Inactivation of the API by Radiation Treatment

By using radiation as an effective method of pathogen inactivation in drugs one would deal with the main disadvantage of API stability.

Under irradiation treatment, the main problem results in unacceptable losses in functional activity of the APIs. There are two mechanisms by which irradiation can damage therapeutic molecules. One is the direct result of a radiation depositing energy into the target. The transfer of this energy results predominantly in the dislocation of outer electrons from molecules and breakage of covalent bonds. The second type of damage has been termed "indirect" and is the result of chemical attack by free radicals and reactive oxygen species typically generated by the interaction of radiation with water molecules and oxygen. Although these free radicals and reactive oxygen species are very short lived, they are responsible for the majority of the damage that occurs in therapeutics irradiated by conventional irradiation procedures.

During irradiation, the damaging secondary effects can be controlled by combinations with freeze-drying to minimize the potential for generating free radicals, protection from free radical damage through the use of free radical scavengers, and reduction of the free radical mobility by irradiation at cryogenic temperatures.

It is difficult to predict a general behavior of the active ingredients against irradiation. Each API is unique and may react differently depending on its own physicochemical characteristics and the characteristics of the environment in which it resides. Later in this chapter the impact of irradiation and its application conditions on the stability of a protein of human origin is presented as an example.

IMPACT OF IRRADIATION ON THERAPEUTIC PROTEINS

Proteins are composed of one or multiple linear amino acid sequences that interact with each other to form secondary structures (sheet, helix, etc.), which are then folded back in space by hydrophobic links to form tertiary structures. The protein may be subjected to several changes that could lead to different levels of degradation such as (*i*) important secondary changes of conformation that can lead to aggregations in liquid environment (irreversible modifications) (12), (*ii*) modification of the secondary structures not leading to aggregates formation in the liquid state but which can affect the stability of the protein during storage (13), and (*iii*) denaturations ending with the fragmentation of proteins.

The impact of irradiation on the therapeutic proteins is affected by a number of parameters such as the structure and state of the proteins (globular, native, or denaturated), the medium composition (presence of other substances, wet, dry, in solution, or whether liquid or frozen), and the irradiation conditions (absorbed dose, dose rate, temperature, presence of oxygen, etc.)

The irradiation process initiates a series of reactions leading to ionic and free radical intermediates and ultimately to stable products that under certain conditions might lead to permanent modifications in proteins. The chemical changes in irradiated proteins include deamination, decarboxylation, reduction of disulfide linkages, oxydation of sulfhydryl groups, peptide-chain cleavage, aggregation, cross-linking, and aggregation (14–17). Hydroxyl radicals and superoxide anion radicals generated by radiation modify the primary