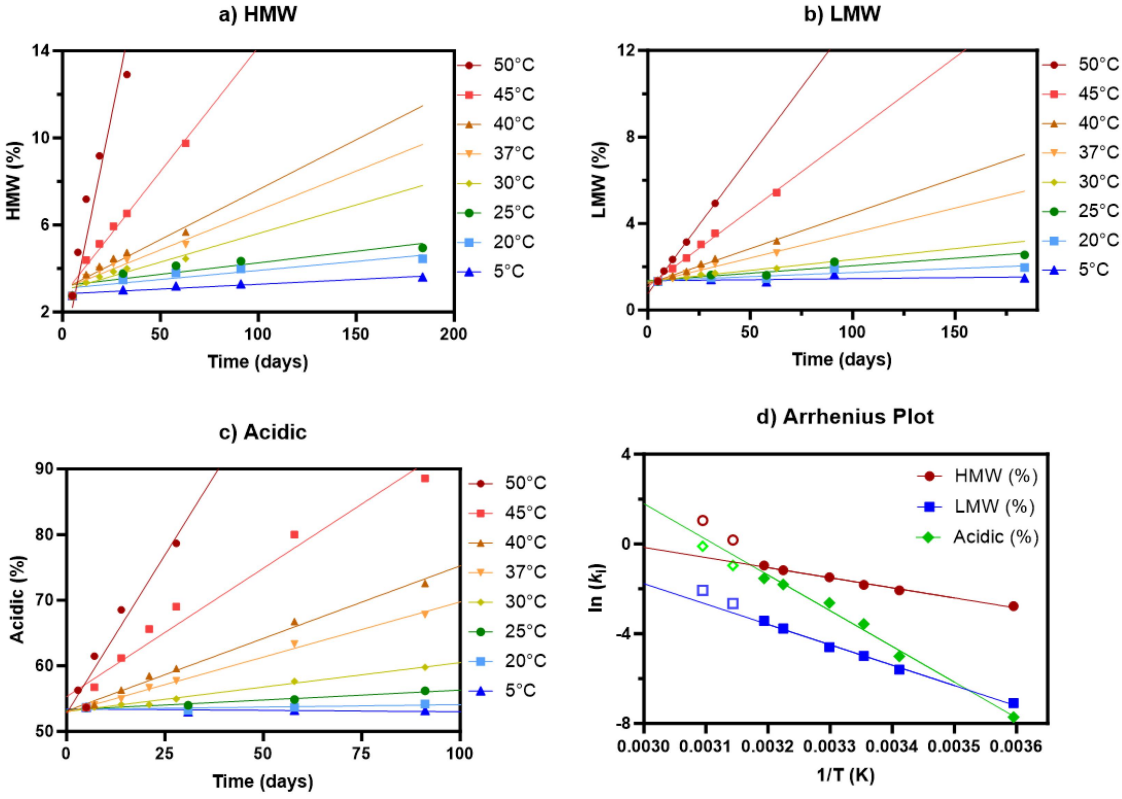

Figure 3. Stability modeling of mAb* at 5–50°C, including a) HMW increase fitted by square root of time kinetics over 6 months at 5–50°C, b) LMW increase linearly over 6 months at 5–50°C, and c) acidic species increase linearly over 3 months at 5–50°C. d) Arrhenius plots for HMW, LMW, and acidic species show a linear relationship at 5–40°C.

oligomer formation was observed at 45°C and 50°C (Figure 2a). The apparent rate constant from empirical fitting (square root of time kinetics) for HMW formation at each temperature was obtained (Table S1).

To examine if the apparent degradation rate constants of the key stability attributes, HMW, LMW, and acidic, have an Arrhenius temperature-dependence, the $\ln(k)$ of each attribute was plotted against inverse temperature (Figure 3d). Visual inspection of the linearity of the Arrhenius plots for HMW, LMW, and acidic species clearly indicates that the $\ln(k)$ for LMW and acidic species follows a linear relationship with inverse temperature for all the temperatures studied, including 5–50°C, with R^2 values ≥ 0.98. Additionally, the $\ln(k)$ values for HMW species from 5°C to 40°C also follow a linear relationship with inverse temperature (R^2 values > 0.99), while extending the temperature range to include 45°C and 50°C led to a deviation from the linear relationship ($R^2 = 0.88$). This is likely due to the activation of different aggregation/degradation pathways and the formation of higher-order HMW species as compared to those observed at lower temperatures (5–40°C).

Thus, the apparent rate constants of HMW, LMW, and acidic species formation follow an Arrhenius relationship in the temperature range of 5–40°C. Arrhenius plots were

obtained by plotting $\ln(k)$ against the inverse temperature and apparent rate constants at 5°C can be calculated using the linearized Arrhenius equation (Equation 4), where A is the pre-exponential constant, E_a is the activation energy, R is the universal gas constant, and T is the temperature (K) and which has been well-established.[19,20]

$$\ln(k) = \ln(A) + \left(\frac{E_a}{R}\right)\left(\frac{1}{T}\right) \quad (4)$$

The goodness-of-fit (R^2) values for HMW and LMW are >0.99, and 0.96 for acidic species, indicating a good quality of Arrhenius fit in the 5–40°C temperature range. The robustness of the Arrhenius kinetic model is further demonstrated by fitting sub-sampled data (i.e., from three temperatures including 5°C) and obtaining kinetic rate constants and activation energies (Equation 4) for sub-sampled data. Similar activation energies (~8 kcal/mol) for the full dataset (all temperatures), and for different subsets, indicate that the kinetic model is robust over the temperature range assessed (5–40°C).

The analysis of LMW and acidic species yielded similar activation energies (~20 kcal/mol and ~30 kcal/mol for LMW and acidic, respectively) for all temperature sub-sampling combinations (Figure 4). This clearly demonstrates that the linear degradation kinetic model for LMW and acidic species follows the Arrhenius relation across the whole-tested temperature range (5–50°C). HMW formation is more complex, involving additional aggregation mechanisms at temperature ≥ 45°C as indicated by the increasing calculated activation energy above 40°C. The calculated activation energy for HMW formation is ~8 kcal/mol for all temperature sub-sampling combinations and the full set of data at 5–40°C, excluding data at 45°C and 50°C (Figure 4). However, when the data at 45°C and 50°C are included the calculated activation energy increases significantly to ~13 kcal/mol, suggesting a change in HMW formation kinetic mechanism at temperatures ≥45°C.

Prediction of key stability attribute trends at storage conditions

Arrhenius plotting was used to predict the reaction kinetics at storage conditions of 5°C. Briefly, apparent rate constants at a defined temperature range (5–40°C) were obtained by empirical fitting (square root of time fit for HMW, linear fit for LMW and acidic species) using short-term data and used to obtain the Arrhenius equation to calculate the apparent rate constant at 5°C. The calculated rate constants and initial values of each attribute were used to predict stability over long-term storage at 5°C.

The Arrhenius equations established for mAb* in the temperature range of 5–40°C using available data up to 6 months was applied to predict the apparent rate constants at 5°C. Stability-indicating attributes, HMW, LMW, and acidic species can be calculated at selected time points using their respective predicted rate constants (Equation 4) at 5°C and the initial values (i.e., measured at time zero). For HMW, the predicted rate constant at 5°C is 0.059%/sqrt(d), the measured rate constant based on the 12-month stability data obtained at 5°C is 0.066%/sqrt(d). The measured HMW after 1 y is 3.98%, which agrees well with the predicted value of 3.87%. There is no meaningful difference after considering the assay variability of 0.3%. For the LMW, the predicted rate constant at 5°C is 0.00077%/d (0.28%/y). The measured LMW after 1 y is 1.57%, which agrees well with the predicted value of 1.61% (assay variability of 0.5%). For acidic species, where the predicted rate constant at 5°C is 0.00054%/d (0.20%/y), the calculated increase of acidic species is ~0.2% after 1 y, which is not considered to be a meaningful change (assay variability of 4%) and agrees well with the measured increase of 0.7%.

