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Evaluation of multilayer film stability by Raman spectroscopy after gamma-irradiation sterilization process

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ABSTRACT

Polymers such as polyethylene (PE) and ethylene vinyl alcohol (EVOH) are primary constituents of single use plastic systems in the biopharmaceutical and biotechnology industries. These devices are sterilized by gamma-irradiation prior to be used, the usual dose being between 25 and 45 kGy. Optical spectroscopies are of great interest for chemical analysis and are used to obtain information on the composition of materials such as polymers. Raman spectroscopy provides information on the fundamental vibrations of molecules, using excitation in the visible wavelength range. The purpose of this study is to unveil the impact of gamma-sterilization on polymers in industry-like experimental conditions. Cross-sections of films are analyzed before and after sterilization using different radiation doses: their compositions and chemical evolution of the material are examined using micro-Raman spectroscopy. As the chemical composition of the layers is complex, due to the presence of additive compounds, there is considerable overlap between the spectral data. In this case, the use of spectral curve resolution chemometric methods is unique for unravelling the complex identification of the layers and to study the degree of chemical modifications.

1. Introduction

The preparation, storage, mixing, freezing, transportation, formulation, and filling of biopharmaceutical solutions are performed in sterile single-use plastic bags. The sterility is achieved through gamma-irradiation, which generates material modifications, as reported in the literature [1]. The integrity and security of packages rely on the appropriate flexibility and barrier property of polymeric materials such as polyethylene and polyethylene-co-vinyl alcohol, respectively [2]. Gamma-sterilization of single-use systems initiates chemical reactions inside the plastic material, leading to either an increase or a decrease in the molecular weights of polymers [3,4]. In our work, we focus on the effects of gamma-irradiation on the solid state of a multilayer polymer film (PE/EVOH/PE), made of polyethylene (PE) – a polymer with interesting water barrier properties and mechanical properties [5] – and ethylene vinyl alcohol (EVOH) – remarkable for its barrier properties to CO_2 and O_2 molecules [2].

Gamma-sterilization of these systems affords complex modifications inside the material, leading to the modifications of additive compounds or to the damage of the polymers themselves [6–8]. Irradiation of polymeric materials has been proven to initiate radical chemical reactions inside the polymeric material [9] leading to either an increase or a decrease in the polymer molecular weight [3,4]. The effects of gamma-irradiation on polymers are well known [6–8,10–12], whereas the effects of gamma-irradiation on multilayer films have been little investigated [2]. The Scheme 1 displays the generation of first and second generation radicals upon gamma-irradiation.

Nevertheless, we expect that the initial reaction will be the same in multilayer film than in PE and EVOH monolayer film. Moreover, very similar chemistry is also expected for the decay of first and second generation of radical species as cross-linking and scission (bond breaking) induce modifications to the matrix environment by introducing connections or branching in the polymer chains (decrease in $—CH_2—$ groups) and breaking polymer chains (increase in $—CH_3$ end groups), respectively.

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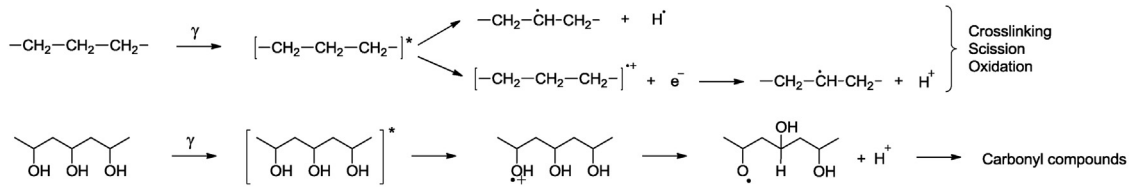

Scheme 1. First and second generation of radicals upon gamma-irradiation of PE and EVOH.

Modifications in polymers can be observed by spectroscopic methods and especially Raman spectroscopy, which is a powerful optical technique spectroscopy with several advantages to investigate the molecular deformations behavior of opaque and thick polymeric material samples [13–20]. Raman spectroscopy provides a complementary approach to infrared absorption spectroscopy for which transparent and very thin films are required. Micro-Raman spectroscopy makes possible to reach the intercalated layer with a better resolution than μ-ATR (Attenuated Total Reflectance) infrared spectroscopy, and offers the possibility to observe the vibrations of the C—C bond skeleton in the main chains [16,21,22], with a better signal than in FTIR. As the C—C bond stretching vibration is strongly Raman active, microenvironments of the polymer chains are probed by the wavelength shifts [23–25]. Degradation of polymers under gamma-irradiation investigated by Raman spectroscopy is well known [26,27]. However, the degradation of multilayer films has been little investigated and even less using chemometric methods [28,29]. Thus it is possible to perform a mapping of three layers film using Raman spectroscopy and to identify the modification in each layer with the help of chemometrics

