High Throughput Prediction of the Long-Term Stability of Pharmaceutical Macromolecules from Short-Term Multi-Instrument Spectroscopic Data

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ABSTRACT: Changes in the measurements of a macromolecular biopharmaceutical's physical form are often used to predict changes in the drug's long-term stability. These can in turn be used as important markers of changes to a drug's efficacy and safety. Such stability estimates traditionally require human judgment and are frequently tentative. We introduce methods for developing mathematical models that predict a drug's long-term storage stability profile from measurements of short-term physical form and behavior. We measured the long-term (2 year) chemical and colloidal stability of Granulocyte Colony Stimulating Factor (GCSF) in 16 different liquid formulations. Shortly after formulations were placed on stability, we also employed various spectroscopic techniques to characterize the short-term thermal unfolding response of GCSF in the 16 formulations. The short-term data were processed using several data reduction methods, including reduction to spectra at low temperature, to melt curves, and to transition temperatures. Least squares fitting was used to predict the long-term stability measurements from the reduced short-term spectroscopic measurements. On the basis of the cross-validation and a permutation test, many of the long-term stability predictions have less than 1% probability of occurring by chance. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:828–839, 2014

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INTRODUCTION

The formulation development process for new protein drug candidates typically uses experimental methods covering a wide range in complexity. Techniques range from those that are expensive, time consuming, and difficult to perform, such as those that assess biological attributes during long-term stability studies, to techniques that are relatively inexpensive, fast, and easy to perform, such as those that primarily use spectroscopic or chromatographic instruments.

Slower, more expensive techniques typically produce information that is more directly relevant to the optimization of formulations and manufacturing processes. These complex methods include time consuming and labor intensive projects such as studies of drug efficacy using animal models and studies of the long-term stability of drugs. A protein drug's long-term stability is typically investigated simultaneously in many formulations, at many timepoints and under several storage conditions. Both intact and degraded forms of the protein are detected and characterized at regular intervals over a period of up to 3 years, using a panel of experimental techniques for each formulation, storage condition, and time point. Such a study can easily require several thousand experimental runs.

Faster, less-expensive techniques typically produce information that is only indirectly indicative of the differences observed in more complex, biologically relevant studies. Examples of faster analytical techniques include immunological binding assays, chromatographic studies during forced degradation studies of drug stability, and various spectroscopic techniques such as circular dichroism (CD), absorbance, and fluorescence spectroscopy. Although the information obtained may be more difficult to interpret, these methods allow one to explore drug formulation design spaces more completely and with greater freedom. More rapid experiments are also necessary during manufacturing process development and maintenance. For example, when a production issue or question arises, one cannot wait years for an answer that is based on long-term stability data.

As fast and slow methods have separate strengths, it is desirable to combine strengths in a manner that achieves optimum speed, cost, and ease of use while producing reliable assessments of changes in long-term stability.

A number of traditional and newly developed methods exist to bridge the gap between slower, more expensive techniques and faster, less expensive ones. Melting temperature assays, for instance, use thermal transition temperatures as a rough qualitative indication of differences in stability of different protein formulations. This imprecise knowledge may then be used to determine the focus and extent of long-term studies. Another qualitative method is the comparison of CD spectra to determine whether two preparations of a protein are sufficiently

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similar for therapeutic use. Qualitative methods require human judgment, which hinders automation and the quantification of model scope and errors. This in turn may impede the use of qualitative methods in high-throughput exploration of formulation spaces.

A common quantitative approach to inferring the long-term behavior of proteins between different formulations is by interpolating between the formulations via polynomial or neural network models. Interpolation is problematic because combinations of formulations must also be tested to develop the interpolational model. Therefore, to perform a full exploration of a formulation space using traditional methods, the number of formulations that would need to be tested with slower, more expensive techniques is exponentially large.