Predictive stability of high-concentration IgG1 formulations to support anti-SARS-CoV-2 mAb development

Development of casirivimab and imdevimab

Two anti-SARS-CoV-2 IgG1 antibodies, cov-mAb1 (casirivimab) and cov-mAb2 (imdevimab), were developed as a fast-track program during the initial phase of the COVID-19 pandemic. To enable rapid speed to clinic, an Arrhenius plotting approach was applied to support shelf life justification of the drug products. Specifically, at the beginning of the development, a formulation stability study at 5°C, 25°C, 30°C, and 40°C was set up to generate 1 month of stability data to predict long-term (12–36 months) stability at 5°C of cov-mAb1 and cov-mAb2. As both cov-mAb1 and cov-mAb2 are IgG1 and have similar biophysical properties and formulations compared to mAb* (Table S2) and are thus expected to follow a comparable degradation pathway, we focused stability modeling on the key stability-indicating attributes, HMW, LMW,

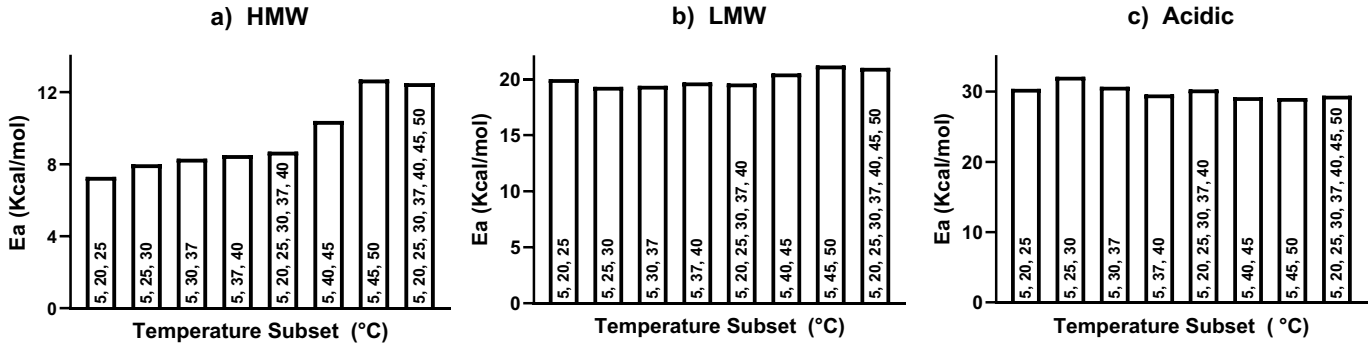

Figure 4. Robustness of kinetic model by the analysis of calculated activation energy using data subsets of different temperatures for a) HMW, b) LMW, and c) acidic species. Temperatures used in activation energy calculation are indicated in their respective bars. Each subset contains at least three temperatures.

and acidic, previously identified for mAb*. With a very limited development time and to ensure sufficient data generation to support rapid regulatory filings, short-time points, such as 0, 1, 3, 5, 14 and 28 d, were designed in the sample plan to determine the reaction kinetics and predict long-term storage stability at 5°C.

One month of stability data at 25°C, 30°C, and 40°C, consisting of six time points at each temperature, for cov-mAb1 and cov-mAb2 were used for kinetic modeling. The data at 5°C were not included for initial evaluation due to the lack of meaningful changes compared to method variability observed within 1 month of incubation, which resulted in the poor fitting for calculating rate constants. The HMW changes over time fit well with the square root of time kinetics with 1-month data, for both cov-mAb1 and cov-mAb2 the R^2 of the square root of time fit was >0.99 (Table S3). The LMW increases over time linearly with reasonably good fitting with all $R^2 > 0.99$, except at 25°C (the lowest $R^2 = 0.92$). The changes in the acidic species showed a good fit to the linear kinetics ($R^2 > 0.97$) at 30°C and 40°C, with a less desirable linear fitting at 25°C. Linear fit R^2 for 25°C data are 0.86 and 0.85, for cov-mAb1 and cov-mAb2, respectively. This is mainly because of the small changes that are within method variability of LMW and charge variants at lower temperatures.

With the rate constants determined from the accelerated temperature conditions of 25°C, 30°C, and 40°C, the Arrhenius plot was obtained by fitting $\ln(k)$ linearly with the inverse temperature (Figure S1). The long-term behavior of HMW, LMW, and acidic species at 5°C is predicted by using these apparent rate constants at 5°C to predict the quality attribute increase from time zero (Table S4). Using the predicted apparent rate constants at 5°C, after 36 months storage, the increases in HMW for cov-mAb1 and cov-mAb2 are projected to be 0.61% and 1.26%, the increases in LMW for cov-mAb1 and cov-mAb2 are projected to be 0.53% and 0.29% and the projected increases in acidic species for cov-mAb1 and cov-mAb2 are projected to be 2.4% and 0.99%, respectively. These predicted changes are small with respect to the stability acceptance criteria for these attributes, indicating that the formulation should be stable for up to 36 months. Notably, this data was also used to provide justification for the initial assignment of drug product shelf life of 12 months, demonstrating that limited, accelerated stability data can be used for shelf life justification in rapid response filings.

Global fitting of Arrhenius kinetics and bootstrapping were applied to generate prediction intervals for cov-mAb1 and cov-mAb2. Compared to Arrhenius plotting, where time- and temperature-dependent behavior are solved in separate steps, time and temperature in global fitting were simultaneously fit by combining the Arrhenius equation (Equation 5) with empirical equations for each attribute (Equation 1-3), producing a global fit of Arrhenius kinetics (Equations 6–8). While 5°C data within 1 month were not included for the Arrhenius plotting approach, the use of global fitting allowed for data at temperatures where minimal changes within method variability were included. Kinetic parameters were determined using stability data in the temperature range of 5–40°C where the same degradation kinetics are expected to apply (**Table S4**). Linear kinetics were applied for LMW and acidic species, while square root kinetics were applied for HMW. The predicted rates at 5°C were found to be comparable across Arrhenius plotting and global fitting approaches, demonstrating the potential use of global fitting to assess kinetics in a single step.

$$k(T) = A * \exp\left(-E_a/RT\right) \tag{5}$$

$$HMW(t,T) = HMW_0 + A_{HMW} * \exp\left(-E_a/RT\right) * t^{1/2} \tag{6}$$

$$LMW(t,T) = LMW_0 + A_{LMW} * \exp\left(-E_a/RT\right) * t \tag{7}$$

$$Acidic(t,T) = Acidic_0 + A_{Acidic} * \exp\left(-E_a/RT\right) * t \tag{8}$$

Prediction intervals were generated using bootstrapping.[12,20] Briefly, data was resampled from the original dataset to generate 1,000 bootstrap samples. Predictions were made for each bootstrap sample and the 95% confidence interval was determined as the central 95% of the dataset predictions. From the lower and upper confidence widths, the prediction width was calculated to incorporate variability of individual measurements as done previously.[21] Using 1 month of stability data, predictions of HMW, LMW, and acidic at 5°C were made up to 36 months using globally fit Arrhenius kinetics with prediction intervals generated via bootstrapping. Long-term storage stability data was used to validate the predictions of HMW, LMW, and acidic species. For HMW, LMW, and acidic species, validation data that were not used in the prediction were found to fall within the constructed 95% prediction intervals, confirming the ability to predict stability up to 36 months using 1 month of stability data (Figure 5). While HMW species retained a narrow 95% prediction intervals over the long-term prediction, LMW and acidic species had noticeably wide prediction intervals with increasing time. A potential reason for this is the higher method variability in determining LMW and acidic species, which leads to increased variability in the predictions at 36 months. Although there is wide variability at 36 months for LMW and acidic species, the predictions with the variabilities can be used to support an initial shelf life of 12 months.

