Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

ASSESSING THE MALDI-TOF MS SAMPLE PREPARATION PROCEDURE TO ANALYZE THE INFLUENCE OF THERMO-OXIDATIVE AGEING AND THERMO-MECHANICAL DEGRADATION ON POLY (LACTIDE)

J.D. Badia[1], E. Strömberg[2], A. Ribes-Greus[1], S. Karlsson[2,*]

DOI: https://doi.org/10.1016/j.eurpolymj.2011.05.001

Please, cite it as: Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

[2] School of Chemical Science and Engineering,

Fibre and Polymer Technology,

KTH - Royal Institute of Technology,

Teknikringen 56-58, SE-10044 Stockholm, Sweden

[*] Corresponding author: Sigbritt Karlsson

Presently, Vice Chancellor Skövde University, SE-541 28 SKÖVDE, Sweden.

e-mail: sigbritt.karlsson@his.se

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

ASSESSING THE MALDI-TOF MS SAMPLE PREPARATION PROCEDURE TO ANALYZE THE INFLUENCE OF THERMO-OXIDATIVE AGEING AND THERMO-MECHANICAL DEGRADATION ON POLY (LACTIDE)

J.D. Badía[1], E. Strömberg[2], A. Ribes-Greus[1] , S. Karlsson[2,*]

[1] Instituto de Tecnología de Materiales (ITM),

Universitat Politècnica de València

Camino de Vera s/n, E-46022 Valencia, Spain

[2] School of Chemical Science and Engineering,

Fibre and Polymer Technology,

KTH - Royal Institute of Technology,

Teknikringen 56-58, SE-10044 Stockholm, Sweden

[*] Corresponding author: Sigbritt Karlsson

Presently, Vice Chancellor Skövde University,

Högskolan i Skövde

Box 408

541 28 SKÖVDE

+46 0500-44 81 43

e-mail: sigbritt.karlsson@his.se

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal, 47*(7), 1416-1428.

ASSESSING THE MALDI-TOF MS SAMPLE PREPARATION PROCEDURE TO ANALYZE THE INFLUENCE OF THERMO-OXIDATIVE AGEING AND THERMO-MECHANICAL DEGRADATION ON POLY (LACTIDE)

J.D. Badía, E. Strömberg, A. Ribes-Greus, S. Karlsson

Abstract

Multiple processing by means of successive injection cycles was used to simulate the thermo-mechanical degradation effects on the oligomeric distribution of PLA under mechanical recycling. Likewise, an accelerated thermal ageing over PLA glass transition was performed in order to simulate its service life. MALDI-TOF MS was used for the analysis and the sample preparation procedure was assessed by means of a statistical Design of Experiments (DoE). The quality effects in use for the analysis were Signal-to-Noise ratio and Resolution. Different matrixes, analyte/matrix proportions and the use of NaTFA as cationization agent were considered. A deep inspection of the statistical results provided a better understanding of the influence of the different factors, individually or in combination, to the signal. The application of DoE for the improvement of the MALDI measurement of PLA stated that the best combination of factors (levels) was the following: matrix (s-DHB), proportion analyte/matrix (1/5 V/V), and no use of cationization agent. Degradation primarily affected the initially predominant cyclic $[LA_C]_n$ and linear $H\text{-}[LA_L]_n\text{-}OH$ species, where LA stands for a PLA repeating unit. Intramolecular and intermolecular transesterifications as well as hydrolytic and homolytic reactions took place during the formation and disappearance of oligomeric species. In both degradation mechanisms induced by thermal ageing and thermo-mechanical degradation, the formation of $H\text{-}[LA_L]_n\text{-}O\text{-}CH_3$ by intermolecular transesterifications was highlighted.

Keywords: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), Design of Experiments (DoE), polylactide (PLA), thermo-mechanical degradation, thermo-oxidative ageing

1. Introduction

The packaging industry is a highly important economic sector that involves big quantities of plastic materials. One-use applications critically reduce the service life of these products, being rapidly drawn to the disposal step with all their properties almost intact. Packages made of commodities such as polyethylene (PE), polypropylene (PP), polystyrene (PS) or poly(ethylene terephthalate) (PET) are usually immediately discarded after the first use, and their elimination and reintegration into the carbon cycle can require hundreds or even thousands of years. Therefore, the interest on plastic materials that accomplish the two-fold benefit of being biodegradable and come from renewable resources has gained much attention. The most popular biodegradable polymers are poly (lactic acid) or polylactides (PLA), polycaprolactone (PCL), poly (butylene adipate terephthalate) (PBAT) and polyhydroxybutyrate (PHB) [1].

