

Table 2.2 Comparison of rate constants for Pulmozyme deamidation at 5 °C obtained from Arrhenius kinetics and real-time stability data after 88 days at 2–8 °C storage

Formulation ^a	k88 days (days ⁻¹)	Karrhenius (days ⁻¹)	ΔE [≠] (kcal/mol)
Tris, pH 8	1.1 ± 0.1 × 10 ⁻²	9.3 × 10 ⁻³	14
Tris, pH 7	1.9 ± 0.5 × 10 ⁻³	2.5 × 10 ⁻³	13.4
Succinate, pH 6	+	7.3 × 10 ⁻⁶	28
Maleate, pH 6	+	3.6 × 10 ⁻⁵	29.9
Histidine, pH 6	3 ± 3 × 10 ⁻⁴	3.8 × 10 ⁻⁴	19.0
Succinate, pH 5	3.7 ± 10 × 10 ⁻⁵	3.6 × 10 ⁻⁵	29.3
Citrate, pH 5	+	3.1 × 10 ⁻⁵	26.0
Acetate, pH 5	+	3.2 × 10 ⁻⁵	25.5

ΔE[≠], energy of activation. Succinate at pH 6 and 5 was evaluated without the 15 °C data because of the large error in the values at 15 °C.

+, indicates that slope was positive. The origin and significance of such a result is discussed in the text.

^aBuffers consist of 5 mM buffer salt, 150 mM NaCl, and 1 mM CaCl₂.

From [Shire \(1996\)](#).

to different linear heating rates. In modulated DSC the heat flow and heat capacity are measured in a single experiment by superimposing a changing heat rate (modulated) on top of a linear heating rate ([Gill, Sauerbrunn, & Reading, 1993](#)). Application of modulated DSC for characterization of a mAb freeze-dried formulation with high sugar content has been reported ([Breen, Curley, Overcashier, Hsu, & Shire, 2001](#)).

Field flow fractionation

This method originally invented by [Giddings \(1993, 2000\)](#) is useful for analyzing aggregates ([Liu et al., 2006](#); [Rambaldi, Reschiglian, & Zattoni, 2011](#)) and also large particles, which are difficult to separate using standard chromatographic and electrophoretic techniques ([Williams, Runyon, & Ashames, 2011](#)). Thus, this method allows for characterization of aggregates over a wide size range, from 0.001 to 50 μm ([Giddings, 2000](#)). The separation of biomolecules is done within a buffer-filled channel without any column matrix ([Rambaldi et al., 2011](#)). The separation occurs by applying an external field that is perpendicular to the laminar flow. This field can be gravitational, centrifugal, magnetic, electrical, temperature, or flow-based ([Giddings, 2000](#)). The effluent from the channel can be monitored using UV, refractive index, and light scattering detection. In particular, online UV, RI, and LS detectors allow for protein characterization and determination of the molecular weight of each species.

The most commonly used configuration of field flow fractionation (FFF) used for therapeutic proteins is asymmetrical field flow FFF (AF4), where the orthogonal external field is a cross flow. Larger protein species remain in the center of the laminar flow, whereas the smaller species are driven to the bottom of the channel which is equipped with a membrane to allow for the cross flow to exit. Although the technique does not use a column matrix, interaction of protein molecules with the bottom membrane can