Arrhenius plotting and global fitting approaches in applying empirical and Arrhenius kinetics were assessed for cov-mAb1 and cov-mAb2, with no meaningful difference in the predicted increases at 5°C from either approach (**Table S4**). After 36 months storage, the difference in predicted increases in HMW for cov-mAb1 and cov-mAb2 are 0.12% and 0.24%, the difference in LMW for cov-mAb1 and cov-mAb2 are 0.04% and 0.30%, and the difference in acidic for cov-mAb1 and cov-mAb2 are 0.99% and 0.02%, respectively. These predicted differences are within method variability for these attributes, indicating that both approaches have comparable prediction capabilities of long-term shelf life stability up to 36 months.

To support preclinical and phase-appropriate clinical trials, regulatory agencies require the production and testing of material to demonstrate that the drug remains stable over its

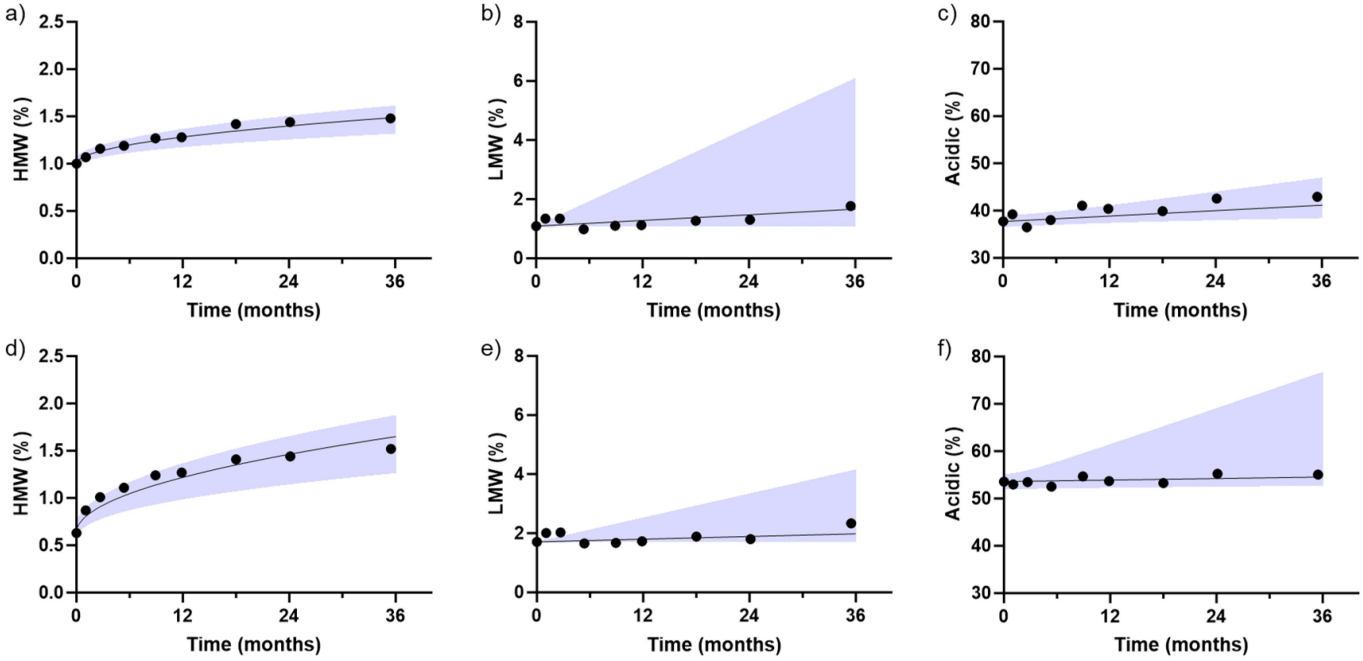

Figure 5. Quality attributes (HMW, LMW, and acidic) for (a-c) cov-mAb1 and (d-f) cov-mAb2 predicted over 36 months using 1 month of accelerated stability data and global fitting. Long-term prediction of HMW, LMW, and acidic species were made using 1 month of accelerated stability data from a separate development lot of material for cov-mAb1 and cov-mAb2. Solid lines indicate predicted values and shaded regions indicate 95% prediction intervals for each attribute.

shelf life. To this end, seven stability studies, encompassing multiple-manufacturing lots including early development and Good Manufacturing Practice (GMP) lots, were set up as part of the development of cov-mAb1 and cov-mAb2. GMP stability data are particularly important in regulatory submissions to ensure compliance with regulatory standards since the GMP drug products are administered to patients in clinical studies.

The predicted HMW, LMW, and acidic values for the seven long-term storage stability studies at 5°C for 12, 18, 24, and 36 months were calculated using the predicted apparent rate constants at 5°C by Arrhenius plotting and by global fitting (Table S5) and agreed remarkably well with the experimentally determined values for 12, 18, 24, and 36 months for cov-mAb1 and cov-mAb2 (Figure 6). The differences between the predicted and measured values at 5°C for 12, 18, 24, and 36 months by both approaches are mostly within the method variability and are small with respect to stability acceptance criteria for these attributes. There is no significant difference in terms of prediction accuracy by Arrhenius plotting and global fitting approaches. Additionally, there is no significant difference in prediction accuracy for stability periods of 12, 18, 24, and 36 months. The results demonstrated that both approaches can accurately predict the long-term stability up to 36 months. Therefore, the predicted apparent rate constants for cov-mAb1 and cov-mAb2 at 5°C, derived from the initial phase of the formulation development study using 1-month stability data of HMW, LMW, and acidic, can be used to justify the long-term storage stability of the same formulation with different manufacturing lots and product presentations (different containers and fill volumes), and can aid in shelf life justification of the drug product, facilitating rapid regulatory filing.

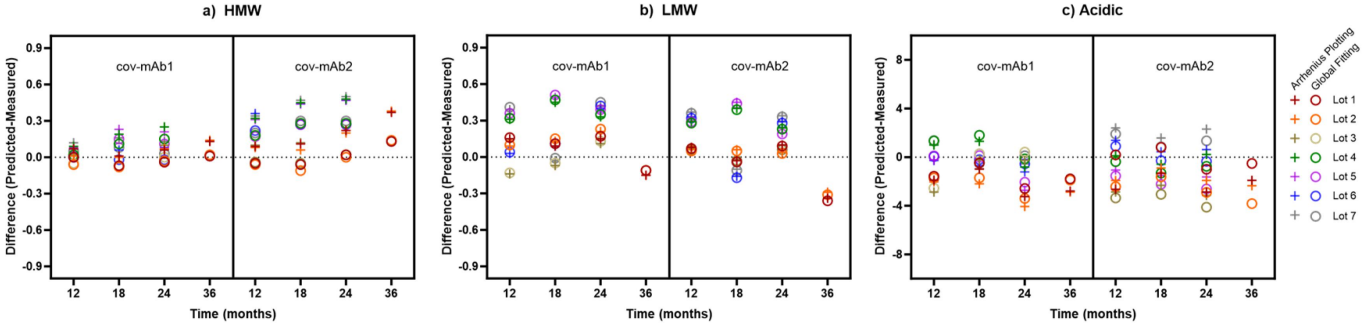

Figure 6. The difference of the predicted and measured values for cov-mAb1 and cov-mAb2 at 5°C for 12, 18, 24, and 36 months for 7 lots by Arrhenius plotting and global fitting approaches. (a) HMW species, (b) LMW species, (c) acidic species. Data up to 36 months are available for lots 1 and 2; data up to 24 months are available for lots 3 to 7. Arrhenius plotting (crosses, +) and global fitting (open circles, ○), lot 1 (red), lot 2 (orange), lot 3 (bronze), lot 4 (green), lot 5 (purple), lot 6 (blue) and lot 7 (gray) are displayed.

