Given the great variety of techniques for analyzing and characterizing proteins (Chapter 2), it is a challenge to assess which of the assays are relevant to developing a stable formulation and delivery system, i.e., stability-indicating assays. Since many of the chemical degradations in proteins are fairly well understood, particularly in regard to how conformation and primary structure impact degradation routes, it is often possible to determine which degradation routes may be prevalent for a particular protein under development. These assessments can then lead to a more rational and directed approach for formulation development. Examples of such assessments are now discussed.

In silico methods to assess protein degradation routes

Deamidation and isomerization reactions as previously discussed are highly dependent on amino acids adjacent to the potential deamidation/isomerization site. Identification of such hot spots suggests whether this may be a major degradation route and also enable careful peptide mapping around the potential sites to determine how susceptible those sites are to alteration. If there is information on the conformation from either X-ray crystallography or nuclear magnetic resonance studies the accuracy of the predictions of these "hot spots" can be increased (Robinson & Robinson, 2001).

Computational methods to assess propensity to aggregate have also been developed. Many of the computer algorithms based on aggregation of proteins in neurodegenerative diseases have been recently reviewed (Kumar, Wang, & Singh, 2010). The specific aggregation-prone regions (APRs) in these proteins appear to be involved with association of β-strand motifs. Many of these algorithms can predict correctly >80% of the sequences involved. An important principle that comes out of these analyses is that not all regions of a protein contribute equally to aggregation, but rather short peptide sequences within the protein may predominate in the aggregation process. It was suggested that since mAbs have a great deal of β-structure, these algorithms could be useful in predicting APRs in mAbs. The computations have shown that APRs are mainly found in the CDR loops and adjoining β-strands of mAbs (Kumar et al., 2010; Wang, Das, Singh, & Kumar, 2009; Wang, Hu, et al., 2009).

Complexity of stability determinations during formulation development: real time versus accelerated stability

Generally during development of formulations for small molecules high temperatures are used to assess changes since the kinetics of various reactions increase with temperature. The temperature dependence of the kinetic rate constants generally follows Arrhenius kinetics and can be used to determine shelf life at the lower temperature storage conditions. Thus, this approach greatly speeds up the development process and precludes the need for real-time stability analysis (Fagain, Sheehan, & O'Kennedy, 1991). This approach can work with small-molecule drugs since usually only one