

# Monitoring of peroxide in gamma irradiated PE/EVOH/PE multilayer film using methionine probe

Nina Girard-Perier, Magalie Claeys-Bruno, Sylvain R.A. Marque, Nathalie Dupuy, Fanny Gaston, Samuel Dorey

# ▶ To cite this version:

Nina Girard-Perier, Magalie Claeys-Bruno, Sylvain R.A. Marque, Nathalie Dupuy, Fanny Gaston, et al.. Monitoring of peroxide in gamma irradiated PE/EVOH/PE multilayer film using methionine probe. Food and Bioproducts Processing, 2022, 132, pp.226 - 232. 10.1016/j.fbp.2022.02.001 . hal-03580549

# HAL Id: hal-03580549 https://hal.science/hal-03580549

Submitted on 18 Feb2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

# Monitoring of peroxide in gamma irradiated PE/EVOH/PE multilayer film using methionine probe

Nina Girard-Perier<sup>a,\*</sup>, Magalie Claeys-Bruno<sup>b</sup>, Sylvain R.A. Marque<sup>c,\*</sup>, Nathalie Dupuy<sup>b,\*</sup>, Fanny Gaston<sup>a</sup>, Samuel Dorey<sup>a,\*</sup>

<sup>a</sup> Sartorius Stedim FMT S.A.S, Z.I. Les Paluds, Avenue de Jouques CS91051, 13781 Aubagne Cedex, France

<sup>b</sup> Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France

<sup>c</sup> Aix Marseille Univ, CNRS, ICR, case 551, 13397 Marseille, France

#### ABSTRACT

Methionine oxidation is used as a proxy to model the gamma radiation induced changes in single-use bags. These changes can be the formation of acids, radicals and hydroperoxides due to the irradiation of the multilayer film the single use bag is made from. Biopharmaceutical multilayer polyethylene bags irradiated from 0 to 260 kGy and aged from 0 to 36 months were filled with a methionine solution to follow the oxidation of methionine. Methionine sulfoxide was measured using high performance liquid chromatography after 21 days of storage. The maximum methionine sulfoxide concentration detected after 21 days of storage was analyzed through a design of experiments. The results to the design of experiments were considered to obtain the desirability domain for the optimization of the conditions of use for the single-use bags, limiting the oxidation of methionine as well as the release of reactive species thereof.

© 2022 Institution of Chemical Engineers. Published by Elsevier Ltd. All rights reserved.

#### 1. Introduction

Methionine oxidation

Liquid chromatography

Design of experiments (DOE)

Keywords:

Single use bag

Gamma irradiation

Single-use bags have obtained rapid acceptance in biopharmaceutical manufacturing worldwide as they offer a cost-effective alternative to traditional stainless-steel systems (Shukla and Gottschalk, 2013; Gaston et al., 2021). Disposable bag technology is well established for cell culture media, buffer, and intermediate storage and most recently also for cell culture single-use bioreactors used to produce vaccines, therapeutic antibodies and proteins. Single use bags are mainly manufactured from appropriate multilayer polymer films with a gas barrier layer such as ethyl vinyl acetate/ethyl vinyl alcohol/ethyl vinyl acetate (EVA/EVOH/EVA) or polyethylene/ethyl vinyl alcohol/polyethylene (PE/EVOH/PE). For several applications, these bags must be sterilized before use. According to pharmaceutical standards (Kátia Aparecida da Silva Aquino, 2012), gamma irradiation is the conventional method of sterilization. The gamma irradiation dose range usually used for biopharmaceutical industries is 25–45 kGy (ISO 11137-1 International Organization for Standardization, Sterilization of Health Care Products - Radiation - Part 1: Requirements for Development, Validation and Routine Control of a Sterilization Process for Medical Devices, 2006). An important aspect to consider is the degradation of the polymer due to the sterilization process. The chemical modifications due to gamma irradiation of a PE/EVOH/PE multilayer film have been thoroughly studied using various analytical techniques (FTIR spectroscopy (Gaston et al., 2016; Girard-Perier et al., 2022), pH analysis (Dorey et al., 2018a), X-Ray Photoelectron Spectrometry (Dorey et al., 2018b) for the in-depth and surface detection of oxidized species in materials). Other articles report on the impact of gamma irradiation on common polymers (Montanari et al., 1998; Hyun Kang, 2001; Buttafava et al., 2005; Abdel Tawab et al., 2013). The generation of radicals (Audran et al., 2015), and thus the modifications of the polymers, can induce aggregation and oxidation of the proteins (Gaber, 2005; Štajner et al.,