Application of the platform model to 5 additional IgG1 high-concentration formulations

The kinetic model using 1 month of accelerated stability data at multiple temperatures to predict long-term stability was applied subsequently to 5 additional anti-SARS-CoV-2 IgG1 high-concentration formulations, namely for cov-mAbs 3–7. These molecules were developed as the next-generation mAbs to treat emerging SARS-CoV-2 variants. To investigate if the inclusion of more time points and/or temperatures would improve the robustness of prediction, accelerated stability studies for each of the 5 cov-mAbs were set up with additional time points and temperatures compared to the study designs of cov-mAb1 and cov-mAb2. In addition to long-term storage stability at 5°C, three accelerated temperature conditions (25°C, 30°C, and 40°C) were tested for cov-mAbs 3, 4, and 5, while an additional accelerated temperature condition (37°C) was included for cov-mAbs 6 and 7. Four time points were included in the sampling plan for the first month, more data was collected at accelerated temperature conditions up to 6 months. Measured values of HMW, LMW, and acidic species up to 36 months were available to compare with the predicted values.

Predictions of long-term storage stability data were first made by global fitting using 1 month of stability data. HMW increase showed a good fit to the square root of time kinetics and LMW and acidic increases largely showed good fits to linear kinetics as was the case for cov-mAb1 and cov-mAb2 (Table S6). The projected HMW increases for cov-mAb 3, 4, 5, 6, and 7 are not greater than 3.2% after 36 months storage at 5°C. The projected HMW increases are acceptable with respect to the stability acceptance criteria for HMW of drug products. The projected LMW increases for five mAbs are not greater than 0.4%, and increases for acidic species are not greater than 2.6%. These predicted LMW and acidic increases are not considered to be meaningful and are within assay variability. Overall, the predicted values indicate that the formulations should be stable and can support the assignment of an initial drug product shelf life of 12 months, with the potential to support up to 36 months.

The predicted HMW, LMW, and acidic values at 5°C were comparable to the measured values for both approaches for cov-mAbs 3–7, with Figure 7 showing cov-mab3 stability predictions generated through global fitting as a representative case. For all five mAbs, these differences of predicted and measured values are within assay variability and are small with respect to stability acceptance criteria for these attributes. Measured long-term stability values are well aligned with the predicted values (Table S7) and are within the generated prediction intervals (Figure 7). Since the formation of HMW, LMW, and acidic species at 5°C can be small or near-zero, the attributes were also modeled at 25°C for up to 3 months to demonstrate the model capability at conditions where higher degradation rates are expected. Predicted stability attributes agreed well with measured data at 25°C (Figure S2, Table S8). Furthermore, the values predicted using globally fit Arrhenius kinetics are comparable to the values predicted using Arrhenius plotting (Figure 8, Table S7), further confirming the ability to use either approach for long-term stability predictions.

Model robustness and considerations

Global fits and bootstrapping were applied to different combinations of accelerated stability data to assess the accelerated stability study length that can be used to accurately predict long-term stability and the number of data points needed to generate accurate predictions. For cov-mAbs 3–7, up to 6 months of stability data were available at 25°C, 30°C and 1 month of stability data were available at 37°C, 40°C in addition to the long-term stability data at 5°C. Predictions of long-term

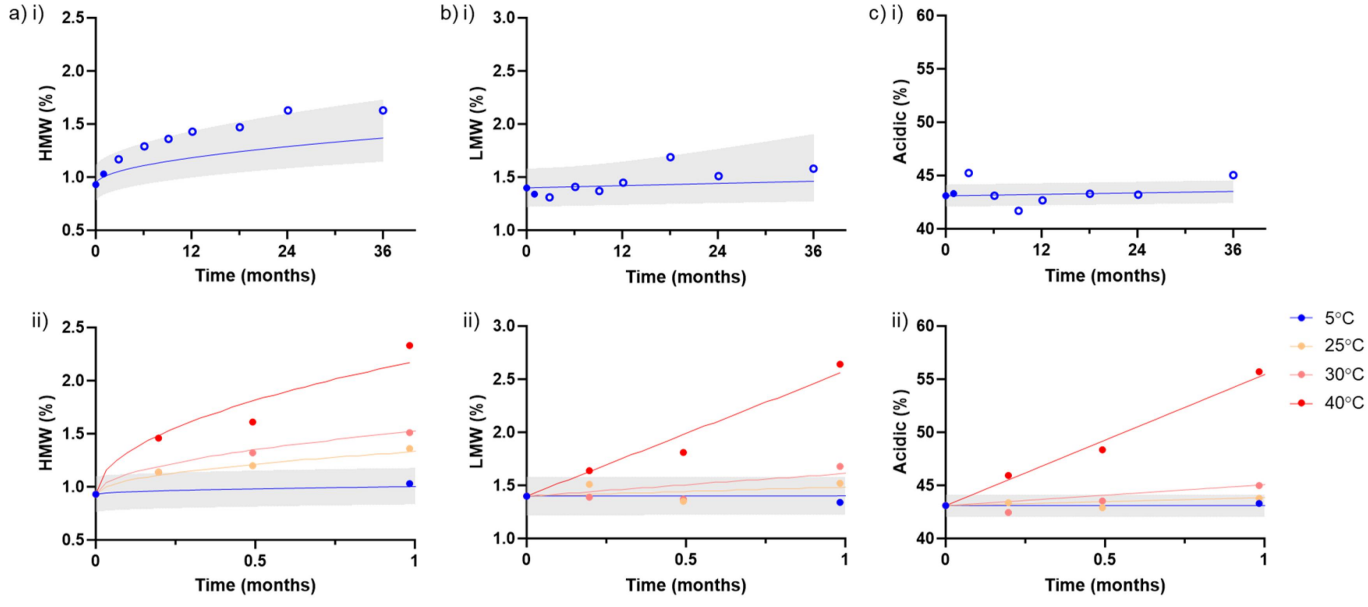

Figure 7. (a) HMW, (b) LMW, and (c) acidic species formation (i) predicted up to 36 months using (ii) 1 month of stability data at accelerated conditions and storage conditions for cov-mAb3. Prediction lines are plotted at different temperatures based on kinetics determined from 1 month of data. Filled markers represent 1 month of stability data that are used to generate the predictions, while open markers represent measured data exceeding 1 month that were not used in making predictions. cov-mAb 3 is shown as a representative example, with similar trends also observed for cov-mAbs 4–7.

