Data analysis in stability studies of biopharmaceutical drugs with isothermal and non-isothermal assays

Alexis Oliva, Matías Llabrés, José B. Fariña

Stability is a particular problem for biopharmaceutical products because the efficacy of peptides and proteins as therapeutic or diagnostic agents can be affected during preparation, shipping, and storage. A particular formulation may have no immediately apparent effect on physical or chemical stability, and the time required for these studies at ambient temperature can be very lengthy because chemical reactions proceed relatively slowly at low temperatures. Undoubtedly, accelerated and stress testing of stability can provide useful information for future product development. The many methods used to study kinetics in aqueous solution may be experimental or computational. Experimental approaches may be isothermal or non-isothermal. Non-linear and linear regression methods can be used to analyze data from these experimental approaches, and the Monte Carlo method could be useful to obtain information about uncertainties in experimental data.

The purpose of this review is to describe and to discuss options for the accelerated study of peptide and protein drugs. These options are not necessarily the same as those used for regulatory testing to set expiration dates. We also review statistical techniques to estimate kinetic parameters (rate constant, activation energy, and pre-exponential factor). Further, we establish the advantages and the limitations of both thermal approaches. We analyze and discuss all aspects using the most recent examples of peptide and protein stability.

Keywords: Arrhenius; Biopharmaceutical; Bootstrap; Data analysis; Isothermal assay; Non-isothermal assay; Peptide; Protein; Regression method; Stability

Alexis Oliva*, Matías Llabrés, José B. Fariña
Departamento de Ingeniería Química y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de La Laguna, 38200, Tenerife, Spain

*Corresponding author.
Tel.: +34 922 318452.
E-mail: amoliva@ull.es

1. Introduction

Many protein drugs have recently been discovered and it is expected that about a dozen therapeutic proteins per year will gain regulatory approval in the coming decade. In an optimized protein formulation, the protein should remain stable for several years, maintaining its active conformation, even under unfavorable conditions that may occur during transport or storage. Practical experience has shown that there are no general approaches to stabilization for proteins [1] and that a customized formulation needs to be developed for each. The shelf-life for an economically viable protein drug is 18–24 months [2], which is difficult to maintain due to its chemical and physical degradation.

To achieve this value, a number of specific challenges have to be met [3]. The International Conference on Harmonization (ICH) Guidelines Q5C on stability testing of biotechnological products advises that the drug manufacturer should provide data on the stability of the biopharmaceutical drug, incorporating information on the many external conditions that can affect potency, purity and quality [4]. First, it is necessary to study the inherent stability of this type of product and identify the main problems likely to be encountered in developing a stable formulation. A particular formulation may have no immediately apparent effect on physical or chemical stability, and the time required for these studies at ambient temperature can be very lengthy because chemical reactions proceed relatively

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slowly at low temperatures. Undoubtedly, accelerated and stress testing of stability can help determine the most suitable excipients and concentrations [5,6], allowing for a significant reduction in testing time; however, preparation and assay of a large number of samples produced for the multiple-temperature accelerated test may offset this benefit.

The Tripartite Guideline on stability describes the storage conditions for accelerated and stress studies [7]; second, a validated stability-indicating method is often required to meet the strict guidelines set by the regulatory authorities [4]. The advent of more sensitive analytical methods in biopharmaceutical development has resulted in a concomitant decrease in the allowable levels of impurities and degradation products. It is important that these methods be effective enough to predict even slow rates of formation of degradation products. Although a variety of analytical methods have been used to characterize physical and chemical stability of peptides and proteins [8], it is crucial to continue data evaluation for the development of stable formulation since failure can be due to lack of efficacy or a poor formulation, but sometimes the end results are unsatisfactory due to the experimental design and data evaluation being inappropriate.

In spite of a great deal of research into peptide and protein stability, few published studies have taken into account different experimental approaches and statistical data analysis, and it is also difficult to compare data because of:

(1) a different choice of analytical procedure;
(2) variations in experimental set-up (e.g., different temperature range);
(3) variations in the applied kinetic model;
(4) non-provision of uncertainties; and,
(5) lack of the data sets needed to analyze and to compare approaches.

It is not the intention of this article to provide an exhaustive literature review about the analytical methods used to study the stability of a biopharmaceutical drug, but to perform more intense investigation on data analysis. Although the basic science behind current methodology has not changed significantly, the methods available for predicting stability have evolved over the years, so the purpose of the current review is to describe and to discuss options for the accelerated study of peptides and proteins. These options are not necessarily the same as those used for regulatory testing to set expiration dates (Table 1). This review first describes the general use of thermal acceleration methods, which are broadly applicable to many degradation mechanisms. Second, we review statistical techniques to estimate kinetic parameters (rate constant, activation energy, pre-exponential factor). We also establish differences between non-linear and linear regression. In this situation, the Monte Carlo method has been shown to be useful to obtain information about uncertainties in experimental data. Finally, we analyze and discuss all aspects using the most recent examples of peptide and protein stability.

2. Isothermal assays

At the end of the nineteenth century, several equations were developed to describe the temperature dependence of reaction rates. One of the most important, well-known equations in physical chemistry came from that period: the Arrhenius equation (see Table 1).

The most frequent difficulty associated with the application of the Arrhenius equation is the proper estimation of its two parameters [i.e. activation energy (Ea) and pre-exponential factor (k_0)] from experimental data. The Arrhenius equation involves exponentiation of the reciprocal of the absolute temperature, which introduces a high correlation between the estimates of the two parameters. This makes it very difficult to estimate them, particularly during numerical minimization of the residual sum of squares (RSS), also called the objective function.

The usual way to overcome this is to represent the Arrhenius equation in the linear form. This procedure estimates k values at some different temperatures and then computes the best linear fit for $\ln(k)$ in the form described by Equation (2) (Table 1). This way, the parameter estimates are obtained as the slope (-Ea/R) and the intercept [$\ln(k_0)$] of the linear fit. As the linear least-squares problem has an analytical solution, the difficulties associated with numerical minimization of the objective function are avoided.

Several authors have studied the suitability of the Arrhenius relationship in different chemical-degradation pathways of peptides and proteins (e.g., deamidation, hydrolysis, racemization, and polymerization). However, one of the main problems found was to determine a suitable temperature range for stability studies, where the degradation mechanism is the same and not susceptible to unfolding, so that the Arrhenius plots would then be valid.

For this reason, Pearlman and Nguyen [9] proposed that only temperatures below 40°C be considered valid. However, other authors have used higher temperatures {e.g., Brange et al. [10] used 4–45°C for insulin and Oliva et al. [11] used a broader interval, 20–60°C for pharmaceutical preparations of human insulin}. Yoshioka et al. [12] used temperatures of 40–70°C for α-chymotrypsin, whereas Oliva et al. [13,14] used 25–80°C for various cholecystokinin fragments.

Linear Arrhenius plots and the activation-energy values calculated from the slopes have been reported for chemical degradation of various peptides and proteins in aqueous solution, first-order or pseudo-first-order kinetics being the most common. Reported values were