2007). Importantly, it has been found that the sterilization method used for biopharmaceutical bags could play a critical role in protein product stability. The aim of this study is to highlight the presence of reactive species by investigating the oxidation of methionine stored in single use bags made from a PE/EVOH/PE multilayer film. Methionine is very sensitive to reactive oxygen species (ROS) and is most of the time oxidized to methionine sulfoxide (Dalle-Donne et al., 2002). This oxidized form can inactivate proteins and cause a decrease in the biological activity (Dalle-Donne et al., 2002).

Although methionine oxidation has been comprehensively studied (Li et al., 1995; Schöneich, 2005; Stadtman et al., 2005; Drazic and Winter, 2014; Folzer et al., 2015; Masato et al., 2016), there is no literature published on the oxidation of methionine in biopharmaceutical bags. In a recent paper (Girard-Perier et al., 2020) we have reported the monitoring of peroxides in EVA/EVOH/EVA biopharmaceutical bags using methionine probe.

The concentration in methionine sulfoxide is quantified using High Performance Liquid Chromatography (HPLC). The final step is then to optimize the usage of single-use biopharmaceutical bags in terms of bag ageing and gamma irradiation doses to limit the oxidation of methionine. As mentioned above, for industrial applications, sterilization at doses below 60 kGy are suitable. However, we performed irradiations at doses of 106 kGy and 260 kGy for two reasons: i) for academic purposes to enhance effects observed at lower doses, and ii) for industrial purposes to know how accidental irradiations at higher doses may alter the properties of the materials.

# 2. Materials and methods

#### 2.1. Sample bags

The two lots of PE single-use plastic bags investigated were made from a multilayer film composed of one layer of ethylene vinyl alcohol (EVOH) sandwiched between two layers of polyethylene (PE), with a total thickness of about 400  $\mu$ m (Fig. 1).

# 2.2. Storage conditions

Before irradiation each sample bag was placed in over pouches and a partial vacuum (not controlled) has been performed according to internal industrial procedures. Thus, materials contain some level of O<sub>2</sub>. The overpouches were placed in the cardboard and then irradiated. After irradiation and before analysis, cardboards were stored in dark, in an air-conditioned room at 20  $\pm$  2 °C.

#### 2.3. Gamma irradiation

PE bags were irradiated with a <sup>60</sup>Co gamma source, at Ionisos, Dagneux, France. The dose rate provided was of 1–2 kGy/h. The target dose average reached was 29 kGy, 59 kGy, 106 kGy and 260 kGy. For more information see supporting information file.

#### 2.4. HPLC system conditions

After the end of each ageing time, bags were filled with a solution of methionine (more information in supporting information file). Sampling was performed after 21 days of storage in the dark, in an air-conditioned room at  $20 \pm 2$  °C. The sample was analyzed with an Agilent 1260 HPLC equipped with a quaternary pump (G1311C), an autosampler (G1329B), and a fluorescence detector (G1321B) (HPLC system conditions have been described in a previous article (Girard-Perier et al., 2020) and are reported in supporting information file).

# 2.5. Chemicals and reagents, methionine solution filling

The details about chemicals, reagents, methionine solution filling have been described in a previous article (Girard-Perier et al., 2020) and are reported in supporting information file.

#### 2.6. Ageing time

Each sample bag was analyzed at different times after gamma irradiation. Five ageing times were considered: A0 (when received), A6 months at room temperature and 130 days, 235 days and 365 days at 40 °C to simulate A12, A24 and A36 months, respectively. These corresponding times were calculated based upon the ASTM F1980 regulation (F02 Committee) using Q10 = 2. After the end of each of these ageing periods, a fresh methionine solution was stored in the bag and analyzed with HPLC after 21 days of storage.