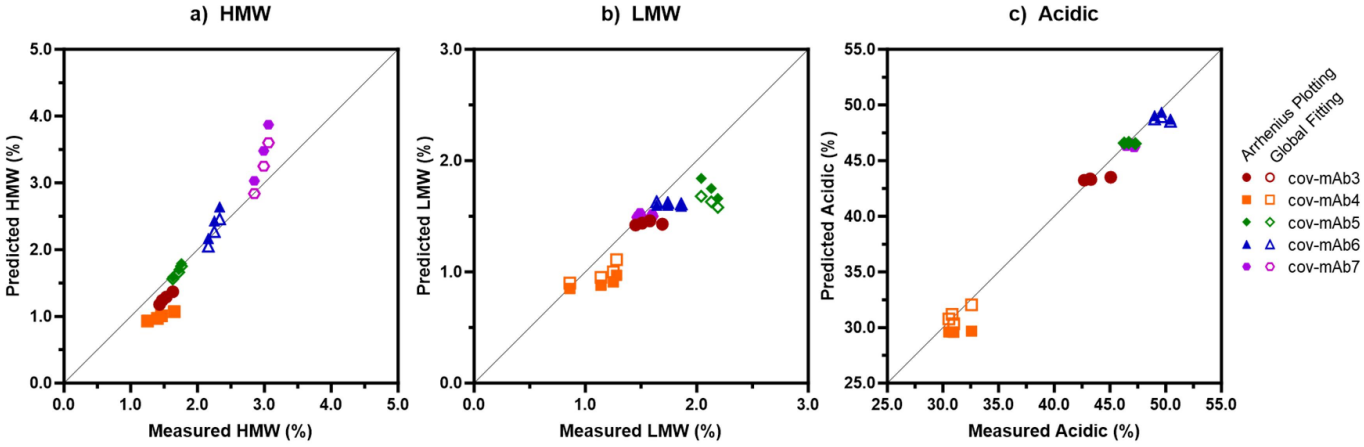

Figure 8. The predicted and measured values for cov-mAb 3–7 at 5°C for 12, 18, 24, and 36 months (a) HMW species, (b) LMW species, (c) acidic species. Datapoints of 12, 18, and 24 months are available for cov-mAb5 (green diamond), cov-mAb6 (blue triangle) and cov-mAb7 (purple hexagon); 12, 18, 24, and 36 months are available for cov-mAb3 (red circle) and cov-mAb4 (orange square). Global fitting of Arrhenius kinetics (open markers) and Arrhenius plotting (filled markers) approaches are displayed. MAbs with results from only one approach appearing on the plot have overlapping results for Arrhenius plotting and global fitting.

shelf life stability were made using up to 6 months of data, while limiting the accelerated stability data at temperatures ≥37°C used to 1 month (Figure 9). Stability data at temperatures ≥37°C at 3 months were not used due to the formation of HMW species of higher order than dimers that predominantly occurred at 5°C. Of the three attributes assessed, HMW predictions made were found to be comparable when using 1, 3, or 6 months of data ranging from 5°C to 40°C. Predictions of LMW and acidic species formation made using 1 month of data were found to be more sensitive to method variability due to the small change or lack thereof that is observed for both attributes within 1 month. While the predictions for attributes were similar when using 1, 3, or 6 months of data, the presence of out-of-trend points, even if still within method variability, can lead to differences in prediction interval width as the resampled datasets during bootstrapping account for future instances of the out-of-trend points. This is seen in Figure 9c, where the out-of-trend point at 3 months leads to noticeably wider prediction intervals for predictions using 3 months of data compared to the predictions using 1 or 6 months of data.

To inform future study designs, the minimum number of datapoints sufficient to generate long-term predictions was assessed. Kinetic parameters at 5°C were determined using the mAb* stability dataset (21 data points for HMW, LMW and 19 data points for acidic species up to 1 month). To assess the minimum data points needed for an accurate determination of kinetics, subsets were randomly drawn (n = 1,000) from the full dataset and used to calculate kinetic parameters for HMW, LMW, and acidic species formation. From visual inspection of Figure 10, kinetics for HMW formation can be reasonably predicted using a total as low as 8 datapoints, and LMW and acidic species can be reasonably predicted using at least 12 datapoints. For LMW and acidic species formation, more points may be considered due to the variability in LMW and acidic species measurements. Using these results, guidelines for accelerated stability study design can be developed to generate sufficient data for long-term stability prediction. Our results indicate that a set of 12 data points can adequately predict kinetics as opposed to the full set of 21 data points reported in Figure 10. This can be accomplished by relatively simple study designs, such as incorporating four timepoints at three accelerated temperature conditions in addition to data collected at storage conditions, if the study is well-designed, and time points are spaced out to provide a good dataset to generate predictions. Based on our results, this can be incorporated within the first month of a stability study consisting of long-term storage and accelerated conditions. While a minimum of 12 points can be used to predict the kinetics of HMW, LMW, or acidic species formation, studies need to be

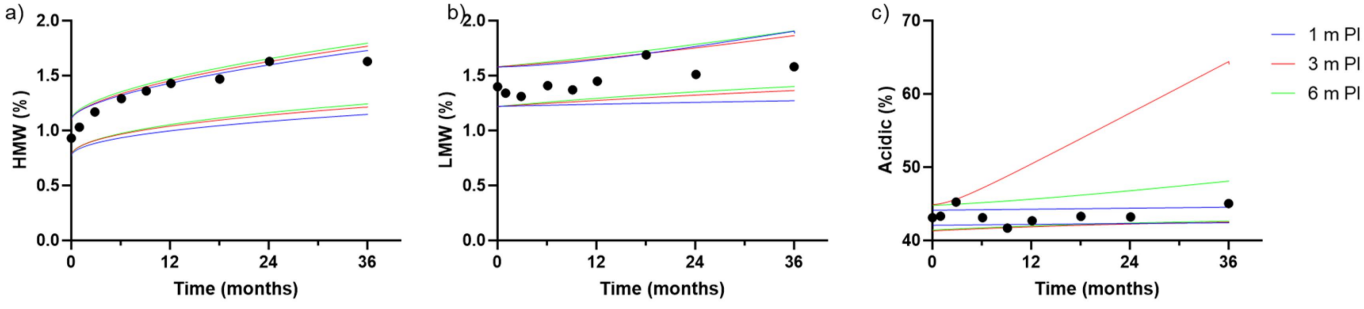

Figure 9. Generated prediction intervals (PIs) of cov-mAb3 using 1 month of data (blue), 3 months of data (red), and 6 months of data (green) for (a) HMW, (b) LMW, and (c) acidic species.

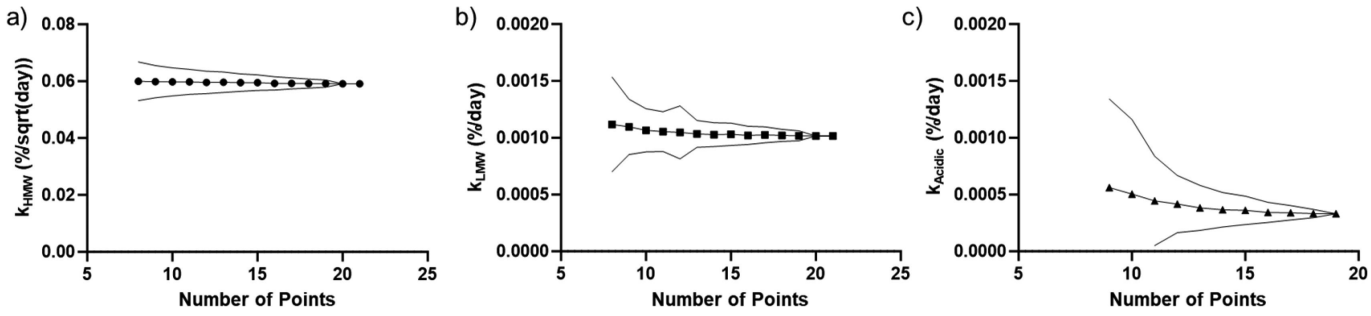

Figure 10. Analysis of statistical convergence of kinetic parameters for HMW, LMW, and acidic species formation using subsets of varying size. Solid lines with markers represent average kinetic rate constants for a dataset of the specified size and solid lines without markers represent ± 1 standard deviation. Subsets were randomly sampled from the full dataset 1,000 times.

carefully designed to space out the points at which data is collected, as well as to consider the quality attributes being modeled and how susceptible their predictions may be to method variability. Improperly designed studies with few data points can lead to high variability in the predicted rate constants, depending on the quality of the collected data.