Gamma-irradiation on multilayer films leads to Raman spectra variations, which are highlighted using chemometric methods. The chemometric tool used in this study and applied to Raman spectra is a curve resolution method named SIMPLISMA (SIMPLe-to-use Interactive Self Modeling Analysis). The SIMPLISMA approach [30] is used to define chemical species rising during gamma-irradiation and to monitor their evolution. Retrieving reconstructed Raman spectra may afford identification of the compounds present in the polymer film before and after irradiation. The concentration profiles give relative quantitative information highlighting the variation in polymer modification after the different gamma-doses. The classical gamma-irradiation dose range used in biopharmaceutical industries is 25–45 kGy [31]. The gamma-irradiation doses investigated in this study are up to 270 kGy in order to increase and exaggerate the gamma-irradiation effect and thus to better emphasize and to investigate the gamma-ray induced modifications. Briefly, the most significant changes are observed on the stabilizer and on the tie layer, which corresponds to the interface between the EVOH layer and the PE layer and which allows bonding between these two layers.

2. Materials and methods

2.1. PE-film

The structure of the multilayer PE-film, provided by Sartorius Stedim FMT (Aubagne, France), is depicted in Fig. 1. This film was prepared by extrusion.

The different layers in this film contain additive compounds including antioxidants (especially phenol, phosphite [32–34], and other aromatic compounds), antiblocking agents and plasticizers. The corresponding molecules cannot be disclosed here due to protection by confidentiality agreement. The EVOH and PE layers contain additive compounds for their stabilization during the manufacturing process and during their shelf life. In this article, we

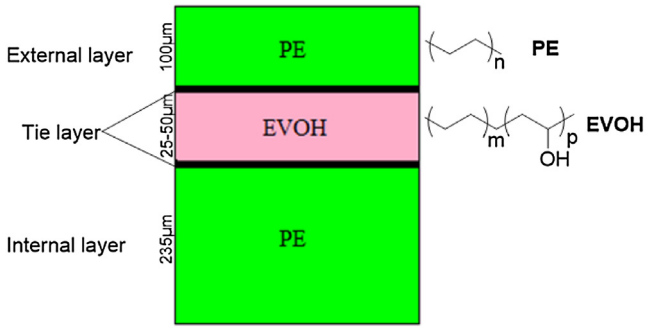

Fig. 1. Molecular structure of PE-film. The internal layer is the side of the film in contact with the solution when the bag is filled. The external layer is the side of the film in contact with air. The tie layer is used to glue PE and EVOH layers together.

distinguish the PE or EVOH polymer alone and the PE or EVOH resin, which is the mixture of the PE or EVOH polymer plus the additives. The different layers are necessarily made of the resin. The two PE layers which constitute the internal and external layers of PE-film are both LDPE and differ in their branching degree. The internal layer is the side of the film in contact with the solution when the bag is filled with biological solution, and the external layer is the side of the film in contact with air. There is a tie layer between EVOH and the internal and external PE layers (bold lines in Fig. 1). This tie layer is made of maleic anhydride-PE modified. The anhydride function grafted on PE reacts with the EVOH hydroxyl group to generate an ester function that ties the two layers together (Scheme 2). The proportion of maleic anhydride is <1% in the tie layer and should not be detectable by Raman spectroscopy.

The Raman analyses were performed on four batches of PE-film, to check if there are changes between the different manufacturing batches. After processing of data, there is no difference between each batch, thus for the sake of simplicity, only results of one batch are discussed hereafter.

2.2. Gamma irradiation

Sheets of film (thickness of about 400 μm) were packed and wrapped in specific packaging (PE) and irradiated at room temperature in a ^{60}Co gamma-source. The ^{60}Co gamma-source provides a dose rate of 8–13 kGy/h, as given by the Synergy Health company (Marseille, France), affording doses at 30 (±1), 50 (±1), 115 (±2) and 270 (±5) kGy. A sterilization cycle corresponds approximately to 25 kGy. To obtain the targeted dose, it is necessary to perform several sterilization cycles, including non-controlled waiting time between cycles under non-controlled storage conditions. Two irradiation campaigns were performed with four batches each to check whether the irradiation has an impact on our results. The Raman spectroscopy analyses of the film samples obtained during the two campaigns of irradiation lead to similar results which are thus not affected by these non-controlled conditions. The samples were analyzed by Raman about 10 days after gamma-irradiation and the recording of the large number of