#### 2.7. Fourier transform infrared spectroscopy (FTIR)

PE/EVOH/PE film samples (12  $\times$  10 cm) were analyzed onto the Bruker "Golden Gate" attenuated total reflectance accessory provided with a diamond crystal. The spectra for each sample were recorded from 3100 to 700 cm<sup>-1</sup>, with 4 cm<sup>-1</sup> resolution and 64 scans, on a Thermo Nicolet Avatar spectrometer equipped with an Mercury Cadmium Telluride (MCT/A) detector, an Ever Glo source, and a KBr/Ge beam splitter. The spectrometer was placed in an air-conditioned room at 21 °C. Five spectra were recorded at different locations on the film for each sample to average the potential film inhomogeneity and measurement variability. A background scan in air (under the same resolution and scanning conditions as those used for the samples) was carried out before the five sets of acquisition. The ATR (Attenuated Total Reflectance) plate was cleaned in situ by scrubbing with an ethanol solution to remove any residual traces of the previous sample. Cleanliness was checked by recording a background spectrum. The penetration depth in the sample was of few micrometers, depending on the IR wavelength.

## 2.8. Principal component analysis (PCA)

PCA is a multivariable technique used to reduce a large set of variables to a small set of variables named "principal components" (Martens and Naes, 1989). The principal components represent the largest variance in the original variables. The variations in intensity and shift of the FTIR peaks were investigated. Spectra were adjusted prior to PCA investigation using baseline and Standard Normal Variate (SNV) correction. The derivative was performed using Savitzky Golay algorithm. All computations were performed using the Unscrambler X 10.5 software.

#### 2.9. Design of experiments

To compare the effect of the different ageing conditions and those of the different doses affording methionine oxidation into methionine sulfoxide, a full-factorial design of experiments was performed with the Azurad software (Azurad: Intuitive and Powerful Software for the Construction and Processing of DOE, 2019) with a  $[4 \times 5]$  matrix.

The gamma irradiation  $(U_1)$  and the ageing time  $(U_2)$  are two main parameters acting on the oxidation of methio-



Fig. 1 - Structure of PE/EVOH/PE (a), structure of PE (b) and EVOH (c).



Fig. 2 – Plots of ageing before filling versus methionine sulfoxide concentration detected at different doses after 21 days of storage. Limit of Quantification = 0.25  $\mu$ M and Limit of Detection = 0.1  $\mu$ M.

nine. Their respective experimental domains are described in Table 1.

Once the sample bag had been gamma irradiated, the dry ageing started (from 0 (when boxes received after irradiation) to 36 months). After each of these dry ageing periods, the bag was filled with a methionine solution and methionine sulfoxide was measured by HPLC after 21 days of storage in the dark, in an air-conditioned room at  $20 \pm 2$  °C.

# 3. Results

Quickly after irradiation (ageing 0), the bags irradiated at 106 and 260 kGy exhibit the highest concentration in methionine sulfoxide ( $\sim$ 5  $\mu$ M, Fig. 2). Oxidation is lower for bags irradiated at 59 kGy with 2.7  $\mu$ M of methionine sulfoxide detected and there is no oxidation observed for bags irradiated at 29 kGy. Excepted for dose at 29 kGy, the overall trend is a decrease in oxidation over time. This means that the longer the bag is

aged after irradiation (dry storage) the less the methionine is oxidized.

After 36 months of ageing, 1  $\mu$ M of methionine sulfoxide is detected in bags irradiated at 29, 59, and 106 kGy while 1.5  $\mu$ M is detected in those irradiated at 260 kGy. The 0 kGy samples show no oxidation.

The concentration of methionine sulfoxide detected in  $\mu M$ (displayed in the Y axis) is then modeled. Based on the experiment domain  $X_1$  and  $X_2$  (coded variable from  $U_1$  and  $U_2$ , in Table 1), we use a polynomial model of degree 2, which can be written as Equation 1:

$$Y = b_0 + b_1 \times X_1 + b_2 \times X_2 + b_{11} \times X_1^2 + b_{22} \times X_2^2 + b_{12} \times X_1 X_2$$

Equation 1: Polynomial model for the concentration of methionine sulfoxide

From the experimental results, the coefficients  $b_i$  and  $b_{ij}$  are calculated using multi-linear regression on the coded variables as given in Equation 2. A calculation example is detailed in supporting information file.

$$\begin{split} Y &= 1.58 + \ 0.92 \times X_1 - \ 1.15 \times X_2 - 0.71 \times X_1^2 + 0.97 \\ &\times X_2^2 - 1.01 \times X_1 X_2 \end{split}$$

Equation 2: Polynomial model with coded variables for the concentration of methionine sulfoxide

Most of the residues have values close to zero and are distributed on either side of the x-axis, which allows the model to be validated with  $R^2$  equal to 0.71 (with degree of freedom = 20). Fig. 3 shows the response surface of the concentration of methionine sulfoxide detected after 21 days of storage. The red area in the top left corner represents the highest maximum concentration of methionine sulfoxide detected, for bags irradiated at doses higher than 150 kGy and used shortly after irradiation (ageing <5 months).