Discussion

In our risk-based predictive stability approach, we characterized the stability behavior and temperature dependence of the key stability-indicating quality attributes (HMW, LMW, and acidic species) of eight high-concentration IgG1 mAb liquid formulations and demonstrated that the attributes have consistent kinetic behaviors. Arrhenius-based empirical kinetics can be used to model the behavior of the quality attributes at storage conditions and accurately predict long-term stability for seven anti-SARS-CoV-2 IgG1 formulations, the results of which were included in assessing drug product stability and justifying product shelf life. Our predicted stability results showed excellent agreement with real-time stability data for up to 36 months using accelerated stability data as early as 1 month obtained from at least three accelerated temperature conditions.

Predictability of stability-indicating attributes

The tested IgG1 mAbs have favorable biophysical properties that are indicative of good developability and long-term stability (**Table S2**).[22–24] The thermal-melting temperatures (T_m) measured by differential scanning calorimetry (DSC) are higher than 60°C, which indicates that the molecules have good thermal stability. At the accelerated conditions up to 40°C, which is more than 20°C lower than T_m, the antibody domain structures are maintained with minimal thermal induced unfolding. The tested mAbs have isoelectric points (pI) between 8.9 and 9.3, making them positively charged at pH 6.0. This minimizes unfavorable protein–protein interactions and ensures good colloidal stability, as indicated by the large positive diffusion interaction parameter, k_D, measured by dynamic light scattering (DLS). This ensures that all the tested IgG1 mAbs follow a comparable degradation pathway under accelerated conditions (up to 40°C) as they do at the storage temperature (5°C), allowing a simple kinetic model to determine degradation rate constants, which is critical for the risk-based stability prediction.

Based on our experience, the key stability-indicating attributes monitored for product release and stability are HMW and LMW species by SE-UPLC, and acidic and basic charge variant species by iCIEF. The formation of HMW, LMW, and acidic species are not caused by a single degradation pathway, but instead by a combination of different chemical reactions. While a detailed mechanistic understanding of the reactions in the liquid formulation is still ongoing and will not be discussed in this work, formation of HMW, LMW, and acidic species are generally well-understood.[25–28] HMW increase is typically caused by aggregation that leads to the formation of dimers and higher-order oligomers, which can be impacted by protein concentration and temperature in addition to intrinsic properties of the mAb.[25,26] LMW increase can be caused by fragmentation of mAbs due to hydrolysis or exposure to extreme conditions.[28] Formation of acidic species is largely caused by deamidation of asparagine to aspartic acid residues, but other pathways such as oxidation can also have an impact on the formation of acidic species.[28] The number of deamidation sites and oxidizable methionine residues are consistent within constant regions of IgG subclasses, but amino acid heterogeneity in variable regions can also impact acidic charge variant formation.[27] As the mAbs discussed here are all IgG1 with similar properties and in the same formulation (**Table S2**), their quality attribute trends can be predicted by a single model. Furthermore, being an empirical model, it allows typically complex and elaborate processes, such as aggregation, to be modeled by a single equation.

Our results show that the dimer peak is the only observed HMW species after storage at 40°C for 1 month, 25°C for 6 months, and 5°C for up to 36 months, therefore dimer formation is the primary degradation pathway under the long-term storage conditions. However, larger oligomer aggregates were observed after incubation at 40°C for 3 months and at 45°C and 50°C for 1 month (Figure 2a). These observations indicate that the larger oligomer aggregates observed at the higher temperature conditions (45°C and 50°C) and 40°C beyond 1 month do not reflect the long-term storage stability. Therefore, data at 40°C beyond 1 month, and at 45°C and 50°C were excluded from the model analysis. The absence of higher-order aggregates simplifies the kinetics. Our empirical model indicates that HMW formation follows apparent zero-order

kinetics with respect to the square root of time, which has been postulated to be the result of a superposition of reactions from multiple substrates, each of which has a different degradation rate.[18,29] The changes in the acidic and LMW species in the temperature range of 5–50°C are well described by the linear fit (zero-order kinetics). As shown in Figure 2b, the charge variant changes for mAb* after 1-month incubation are mainly the increase in acidic species accompanied with the decrease in Main, while basic species did not show meaningful changes, indicating the increase in acidic species is the main pathway for charge variant changes. The LMW species formed at 5–50°C includes fragments, such as Fab and a one-armed antibody. The empirical model of square root of time for HMW and a linear model for acidic and LMW species successfully describe the kinetics. This allows calculation of a single apparent rate constant for all studied IgG1 mAbs, indicating a consistent underlying pathway of HMW, LMW, and acidic species formation.

While this model was established for IgG1 mAbs with similar biophysical properties and formulations, a similar workflow can be implemented to develop models for other formats, modalities, and formulations where prior knowledge is available. In these cases, risk should be assessed when developing a new model. This work was supported by our historical experience in successfully developing >20 IgG1 mAb drug products, which enabled the use of a full stability dataset (i.e., encompassing multiple temperatures and long-term storage stability data) to establish empirical equations for key quality attributes. Empirical equations can then be applied to short-term accelerated data to make stability predictions. Such approaches can be useful not only in early-stage drug product development of multiple mAbs with similar formats, biophysical properties, and formulations, but also in late-stage drug product development where long-term stability data from early-stage can be leveraged to establish the model.

Arrhenius plotting and global fitting for long-term stability predictions

Both Arrhenius plotting and global fitting approaches resulted in comparable calculated rate constants at the recommended long-term storage temperature, with the predicted long-term stability by both approaches also agreeing with experimental results up to 36 months. To ensure the accurate prediction of long-term stability by risk-based predictive stability approach, it is essential to confirm the temperature range over which the same kinetic behavior applies under storage conditions. The Arrhenius plotting approach is able to identify temperatures where Arrhenius kinetics cannot be applied based on the goodness-of-fit. Our design includes four time points within the 1-month testing period with at least three accelerated temperature conditions. For the IgG1 mAbs, the temperature range over which the same kinetic behavior applies is 5–40°C; having rate constant data from three temperature conditions, such as 25°C, 30°C, and 40°C works well when the rate constants obtained have good statistical fits at all three temperatures. However, in cases where changes in the attributes are small (within or close to the method variability) within the 1-month study period, this can lead to large uncertainties in calculating the apparent rate constants. Adding another temperature condition between 30°C and 40°C, such as 35°C, is advantageous in ensuring the quality of fitting and Arrhenius kinetics can be applied to determine kinetic parameters at long-term storage temperature.

While the Arrhenius plotting approach requires temperature conditions where a meaningful change in the quality attribute is detected, one advantage of global fitting is that data at individual temperatures are combined into a single dataset. This dataset is then fitted for Arrhenius kinetics against time and temperature simultaneously, allowing for the inclusion of data where no meaningful change in attributes with respect to method variability over the period studied (e.g., 1 month at 5°C) is observed. This dataset helps to establish the variability in the overall dataset and is useful in subsequent generation of prediction intervals via bootstrapping. Prediction intervals generated in this way represent the predicted kinetics at the storage temperature while also considering the statistical variability in the experimental measurements to generate the dataset and prediction intervals.