Polylactides are thermoplastic polyesters obtained from the ring-opening polymerization of lactide, which may be derived from the fermentation of sugar feedstocks at competitive prices compared to that previously achievable from petrochemical-derived products [2]. PLAs have numerous interesting properties including good processability, mechanical properties, thermal stability and low environmental impact [2-3], which enhance their performance as suitable candidates for replacing commodities at the packaging sector. This solution will therefore imply the increase of a new source of polymeric waste, which would have to be managed. Moreover, with the aim of extending the service life of PLA goods, before finally discarding them, it would be advisable to explore the possibilities of recovering the material. Among all recovery methods, mechanical recycling represents one of the most successful processes and has received considerable attention due to its main advantages, since it is relatively simple, requires low investment, and its technological parameters are controlled [4]. Moreover, life cycle assessment studies have pointed out that mechanical recycling is the most preferable recovery route for relatively clean and homogeneous waste streams in terms of energy saving and emission of gases contributing to global warming [5]. Nevertheless, polymers are subjected to the influence of degradative agents such as oxygen, UV-light, mechanical stresses, temperature and water, which, separately or in combination, during its material loop (synthesis - processing - service life - discarding - recovery), results in chemical and physical changes that alter their stabilization mechanisms and long-term properties [6] . These degradation processes may modify the structure and composition of PLA and consequently change the thermal, rheological and mechanical properties of the recyclates [7-8]. The assessment of the degradation mechanism is therefore necessary to determine the quality of recycled PLA and guarantee its further performance in second-market applications. Simulation of mechanical recycling by multiple processing and service life by accelerated thermal ageing to assess the effects of thermal and thermo-mechanical degradation has been previously performed for commodities [9-15] such as PE [9], PP [9], PS [10-11], poly (vinyl chloride) (PVC) [12] or PET [13-16].

Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) has gained attention during the last years as a potential technique for the analysis of the compositions, end groups and, in some cases, molecular weight distributions of intact synthetic polymers [17]. Characteristics and main applications of MALDI can be found in several reviews [18-19]. Studies on polyesters is an issue of interest among researchers [20-21], but studies of polylactides are still few [22-29], and mainly applied to low molecular weight PLA synthesized under laboratory conditions. As far as we are concerned, studies on the application of MALDI to characterize the effect of multiple-processing and accelerated thermal ageing on specifically commercial PLA have not been published and therefore this is an area of interest for future work.

The difficulty of performing good-quality and reliable MALDI measurements depends on many factors such as the molar mass of the polymer, the choice of solvent, the choice of matrix [30-32], the ratio analyte/matrix, the use of cationization agent, the laser energy or the mode of detection (linear or reflector), among others [30]. Some research groups have reported these difficulties and have found correlations between the crystal and/or molecular structure of matrices and the MALDI spectral data [31]. Advances and contributions in issues such as the choice of the correct solvent for sample crystallization [32], matrix functional groups arrangement [33], improvement of Signal-to-Noise ratio [33], or the influence of crystallinity of polymers on the measurement [34] have been performed. However, there are still no clear criteria enabling the prediction of the optimal conditions for the matrix-polymer sample preparation. Cost-effective methodologies should be therefore implemented, in order to optimize the performance of the MALDI measurement. Despite its straightforward applicability, speediness and ease, statistical methods such as the Design of Experiments (DoE) are not widely applied yet.

DoE plays a fundamental role in the optimization of scientific and industrial problems [35], which involves the study of the influence of multiple input variables (Factors) analyzed at different scenarios (Levels) on the experimental outcomes (Effects). DoE [36] stands as a useful, reliable and immediate procedure that can provide us not only with the best combination of settings for the preparation of the MALDI sample, but also help understand the influence of each factor individually or in combination on the quality of the response. In previous works, the suitability of DoE to understand the influence of matrix, analyte/matrix proportion and amount of cationization agent was reported for the study of poly(ethylene terephthalate) [16].

In the present study, simulation of reprocessing and service life was performed by successive injection moulding cycles and accelerated thermo-oxidative ageing respectively on commercial PLA. In the first part of the study, DoE was applied for determining the best conditions of the MALDI sample preparation for PLA in order to enhance the quality of the signal, taking into account matrix, proportion analyte/matrix and use of cationization agent. In the main analysis, the oligomeric degradation mechanisms that may explain the influence of degradation occurring in PLA during both thermo-mechanical and thermo-oxidative ageing were studied.