# 4. Discussion

The oxidation of methionine in solution when stored 21 days in irradiated bags highlights the release of oxidant species which are likely peracids and  $H_2O_2$ . The oxidation level depends on the irradiation dose and the bag ageing before filling. In previous reports (Audran et al., 2015; Dorey et al., 2019), we showed that hydroxy alkyl radicals in PE/EVOH/PE films are observed several weeks after irradiation and that the  $O_2$  barrier properties of the EVOH layer are enhanced with



Scheme 1 – Chain reactions accounting for the O<sub>2</sub>-barrier enhanced property and for the oxidation process in the EVOH layer.



Scheme 2 - Oxidation process involving the formation of peracetic acid.



Fig. 3 – Response surface of the effect of ageing (months) vs dose (kGy) on the methionine sulfoxide concentration detected ( $\mu$ M).



Fig. 4 – Plots of methionine sulfoxide concentration versus dose for EVA/EVOH/EVA film (pink-data from previous article (Girard-Perier et al., 2020)) and PE/EVOH/PE (green) – 6 months after gamma irradiation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).



Fig. 5 – PCA of all infrared spectra at all absorbed doses and all ageing for zone 3100-700 cm<sup>-1</sup> after baseline and SNV correction.

the increasing irradiation dose. This was accounted for by the coupling of hydroxyalkyl radicals with oxygen, generating peroxyl radicals which in turn abstracts H-atoms from the EVOH chains to regenerate the hydroxyalkyl radicals and yield hydroperoxyl hemiacetal moieties. The latter collapse immediately and spontaneously into keto derivatives and hydrogen peroxides that are involved in the oxidation of the methionine (Scheme 1). As mentioned in article reporting on the oxidation of methionine in bags made of EVA/EVOH/EVA films, for which no radicals are detected by EPR and low level of generation of  $H_2O_2$  is expected from degraded  $O_2$  barrier of EVOH layer, the oxidation is mainly due to the generation of peracids by the reaction of  $H_2O_2$  with carboxylic acids (Scheme 2).

The higher the dose, the higher the oxidation level as expected from a higher generation of A (Audran et al., 2015). The level of oxidation decreases for doses 59, 106 and 260 kGy with time except for doses 29 kGy, for which an increase is observed up to 6 months. As described in Scheme 2, formation of peracid is a bimolecular reaction dependent on the concentration of both species. Taking into account that PE generates less acid than EVA, that migration processes are likely



Fig. 6 – Desirability graphs of ageing (months) vs doses (kGy), a) for case 1 (3 μM), b) for case 2 (1 μM) - The bluish zones represent the highest desirability. The black bold line represents the desirability = 50%.

dependent on the type of polymer layer, and that amount of carboxylic acid is dependent on the irradiation doses. All kinetics make that the generation of methionine oxide at 29 kGy dose is delayed and increases with time up to 6 months<sup>1</sup>. Noteworthily, level of oxidation is close to the LOQ meaning that such a trend is not detected for other doses as larger amounts of carboxylic acids and  $H_2O_2$  are generated.

Moreover, it was reported that the level of organic acid in the PE/EVOH/PE film decreases quickly with ageing, supporting nicely the importance of the generation of peracetic acid in the oxidation process.

The twice lower amount of oxidation observed in Fig. 4 in PE/EVOH/PE films than in EVA/EVOH/EVA films at doses 29 and 59 kGy is discussed in details as it can be due to two reasons:

- A low generation of carboxylic acids in PE/EVOH/PE films impeding the generation of peracids.
- A larger amount of secondary antioxidant(Menzel et al., 2021) in PE/EVOH/PE film than in EVA/EVOH/EVA film reducing the formation of peracids by scavenging H2O2 in the first case, and promoting an inefficient scavenging in the second case due to too low amounts<sup>2</sup>.