Data requirements for robust predictions

The results presented here demonstrate the capability to predict shelf life stability up to 36 months from as little as 1 month of stability data obtained from accelerated stability studies with at least three accelerated temperatures, which extends previously reported results in the literature that predict 36-month stability using 3–6 months of accelerated stability.[12] Notably, inclusion of accelerated stability data greater than 1 month did not lead to a meaningful increase in prediction accuracy for HMW results, suggesting that 1 month of stability data is sufficient for predicting long-term HMW formation if enough datapoints are included in the study.

For storage conditions (at 5°C), where reactions are slower and thus there is a lower measurable change in quality attributes, including greater than 1 month of stability data may have varying effects on prediction accuracy depending on how the measured change compares to method variability. For HMW species, where the change after 3 and 6 months exceeds the method variability, the inclusion of >1-month storage stability data at 5°C can improve the prediction accuracy, but does not drastically impact it. Alternatively, for LMW and acidic species, predictions made including 3- or 6-month storage stability data instead of 1 month are more susceptible to variability in measurements due to the lower expected changes for these attributes at different temperatures relative to method variability. For example, inclusion of 3-month storage stability data in the prediction of acidic species for covmAb3 leads to a larger upper prediction width even though the measured change is within method variability (Figure 9). Alternatively, when 6-month storage data is also included, the prediction interval is more comparable to the prediction using 1 month of data due to the 6-month measurement having a lower measured change compared to 3 months, demonstrating the overall susceptibility to method variability when there are no meaningful changes during the study period. This

means that 1 month of accelerated stability data may be enough to make accurate long-term predictions if data quality is ensured and assessed for outliers.

With accelerated data collection limited to 1 month to enable accurate stability predictions, sufficient data need to be generated within the shortened timeframe to account for data and model variability. In this work, a total of 12 data points within 1 month were determined to be the minimum dataset size that would enable reproducible kinetic predictions, which is lower than the 20–30 data points recommended for AKM and achievable via a simple study design during the first month of a stability study.[17] HMW measurements were predicted with higher accuracy compared to LMW, which is likely due to HMW being more easily resolved from the main peak in SE-UPLC chromatograms and a higher measurable change compared to LMW. Similarly, method variability in resolving acidic species from the main species in electropherograms also leads to wider prediction intervals for acidic compared to HMW species. While a minimum of 12 points was identified to be sufficient for accurate long-term stability predictions, the inclusion of additional data points can help account for variabilities in LMW and acidic measurements. Therefore, including at least 4 time points at three temperatures should yield sufficient data for reliable stability predictions of HMW, which is a typical critical quality attribute for mAbs, but a study using an additional temperature condition may enable better predictions for LMW and acidic species.

Current and emerging practices in predictive stability

Generally, risk-based predictive stability approaches are not an established practice for biological drug product development. Linear extrapolation is more common in assessing drug product long-term stability for clinical trial applications (CTAs) based on ICH Q1.[5] We compared the prediction accuracy of linear extrapolation of 6 months of storage stability (5°C) data with that of a risk-based empirical model using global fitting and 1 month of accelerated data with at least 3 temperatures and 1 month of storage stability (5°C) data for the studied IgG1 mAbs (data not shown). Compared with the linear extrapolated HMW, LMW, and acidic values, the predicted values from the risk-based empirical modeling approach agreed much better with the measured values and resulted in narrower prediction intervals compared to linear extrapolation results. Extrapolation of HMW species generally led to overprediction of the increase over time compared with measured values and a wider 95% confidence interval compared to predictions made using the empirical square root of time model. For LMW and acidic species, because of the minimal changes at 5°C for 6 months that may fall within method variability, linear extrapolation values may not align well with real-time measurements, especially for stability over 1 y, and exhibit a much wider 95% confidence interval compared to risk-based empirical modeling approaches. Incorporating stability data at accelerated temperature conditions led to more accurate predictions with narrower prediction intervals when using only 1 month of stability data compared to the linear extrapolation of 6 months of storage stability data alone, thus enabling an earlier and more robust stability prediction and initial shelf life proposal.

In addition to the empirical models reported in this work, first-order kinetic models and, more broadly, AKM approaches have also been reported to accurately predict long-term stability of biologics using accelerated stability data by fitting reaction orders in kinetic models.[11,12,17] As opposed to emphasizing the reaction order of the degradation kinetics, we instead relied on the empirical modeling based on characterization of kinetic behaviors for each quality attribute assessed. For LMW and acidic species, the predicted long-term stability by a linear fit model shows good agreement with the experimental results. For HMW, the linear fit model may work well with certain antibody formulations (data not shown) but was not a good fit for HMW formation in high-concentration IgG1 formulations (Figure 1a). An empirical model using the square root of time for HMW formation of IgG1 formulations was able to accurately predict HMW formation kinetics and agreed well with long-term stability data, leading to a dramatically shortened required assessment time. While AKM identifies the most statistically appropriate model, it requires at least 20–30 experimental data points and a significant change in the quality attribute being assessed.[17] Thus, an empirical model that has been established using prior knowledge of stability behavior of quality attributes may be more favored for high priority, rapid response programs where only limited datasets are available within 1 month. Specifically, we focused on using a 1-month study with at least 12 total data points across 3 accelerated temperatures to predict long-term shelf life stability. Our approach demonstrated good prediction accuracy while reducing the number of datapoints and the time required.

Applying predictive stability to support product shelf life setting

In addition to HMW, LMW, and acidic charge variants as key stability-indicating quality attributes, additional critical quality attributes of mAb drug products typically include visual appearance, clarity, pH, protein content, potency, and subvisible particulates. While these attributes are important for product quality, they either have high method variability, as in the case of potency and subvisible particulates, or do not typically change significantly during accelerated stability studies or over the product shelf life because they are controlled by well-developed and established drug substance and drug product manufacturing processes and are thus not key stability-indicating attributes for IgG1 mAbs. Therefore, the predictive stability of the key quality attributes, HMW, LMW, and acidic species, as stability-indicators, can be used to support product shelf life justification, together with the manufacturing process controls and the quality data obtained for the manufactured drug product.

Given our approach requires 1-month stability data, it can be applied to developability assessment and formulation development to generate a robust understanding of the shelf life stability of key stability-indicating attributes to enable more informed decision-making in selecting the drug candidate and drug product formulation. More importantly, our risk-based

empirical model can be particularly useful for rapid response programs with limited amounts of research stability and little GMP stability data, as was the case during the development of mAbs for the treatment of COVID-19. In fact, cov-mAb1 (casirivimab) and cov-mAb2 (imdevimab) were selected as the lead candidates to initiate clinical trials rapidly at the early phase of the SARS-CoV-2 pandemic.[30,31] After characterizing their biophysical and physicochemical properties, a 120 mg/mL formulation with 10 mM histidine, 8% (w/v) sucrose, and 0.1% polysorbate 80 at pH 6.0 were selected to enable both intravenous and subcutaneous administrations. A short-term (1 month) accelerated predictive stability study was designed and executed to generate the stability data to be used for predictive stability to assess long-term stability of the formulations. Additionally, the predictive model, which was developed from an early development lot of material, has been effectively applied to subsequent manufacturing lots, including GMP lots (Figure 6). Arrhenius-based empirical kinetics were able to successfully predict that the changes of HMW, LMW, and acidic species are well within their respective acceptance criteria up to at least 24 months. Predictions based on this model were used to justify the product shelf life and to support Investigational New Drug (IND) and CTA filings.

