level of aggregation may be sufficient to challenge the specificity of methods such as SEC or light scattering.

The forced degradation experiments do not necessarily result in product decomposition. The study can be stopped if no degradation is observed after DS or DP has been exposed to a stress that exceeds the conditions of accelerated stability protocol. Protocols for generation of product-related degradation may differ for DS and DP, owing to the differences in matrices and concentrations. For example, sugar additives often present in DP are known to stabilize proteins vis-à-vis denaturing conditions.

9.9.5.2 Selection of Stress Conditions

Forced degradation is normally carried out under more severe conditions than those used for accelerated studies. The choice of stress conditions should be consistent with the product's decomposition under normal manufacturing, storage, and use conditions, which are specific in each case. The ICH guidance recognizes that it is impossible to provide strict degradation guidelines and allows certain freedom in the selection of stress conditions for biologics. The choice of forced degradation conditions should be based on data from accelerated pharmaceutical studies and sound scientific understanding of the product's decomposition mechanism under typical use conditions. A minimal list of stress factors suggested for forced degradation studies must include acid and base hydrolyses, thermal degradation, photolysis, and oxidation and may include freeze–thaw cycles and shear.

Regulatory guidance does not specify pH, temperature ranges, specific oxidizing agents or conditions to use, the number of freeze–thaw cycles, or specific wavelengths and light intensities. The design of photolysis studies is left to the applicant's discretion; however, Q1B recommends that the light source should produce combined VIS and UV (320–400 nm) outputs and that exposure levels should be justified. Consult the appropriate regulatory authorities on a case-by-case basis to determine guidance for light-induced stress.

Degradation products that arise in significant amounts during manufacture and storage should be identified, tested for, and monitored against appropriately established acceptance criteria. Examination of some degradation products generated under stress conditions may not be necessary for certain degradants if it has been demonstrated that they are not formed under accelerated or long-term storage conditions.

The forced degradation studies should be part of impurity characterization. When identification of the impurity is not feasible, incorporate the description of unsuccessful experiments (including those conducted in stress-testing studies) in the text of the application. The most frequently encountered protein variants include truncated fragments, deamidated, oxidized, isomerized, aggregated forms, and mismatched disulfide links.

Degradation pathways for proteins can be separated into two distinct classes that involve chemical and physical instabilities. Chemical instability is any process that yields a new chemical entity, including modification of the protein (via individual amino acid alteration), covalent bond formation, and cleavage. Physical instability refers to changes in the HOSs (secondary and above). Noncovalent aggregation usually results from partial or full unfolding, which enhances the hydrophobic interactions between protein molecules. It may also lead to denaturation, adsorption to surfaces, and precipitation. Aggregation presents a significant patient risk, because protein aggregates are frequently immunogenic; therefore, analytical methods