By chance, due to the experimental conditions in irradiation, the nature of the polymer layer, the chemistry occurring and the migration processes, level of oxidations at dose of 106 kGy are the same for EVA/EVOH/EVA and PE/EVOH/PE films. All in all, concerning PE/EVOH/PE, chemical processes described in account for all results reported.

Fig. 5 shows PCA of the infrared spectra of the internal faces of bags (all irradiation doses and all ageing) before (in blue) and after 21 days of methionine solution storage (in red). A single group is observed, which means that all faces have the same behavior. Importantly, there is no damage observed on the polymer film integrity after methionine contact.

The level of oxidation cannot be predicted, as it depends on parameters such as the irradiation dose, the ageing of the empty bag, the buffering conditions, and the reducing proper-

 $^{1}$  A reviewer mentioned that antioxidant might scavenge generated H<sub>2</sub>O<sub>2</sub>. We can mention that PE layers contain secondary antioxidant which may scavenge generated H<sub>2</sub>O<sub>2</sub> in low amount at dose 29 kGy and hence, delayed its growth.

Table 2 – Requirements values for cases 1 and 2.		
	Case 1	Case 2
Y: Methionine sulfoxide	Target: 0	Target: 0
concentration (µM)	Max: 3	Max: 1

ties of the substrate. As factoring processes require the tuning of several parameters to observe the minimal impact of the generation of hydrogen peroxides, a design of experiments is applied. The minimization of the methionine sulfoxide concentration is the main goal. We apply the desirability function proposed by Derringer and Suich (1980) in which the value of the response is transformed into an individual desirability function d(Y), representing the degree of satisfaction, scaled between 0 and 100%: 0% pointing at an unacceptable value of Y and 100% denoting a completely acceptable value of Y.

Two cross-border cases (case 1 is less stringent than case 2) are selected for the requirements (Table 2).

The non-generation of methionine sulfoxide would be the ideal situation (target = 0  $\mu$ M). For the in-use situation, the minimum concentration of 3  $\mu$ M methionine sulfoxide is still suitable in case 1, and of 1  $\mu$ M in case 2.

When an undesirable value is obtained (methionine sulfoxide concentration > value max), the desirability value is 0% (in red, Fig. 6), and no compromise is found. By sharp contrast, when each requirement is completely satisfied (methionine sulfoxide concentration  $\leq$  target value), the desirability value is 100% (in blue, Fig. 6). Finally, when the desirability value is between 0% and 100%, an acceptable compromise is found, as defined in Table 2. The response surface corresponding to the desirability function is displayed in Fig. 6a for case 1 and Fig. 6b for case 2. The bluish zones represent the highest desirability.

To comply with requirements of case 1 - a maximum concentration of methionine sulfoxide detected lower than 3  $\mu$ M and a desirability >50% - conditions are observed in the right part of the bold line Fig 6a):

- Sterilization of bags from 30 kGy to 50 kGy and storage of bags for aging between 0 and up to 36 months after irradiation.
- Sterilization of bags from 50 kGy to 100 kGy and storage of bags for aging between 10 and up to 36 months after irradiation.

<sup>&</sup>lt;sup>2</sup> Due to industrial confidentiality, the exact amount of secondary antioxidant cannot be disclosed.

- Sterilization of bags from 100 kGy to 260 kGy and storage of bags for aging between 20 and up to 36 months after irradiation.

To comply with requirements of case 2 - a maximum concentration of methionine sulfoxide detected lower than 1  $\mu$ M and a desirability >50% - conditions are observed below the bold line Fig. 6b): the bags are sterilized from 30 kGy to 50 kGy and stored for aging between 13 and 28 months after irradiation.

Whatever the case, the best conditions for the nongeneration of methionine sulfoxide are a 30 kGy dose and 20-month aging.

# 5. Conclusion

To monitor oxidant species (mainly H<sub>2</sub>O<sub>2</sub> and peracids) and to highlight changes in gamma irradiated single use bags, methionine was used as a probe as it generates methionine oxides readily detected by HPLC. The maximum methionine sulfoxide concentration detected in the gamma irradiated PE/EVOH/PE bags was analyzed through a design of experiments. The generation of oxidants due to the gamma irradiation induced modification of the PE/EVOH/PE film was evidenced. From these investigations, an experimental design was developed as a tool to determine the set of conditions to fine tune the most appropriate gamma irradiation dose and storage conditions. Three sets of conditions were proposed. The optimal conditions for the use of these single-use bags minimizing the impact of radical chemistry were highlighted. The answers to the design of experiments were then considered to obtain the desirability domain to optimize the conditions of use of the single-use bags limiting methionine oxidation and the release of reactive species thereof.

