reaction mechanism is being investigated. However, since there are many potential degradation routes in proteins due to the increased number of functional groups the Arrhenius plots may not be linear. In addition, a protein and mAb are susceptible to conformational changes at higher temperatures possibly resulting in greater solvent exposure of amino acid residues that undergo degradation. Thus, the degradation kinetics at the higher temperatures may be significantly different than at the lower recommended storage conditions requiring very different formulations. For these reasons regulatory agencies currently require real-time stability assessment for determination of product shelf life (Werner & Langlouis-Gau, 1989).

Although Arrhenius kinetics are not useful for setting shelf life of proteins this kinetic approach has been used successfully to evaluate enzymatic activity of proteases with appropriate modeling using more than one pathway. This approach may work well for proteases since the enzymatic activity is intimately related to the protein conformation, and essentially only one reaction is being monitored: the unfolding of the proteins prior to any chemical modification. Although there may be several different unfolded forms, these different forms will all be inactive and not distinguishable from each other by activity assays. In the case of the enzyme α-glucosidase isolated from the bacterial thermophile *Bacillus thermoglucosidius*, the incorporation of a reversible and irreversible pathway into the kinetic model resulted in linear Arrhenius plots for the rate constants for each process (Suzuki, Nakamura, Kishigami, & Abe, 1980; Yoshioka, Izutsu, Aso, & Takeda, 1991).

Accelerated rate studies become more complicated when a number of analytical assays are used rather than just final activity loss. As an example, for interleukin 1b it was shown that at or below 30 °C, the primary degradation pathway was deamidation, whereas at or above 39 °C, it was aggregation and precipitation (Gu et al., 1991). Thus, the different degradation routes of this protein precluded shelf life prediction from accelerated studies. As was discussed earlier, it was possible to use accelerated studies as a predictive tool if the degradation is monitored for one specific route over a temperature range where the protein conformation is unchanged (Shire, 1996).

Liquid formulation development

Many of the degradation routes that were discussed are highly dependent on solution pH and ionic strength as well as temperature of storage. Although it is virtually impossible to prevent all the degradations from occurring, minimizing the rates of all the reactions, hence optimizing the stability, can be used to develop a successful liquid formulation of a protein or mAb. An example of this is the pH dependence of the pseudo first-order rate constant for deamidation and Asp isomerization in an IgG_1 mAb (Figure 4.3). From these data it is clear that formulation at ~pH 6 would be optimum for both reactions, and generally many liquid mAb formulations are formulated between pH 6 and 7 (Table 4.1).

Development of stable liquid formulations requires screening of different excipients, and each excipient has a specific purpose. The development of a liquid formulation that uses excipients already present in marketed products may require less safety testing than an excipient that has never been used. However, potential interactions with other excipients as well as the protein drug do not guarantee a fast and