structure of proteins, which results in the distortion of the secondary and tertiary structures.

The negative effect of irradiation on the stability and integrity of proteins can be widely limited if the irradiation conditions are well controlled to minimize the formation of radiolytic products and their diffusion.

More than 40% of currently marketed protein drug products are solids, primarily lyophilized (i.e., freeze-dried) powders for reconstitution. This form is often selected to preserve potency and prolong shelf life. In freeze-dried products, diffusion of radicals will be minimized and movement of large radicals will be virtually eliminated making the impact of irradiation on the proteins stability insignificant.

Impact of the Radiation Treatment Combined to Freeze-Drying on the Stability of a Therapeutic Protein: Human Serum Albumin

Human serum albumin (HSA) is a monomeric protein. It is a polypeptidic chain of 585 amino acids among which 35 cysteines forming 17 disulfures bonds (12). It has a molecular mass of 65 kDa and is mainly constituted by α-helix. It is a globular protein formed by three different but very similar domains in their peptide sequences and their three-dimensional conformations (18,19). Regarding its conformation, hydrophobic sites are internal and hydrophilic sites are external. HSA is the most abundant protein in human blood plasma. It is produced in the liver and is involved in many transports (hormones and fatty acids) and in the osmotic pressure maintenance (18,19). It may be deficient in the case of liver cirrhosis of nephritic syndrome or choleretic enteropathy. It is therefore a key protein that must be administered to deficient individuals.

In our work, the effect of radiation combined to the freeze-drying process is assessed for a pharmaceutical formulation containing 1% of HSA. This formulation is exposed before or after freeze-drying to 15 and 25 kGy (doses usually applied for sterilization) and 40 kGy excessive radiation dose. Radiation doses are delivered using a Van de Graaff electron beam accelerator (2.2 MeV, 150 μA).

Changes in the molecular properties of HSA due to irradiation and freeze-drying are evaluated, thanks to size exclusion HPLC (HP-SEC) (20), SDS-PAGE electrophoresis (21), and Fourier transform infrared (FTIR) spectroscopy (22,23).

HP-SEC and SDS-PAGE electrophoresis allow the separation of molecules according to their molecular weight and, thus, can highlight irreversible degradation such as aggregation and fragmentation. FTIR is based on the excitation of molecules by a laser and on the vibrations of intermolecular links. FTIR equipped with attenuated total reflection (ATR) is used to investigate alteration of the secondary structure of the protein and to learn about changes that may be reversible.

According to the HP-SEC results confirmed by electrophoresis on SDS-PAGE, the HSA protein within the framework of this study is present under monomer form (main form) and dimer and trimer forms (minors forms) (Figs. 1 and 2).

The freeze-drying of the HSA formulation does not seem to affect the stability of the protein (Fig. 3). The same chromatographic and electrophoretic profiles are obtained before and after freeze-drying (Fig. 3).