Another example of the quantitative prediction of long-term behavior is the fitting of aggregation models to short-term data. In some cases, such models allow the extrapolation of protein aggregation behavior at high temperatures to lower temperature long-term storage conditions. Non-Arrhenius models of aggregation behavior are often required due to the multistage aggregation kinetics and complex unfolding behavior of proteins.[1–4]

Predictive methods would ideally possess the following three traits. First, they would have no built-in limitations regarding the type of data predicted and the data from which they predict. For instance, in addition to aggregation rates, one may want to predict the long-term chemical stability or biological activity of a protein. One may want to model these data in terms of information contained in lower cost chromatographic assays, spectroscopic studies, or biological measurements. Second, the models should be quantitative to permit automation and allow the estimation of model scope and error. Third, the method should not require interpolation between formulation conditions.

In this paper, we have achieved the goals just described by combining three independently developed approaches. The approaches existed previously but had never been put together effectively. We combined multidimensional, multi-instrument spectroscopic datasets, and analysis methods, as developed in the empirical phase diagram technique[5–8] with extensive long-term stability data characterizing a protein's response to formulation parameters,[9,10] along with multidimensional predictive modeling techniques. In the current implementation of our approach, the results of long-term stability measurements are predicted from more rapid spectroscopic testing.

Under the new methodology, formulation parameters need not be used in the models. Models are developed by adequately investigating a protein's principal modes of behavior, rather than by exploring a large number of formulations and their combinations. Models may thus be developed using a relatively small number of formulations, and then applied to a much larger formulation space using only rapid measurements of short-term behavior.

Our method is based on the proposal that two protein formulations with similar short-term behavior will have similar long-term behavior. In other words, we interpolate between the long-term behaviors of protein formulations using short-term behavior as a guide. The method can fail when two formulations exhibit similar short-term behavior but have dissimilar long-term behaviors. To prevent or correct this situation, one would need to incorporate short-term experimental techniques that are sensitive to the differences which determine long-term stability.

The use of general purpose modeling techniques in principle allows one to use arbitrary types of data. Spectroscopic methods, for example, are considered to be rich in information concerning the physical state of a protein drug, but the complexity of macromolecular behavior can hide the meaning of patterns found in these measurements.[11–27] By developing predictive models of difficult to obtain data in terms of more easily obtained data, our method automatically discovers and utilizes patterns in the easily obtained data, so that one may extract more information from rapid techniques and potentially reduce the complexity and cost of long-term stability studies.

MATERIALS AND METHODS

Long-Term Stability Studies

Thelong-term stability studies are presented in summary form here. For details of the preparation and testing of formulations, see Refs. 9 and 10. Granulocyte Colony Stimulating Factor (GCSF) was obtained from Roche Diagnostics GmbH (Penzberg, Germany). The protein formulations were prepared following a factorial experimental design, alternating acetate and citrate buffer systems, and varying buffer concentrations, pH, and concentrations of Tween80 and hydroxypropyl-β-cyclodextrin (HP-β-CD). The formulations are numbered 11–26. Parameter values for each formulation are shown in Table 1.

Shortly after formulation, thermal unfolding transition midpoints (T_m) were obtained by differential scanning calorimetry with a 90 K/h heating rate.

Accelerated and real-time stability studies were then performed by storing the formulations at 40°C for 3 months, 25°C for 10 months, and 4°C for 20 or 24 months. (Some of the 4°C formulations were stored for 20 months and some for 24 months because of the large number of formulations that needed to be processed at the beginning of the study.) Chemical and colloidal stability were assessed by various techniques, listed as follows:

Table 1. Formulation Parameters for 16 Formulations of GCSF in a Factorial Design of Experiment

Formulation	Buffer	pH	Buffer Concentration (mM)	Tween 80 (%)	HP-β-CD (%)
11	None	4.5	0	0.05	0
12	None	4.5	0	0.005	0
13	None	5	0	0.005	0
14	None	5	0	0.05	0
15	Citrate	4.5	20	0.005	0
16	Citrate	4.5	50	0.05	0
17	Citrate	5	20	0.05	0
18	Citrate	5	50	0.005	0
19	None	4	0	0	5
20	None	4	0	0	1
21	None	4.5	0	0	1
22	None	4.5	0	0	5
23	Acetate	4	20	0	1
24	Acetate	4	100	0	5
25	Acetate	4.5	20	0	5
26	Acetate	4.5	100	0	1