2. Experimental procedure

Figure 1 schematically shows the procedure followed to simulate degradation induced by multiple processing and service life. Materials, reagents, simulation conditions and MALDI analytical features are stated as follows:

2.1. Materials and reagents

Polylactide (PLA) 2002D is a thermo-forming grade PLA obtained from Natureworks LLC (Minnetonka, MN) as pellets, provided by AIMPLAS (Paterna, Spain). MALDI matrixes, namely 1,8,9-anthracenetriol (dithranol), 2-(4-hydroxyphenylazo) benzoic acid (HABA), 2,5-dihydroxybenzoic acid (s-DHB), as well as the cationization agent, sodium trifluoroacetate (NaTFA), were purchased from Sigma-Aldrich (Stockholm, Sweden). Tetrahydrofurane was purchased from VWR (Sweden).

2.2. Reprocessing simulation

Prior to processing, virgin PLA pellets were dried during 2 h at 80 ºC in a dehumidifier Conair Micro-D FCO 1500/3 (UK), in order to remove as much humidity as possible from PLA flakes. Afterwards, samples were processed by means of injection moulding by means of an Arburg 420 C 1000-350 (Germany) injector, single-screw model (diameter Φ=35 mm, length/Φ=23). Successive processing steps were applied under the same conditions. Temperature gradient set from hopper to die was 160, 170, 190, 200 and 190ºC. Moulds were set at 15 ºC. Cooling time residence was ca. 40 s and total residence time ca. 60s. Samples were dried before each processing cycle. After injection, a fraction of the samples was kept as test specimens and the rest was ground by means of a cutting mill Retsch SM2000 (UK), which provided pellets of size d< 20 mm to be fed back into the process. Up to five processing cycles were applied to obtain the different testing specimens of reprocessed PLA (RPLA-i, with i: 1-5).

2.3. Service life simulation

Thermo-oxidative aging was performed on dehumidified virgin PLA by means of a Heraeus UT 6060 (Hanau, Germany) forced-ventilation oven under air atmosphere at 60 ºC. Samples were removed for analysis after the exposure times of 1, 3, 6, 10 and 12 weeks.

2.4. MALDI sample preparation and analysis

MALDI sample mixtures (Matrix (*M*) + Analyte (*A*) + Cationization Agent (*C*), *MAC*) were prepared in laboratory conditions according to ISO 291, atmosphere 23/50, class 1 [37]. Individual solutions of *M*, *A* and *C* in THF were prepared at a concentration of 10 $g \cdot L^{-1}$. Different proportions (**Table 1**) of *M*/*A*, according to the Design of Experiments (section 3.2) were applied. In case *C* was added to the *MAC*, it was in the same volumetric ratio as the *A*. The mixtures were then vortexed using a Vortex Genie (Scientific Industries, Bohemia, NY). Approximately 0.5 µL of the sample mixture were added on the target plate and the spots were allowed to dry at ambient temperature before insertion into the instrument.

MALDI-TOF/MS experiments were conducted on a Bruker UltraFlex MALDI-TOF mass spectrometer with a SCOUT-MTP ion source (Bruker Daltonics, USA), a gridless ion source and a reflector. All spectra were acquired in the reflector positive ion mode with an acceleration voltage of 25 kV and a reflector voltage of 26.3 kV. The detector *m/z* range was 200–6000 Da in order to exclude high intensity signals arising from the low mass ions and to cover the whole PLA mass spectrum. Ions below 100 *m/z* were removed with pulsed deflection. The laser intensity was set to the maximum value possible, taking care not to burn the *MAC* in order to avoid the appearance of high-intensity background peaks, which could decrease the Signal-to-Noise ratio and the Resolution. Spectra were gathered by irradiating 40-50 different positions at the centre area on the sample spot, with a total of 2500 (5 X 500) shots per sample. Time-to-mass conversion of the time-of-flight mass spectra was achieved using a self-calibration method [38]. All MALDI spectra were treated using FlexAnalysis 2.4 (Bruker Daltonics, USA) software. Interpretation of data was carried out taking into account all decimals of the atomic masses composing the oligomers, but note that *m/z* values are given with only one decimal. Statistical Design of Experiments (DoE) was aided by Minitab® 15.1.0.0. software (Minitab Inc., USA). Measurements were performed by triplicate and the average values were considered as representative.