This initial shelf life strategy with predicted shelf life stability was included in IND and Investigational Medicinal Product Dossiers submitted with 10 regulatory packages. In the context of great public health need to combat SARS-CoV-2, the use of predictive modeling for an initial shelf life of 12 months or less was largely, but not universally, accepted. The application of these models and strategies has not been used outside of rapid response assessments of initial clinical shelf life. In our view, the preliminary review and adoption of initial product shelf life set by kinetics-based predictive stability approach, even in limited cases, is encouraging and suggests that further experience and validation of stability prediction models for biologics may result in broader acceptance of modeling approaches by health authorities. This is consistent with recent recommendations by the Accelerated Drug Development working group of the IQ Consortium, highlighting predictive stability models as an opportunity to accelerate pathways toward regulatory approvals.[32] While modeling of small molecule drug products has long been established, the growing number of examples in the literature,[11–13,17,33] as well as pandemic experience in using predictive stability in regulatory filings, has led to increasing confidence in the ability to model stability behavior of biologics.[32]

Conclusions

The development of predictive stability models allows for the acceleration of decision-making during drug product development. In this work, a modeling approach based on Arrhenius and empirical kinetics was developed for the prediction of key stability attributes of high-concentration mAb formulations for up to 36 months using 1 month of stability data from three or more accelerated temperature conditions. Our risk-based empirical model for long-term stability offers more accurate predictions and has narrower prediction intervals compared to the currently recommended linear extrapolation that is widely adopted by regulatory agencies. This model is particularly useful for rapid response programs, as was demonstrated by our successful experience using it to support shelf life justification in regulatory filings for investigational antibodies during the COVID-19 pandemic.

Recently, the ICH Quality Discussion Group (QDG) recommended the revision of the ICH series of stability guidelines,[3] including ICH Q1 and Q5C. Our kinetic analysis and risk-based stability prediction provide further evidence of available alternative prediction methods and the need to update these guidelines, serving as a notable example of a case where shelf life justification for CTA can be based on predictive stability.

While the model developed here has been shown to be useful for high-concentration IgG1 mAb formulations, the approach can be applied to biological drug product formulations at different concentrations or different formats, such as IgG4, bispecific constructs, or bioconjugated molecules. Additionally, outside of regulatory filing for high priority programs and rapid response settings, this model can be used to accelerate decision-making during drug development based on limited datasets, such as during developability assessments for drug candidates or formulation development studies.

Materials and methods

Materials

All tested therapeutic IgG1 mAbs were produced at Regeneron Pharmaceuticals, Inc. using standard manufacturing processes. Pharmaceutical-grade chemicals were used for formulation preparation, and HPLC-grade chemicals were used for chromatography and electrophoresis. Sodium chloride (catalog # 3628-01), sodium phosphates monobasic (catalog # 3820-01), and dibasic (catalog #3817-01) were purchased from J.T. Baker. Sodium perchlorate (catalog # 310514) and urea (catalog # U0631) were purchased from Millipore Sigma. Histidine (catalog # 32924) and histidine hydrochloride monohydrate (catalog # 32930) were obtained from Ajinomoto. Sucrose (catalog # S-124-2-MC) and polysorbate 80 (catalog # SR40925) were obtained from Ferro Pfanstiehl and Croda, respectively.

Stability studies

MAb* is an IgG1 molecule, which was formulated at 150 mg/mL protein concentration in 10 mM histidine, 10% (w/v) sucrose, and 0.01% polysorbate 20 at pH 6.0. Seven anti-SARS-CoV-2 IgG1 mAbs (cov-mAb 1, 2, 3, 4, 5, 6, and 7) were each formulated at 120 mg/mL protein concentration in 10 mM histidine, 8% (w/v) sucrose, and 0.1% polysorbate 80 at pH 6.0. Stability studies were set up according to the conditions listed in Table S9. Samples were stored at designated accelerated and storage temperature conditions, staged upright, and pulled at specific time points.

A panel of analytic tests was performed to monitor common stability-indicating attributes and understand the molecular degradation pathways (data not reported). SE-UPLC and

iCIEF were used to monitor key stability-indicating and shelf life limiting attributes, specifically, HMW species, monomer, and LMW species by SE-UPLC, and acidic charge variants (acidic), main, and basic charge variants (basic) by iCIEF. The key stability-indicating attributes were identified as HMW, LMW, and acidic species. Minimal changes (within method variability) for basic species were observed for all IgG1 mAbs under the conditions studied; therefore, only acidic species were considered for charge variant analysis and predictions.

Analytical methods

Size exclusion-ultra performance liquid chromatography

A Waters Acquity (Milford, MA) system with Waters Acquity UPLC BEH200 sec column was used for SE-UPLC analysis. A typical experiment was set at a flow rate of 0.3 mL/min, with a mobile phase containing 10 mM phosphate, pH 7.0, 0.5 M sodium chloride for mAb* or 0.5 M sodium perchlorate for cov-mAbs 1–7, and absorbance at 280 nm was monitored for quantification. The HMW, LMW, and monomer species were integrated into three different groups of size variant species and reported as percentages of total peak area.

Imaged capillary isoelectric focusing

Protein Simple iCE3 Charge Variant Analyzer (iCE3^{TM}, Protein Simple, CA) was used for charge variant analysis. Samples were prepared with pH 3–10 Pharmalytes, methyl cellulose, pI markers, and 0.5 mg/mL antibody. iCIEF separation was carried out using an FC-coated Cartridge (Protein Simple #101701), pre-focused at 1.5 kV for 1 min followed by focusing at 3 kV for 7 min. The acidic, basic, and main regions were integrated into three different groups of charge variant species and reported as percentages of total peak area.

Kinetic model

Data analysis, stability empirical model construction, and graph creation were performed using JMP statistical software (version 17). Figures were created using GraphPad Prism (version 9). Two approaches in applying empirical and Arrhenius kinetics are assessed within this work to predict reaction kinetics at storage conditions of 5°C, namely Arrhenius plotting and global fitting. Global fitting of Arrhenius kinetics and bootstrap analyses to generate prediction intervals were performed using functions in the SciPy[34] and Scikit-learn[35] libraries of Python (version 3.9).

Arrhenius plotting

For the Arrhenius-plotting approach, the apparent rate constants (k) were first obtained at defined temperatures range (5–40°C) by empirical fitting (square root of time fit for HMW, linear fit for LMW and acidic) (Equations 1–3). Then, Arrhenius plots were then obtained by plotting ln(k) against the inverse temperature $\left(\frac{1}{T}\right)$. The linearized Arrhenius equation is used to calculate the apparent rate constants at the storage temperature of 5°C (Equation 4).

Global fitting

Global fitting of Arrhenius kinetics was also used to calculate long-term storage stability using accelerated stability data. For long-term stability predictions, accelerated datasets consisted of 1 month of stability data with time and temperature as independent variables that lead to changes in HMW, LMW, and acidic species content.

Parameter fitting for Equations 6–8 was done through nonlinear regression analysis using an in-house based Python script to determine activation energies and pre-exponential factors for each attribute after which R^2 was used to assess the fit of the data to the calculated model. The calculated kinetic parameters were then used to predict the HMW, LMW, and acidic species trends during long-term storage at 5°C.

To accurately assess the prediction of long-term storage stability, bootstrapping analysis was used to generate 95% prediction intervals. For each prediction, a given set of accelerated experimental data consisting of time, temperature, and measured HMW, LMW, and acidic species values was resampled with replacement to generate a total of 1,000 datasets. Kinetic analysis by globally fitting Arrhenius kinetics was applied to each dataset. From the 1,000 predictions, the central 95% of the data were used to generate confidence intervals. Prediction intervals were then generated using the error from the fitted model (confidence widths) and expected variability in the data as done previously.[20]

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