### **Conflict of interest**

Authors state no conflict of interest.

## Data availability

Data will be made available on request.

#### **CRediT** authorship contribution statement

Nina Girard-Perier: Writing – original draft, Writing – review & editing. Magalie Claeys-Bruno: Writing – original draft, Writing – review & editing. Sylvain R.A. Marque: Writing – original draft, Writing – review & editing. Nathalie Dupuy: Writing – original draft, Writing – review & editing. Fanny Gaston: Writing – original draft, Writing – review & editing. Samuel Dorey: Writing – original draft, Writing – review & editing.

## Acknowledgments

We acknowledge Sartorius Stedim FMT S.A.S for the financial support of this work.

N.D, M.C-B and S.R.A.M are thankful to AMU and CNRS for support.

# Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fbp.2022.02.001.

## References

- Abdel Tawab, K., Ibrahim, S.M., Magida, M.M., 2013. The effect of gamma irradiation on mechanical, and thermal properties of recycling polyethylene terephthalate and low density polyethylene (R-PET/LDPE) blend compatibilized by ethylene vinyl acetate (EVA). J. Radioanal. Nucl. Chem. 295, 1313–1319.
- Audran, G., Dorey, S., Dupuy, N., Gaston, F., Marque, S.R.A., 2015. Degradation of γ-irradiated polyethylene-ethylene vinyl alcohol-polyethylene multilayer films: an ESR study. Polym. Degrad. Stab. 122, 169–179, http://dx.doi.org/10.1016/j.polymdegradstab.2015.10.021.
- Azurad: Intuitive and Powerful Software for the Construction and Processing of DOE; Marseille, 2019.
- Buttafava, A., Consolati, G., Mariani, M., Quasso, F., Ravasio, U., 2005. Effects induced by gamma irradiation of different polyesters studied by viscometry, thermal analysis and positron annihilation spectroscopy. Polym. Degrad. Stab. 89, 133–139.
- Dalle-Donne, I., Rossi, R., Giustarini, D., Gagliano, N., Di Simplicio, P., Colombo, R., Milzani, A., 2002. Methionine oxidation as a major cause of the functional impairment of oxidized actin. Free Radic. Biol. Med. 32 (9), 927–937, http://dx.doi.org/10.1016/S0891-5849(02)00799-2.
- Derringer, G., Suich, R., 1980. Simultaneous optimization of several response variables. J. Qual. Technol. 12 (4), 214–219, http://dx.doi.org/10.1080/00224065.1980.11980968.
- Dorey, S., Gaston, F., Dupuy, N., Barbaroux, M., Marque, S.R.A., 2018a. Reconciliation of PH, conductivity, total organic carbon with carboxylic acids detected by ion chromatography in solution after contact with multilayer films after γ-irradiation. Eur. J. Pharm. Sci. 117, 216–226, http://dx.doi.org/10.1016/j.ejps.2018.02.023.
- Dorey, S., Gaston, F., Marque, S.R.A., Bortolotti, B., Dupuy, N., 2018b. XPS analysis of PE and EVA samples irradiated at different γ-doses. Appl. Surf. Sci. 427, 966–972, http://dx.doi.org/10.1016/j.apsusc.2017.09.001.
- Dorey, S., Gaston, F., Girard-Perier, N., Dupuy, N., Marque, S.R.A., Delaunay, L., 2019. Generation of O2-permeation barrier during the gamma-irradiation of polyethylene/ethylene-vinyl alcohol/polyethylene multilayer film. Ind. Eng. Chem. Res. 58 (31), 14115–14123, http://dx.doi.org/10.1021/acs.iecr.9b02145.
- Drazic, A., Winter, J., 2014. The physiological role of reversible methionine oxidation. Biochim. Biophys. Acta 1844, 1367–1382.
- F02 Committee. Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices, ASTM International, West Conshohocken, PA. https://doi.org/10.1520/F1980-16.
- Folzer, E., Diepold, K., Bomans, K., Finkler, C., Schmidt, R., Bulau, P., Huwyler, J., Mahler, H.-C., Koulov, A.V., 2015. Selective oxidation of methionine and tryptophan residues in a therapeutic IgG1 molecule. J. Pharm. Sci. 104, 2824–2831.
- Gaber, M.H., 2005. Effect of γ-irradiation on the molecular properties of bovine serum albumin. J. Biosci. Bioeng. 100 (2), 203–206, http://dx.doi.org/10.1263/jbb.100.203.
- Gaston, F., Dupuy, N., Marque, S.R.A., Barbaroux, M., Dorey, S., 2016. One year monitoring by FTIR of γ-irradiated multilayer film PE/EVOH/PE. Radiat. Phys. Chem. 125, 115–121, http://dx.doi.org/10.1016/j.radphyschem.2016.03.010.
- Gaston, F., Dupuy, N., Girard-Perier, N., Marque, S.R.A., Dorey, S., 2021. Investigations at the product, macromolecular, and molecular level of the physical and chemical properties of a γ-irradiated multilayer EVA/EVOH/EVA film: comprehensive analysis and mechanistic insights. Polymers 13.

