such as *m*-cresol, chlorocresol, phenol, benzyl alcohol, methyl paraben, propyl paraben, benzethonium chloride, benzylalkonium chloride, thimerosal, and chlorobutanol), the chemical and physical properties of these preservatives may make formulation of proteins a challenging task. Potential interaction of preservatives with the protein can diminish the effectiveness of the preservative, and degradation of preservatives can also produce compounds that interact with proteins. As an example, the commonly used preservative benzyl alcohol undergoes oxidation to benzaldehyde (Hewala, 1994) that can react with the primary amines of proteins (Roberts & Caserio, 1967). This problem can be mitigated by development of a lyophilized formulation that is reconstituted with a preservative-containing diluent, hence shortening the time of exposure of the protein to the preservative.

Approaches for formulation development

The most common strategy used for development of protein drug formulations is to set up a study that screens different excipients and solution conditions. However, these screens can be very large, labor intensive, and time-consuming. Often experiments are designed to obtain quickly an understanding of the critical properties of the protein drug. These preformulation studies investigate the response of the protein to a variety of stresses induced by different solution conditions. The studies include a general characterization of the protein that may reveal potential stability issues (Chang & Hershenson, 2002). Preformulation studies can also be simplified by using information on the response of the protein to the solution conditions during upstream process development. During cell culture and recovery and purification process development, observations regarding stability at different temperatures, ionic strength, and pH may help in narrowing the range of conditions that need to be explored.

Development of stability-indicating assays

During the preformulation step assays are also developed that can monitor changes that may impact potency and safety of the product. These so-called "stability-indicating" assays are then used in a full development screening program. Often one assay such as reversed phase HPLC is sufficient to monitor the stability of a small-molecule drug, but for proteins it is necessary to use several assays to monitor stability. An example of this is for a mAb where size exclusion chromatography (SEC) showed small changes in the chromatogram after 1 year of storage at −70, 5, 30, and 40 °C (Figure 4.1(a)). However, analysis by hydrophobic interaction chromatography (HIC) after papain digestion (generating Fabs) after 1 year of storage at the same temperatures showed significant changes in the chromatogram (Figure 4.1(b)). Essentially different assays explore different properties of a complex molecule such as a protein, and thus several assays are required to adequately monitor the stability of a protein. Thus, a SEC assay that is sensitive to changes in the aggregation and fragmentation of a protein may not pick up degradation that results from changes in Asp isomerization as detected by the HIC assay. Using only one assay may explore only one of many degradation routes. This is akin to the famous parable about different blind men touching different parts of an elephant and coming to very different conclusions regarding the animal's appearance (Figure 4.2).