Girard-Perier, N., Claeys-Bruno, M., Marque, S.R.A., Dupuy, N., Gaston, F., Dorey, S., 2020. Monitoring of peroxide in gamma irradiated EVA multilayer film using methionine probe. Polymers 12 (12), 3024,

http://dx.doi.org/10.3390/polym12123024.

- Girard-Perier, N., Dorey, S., Gaston, F., Girard, F., Marque, S.R.A., Dupuy, N., 2022. One-year ageing FTIR monitoring of PE/EVOH/PE film after gamma or electron beam irradiation. Polym. Degrad. Stab. 195, 109790.
- Hyun Kang, P., 2001. The effect of  $\gamma$ -irradiation on ultra-high molecular weight polyethylene recrystallized under different cooling conditions. Radiat. Phys. Chem. 60, 79–87.
- ISO 11137-1 International Organization for Standardization, Sterilization of Health Care Products - Radiation - Part 1: Requirements for Development, Validation and Routine Control of a Sterilization Process for Medical Devices. 2006. Kátia Aparecida da Silva Aquino, 2012. Sterilization by Gamma
- Irradiation. Li, S., Schöneich, C., Borchardt, R.T., 1995. Chemical instability of
- protein pharmaceuticals: mechanisms of oxidation and strategies for stabilization. Biotechnol. Bioeng. 48, 490–500.
- Martens, H., Naes, T., 1989. Multivariate Calibration. Wiley, New York.
- Masato, A., Kiichi, F., Uchiyama, S., 2016. Suppression of methionine oxidation of a pharmaceutical antibody stored in

a polymer-based syringe. J. Pharm. Sci. 105, 623–629.

- Menzel, R., Dorey, S., Maier, T., Pahl, I., Hauk, A., 2021. X-ray sterilization of biopharmaceutical manufacturing equipment-extractables profile of a film material and copolyester Tritan<sup>™</sup> compared to gamma irradiation. Biotechnol. Prog., e3214.
- Montanari, L., Costantini, M., Signoretti, E.C., Valvo, L., Santucci, M., Bartolomei, M., Fattibene, P., Onori, S., Faucitano, A., Conti, B., Genta, I., 1998. Gamma irradiation effects on poly(dl-lactictide-co-glycolide) microspheres. J. Control. Release 56, 219–229.
- Schöneich, C., 2005. Methionine oxidation by reactive oxygen species: reaction mechanisms and relevance to Alzheimer's disease. Biochim. Biophys. Acta 1703, 111–119.
- Shukla, A.A., Gottschalk, U., 2013. Single-use disposable technologies for biopharmaceutical manufacturing. Trends Biotechnol. 31, 147–154.
- Stadtman, E.R., van Remmen, H., Richardson, A., Wehr, N.B., Levine, R.L., 2005. Methionine oxidation and aging. Biochim. Biophys. Acta 1703, 135–140.
- Štajner, D., Milošević, M., Popović, B., 2007. Irradiation effects on phenolic content, lipid and protein oxidation and scavenger ability of soybean seeds. Int. J. Mol. Sci. 8 (7), 618–627, http://dx.doi.org/10.3390/i8070618.