Figure 1

3. Results and discussion

The present work is divided into two different sections. In the first part of the study, the design of experiments, DoE, was applied for determining the best conditions of the MALDI sample preparation for PLA in order to enhance the quality of the spectra. Thereafter, the MALDI analysis of the influence of thermo-oxidative and thermo-mechanical degradation on the oligomeric distribution of polylactide was assessed.

3.1. Determination of the experimental factors and levels considered for DoE analysis of MALDI sample preparation

With the aim of performing a reliable MALDI measurement with good-quality signals, some considerations regarding the Sample Preparation (SP) were taken into account. The followed procedure is explained with more detail in a previous work [16]. Some variables were defined as Factors (*F*) for the DoE: suitable matrix, reasonable proportion analyte/matrix and necessity of cationization agent. Given that each factor could be analyzed for a wide span of Levels (*L*), the study of the interaction of all of them shall not be operative, and thus a preliminary screening was performed in order to reduce this quantity of *F/L* situations: Firstly, S-DHB, Dithranol and HABA were chosen (**Figure 2**) as proper [30] matrixes from an exhaustive list in literature [39]. Tetrahydrofurane (THF) was taken as solvent for all components since it was extremely important that the mixture behaved as one during the evaporation process of the solvent, avoiding thus sample segregation, which could impoverish the quality of the signal [40]. On the other hand, since polyesters are relatively polar polymers, Na^+ and K^+ adducts could be observed in the MALDI spectra, even if they were not deliberately added to the mixture [41-43]. It is know that these cations are present as impurities in matrixes, reagents, solvents or glassware among other sources, and therefore polymers with high cation affinity do not necessarily need a high amount of extra salt in the sample [30]. The presence of specific functional groups such as carboxyl and hydroxyl is very important in the cationization process [44]. On the other hand, the fact that matrixes such as HABA and dithranol are particularly insensitive to impurities [18] justifies the study of the addition of a cationization agent. Sodium Trifluoroacetate (NaTFA) was chosen as a source of ions.

Figure 2

3.2. The Design of Experiments applied to MALDI-VPLA

The levels *L* at which each *F* was analyzed are summarized in **Table 1**. In order to perform the DoE, a significant step is the correct choice of parameters to be considered as reliable effects (*E*). Signal-to-Noise ratio (*S/N*) and Resolution (*RES*) stand out as suitable quality indicators in many different spectrometric studies [35], and therefore were chosen for this study. A schematic summary of the combination of DoE and MALDI is depicted in **Figure 3.**

Table 1, Figure 3

After a first screening, the adducts which provided the signal of highest intensity were cyclic $([LA_C]_n Na)^+$, and thus were chosen for the analysis. With the aim of assuring the reliability of this study, instead of focusing the discussion on the results obtained from the study of an individual peak, a span of 500 *m/z* between 1500 and 2000 *m/z* in which up to seven $([LA_C]_n Na)^+$ peaks (1536.3, 1608.3, 1680.4, 1752.5, 1824.5, 1896.6, and 1968.6 *m/z*), with a separation of a LA repeating unit (72.062 *m/z*) were chosen for characterization. Absolute and relative Effects *E* (being *E= S/N* or *RES*) were analyzed according to the following expressions:

$$E = \sum_i \left(E_i \cdot \frac{I_i}{Imax} \right) \tag{1}$$

$$E_{rel} = \sum_i \left(\frac{E_i}{\mathrm{Emax}} \cdot \frac{I_i}{Imax} \right) \tag{2}$$

where *I* is the intensity of the peak, *Imax* the maximum intensity, *Emax* the maximum of the effect and *i* is the counter of the seven studied peaks. **Table 2** shows the results of the analysis of variance in terms of p-value (*P*) and adjusted regression coefficient (R^2) for each *E* according to the General Linear Model (GLM) [36] applied for the evaluation of the DoE. The statistic *P* measures the significance of a change in level *L* of a factor *F*, individual or in combination with other *F* (interaction), to the *E*. If *P* is lower than or equal to a confidence value (α), then this *F* (or interaction of *F*) is significant, and its assessment is thus worthy. On the other hand, R^2 measures the suitability of the GLM to explain the variability of data. The closer R^2 is to 1, the better is the selection of the *E* to characterize the experiment under consideration. Some conclusions could be drawn from this analysis: Absolute effects were chosen, since those relative offered R^2 far below 95%. In addition, the fact of carrying out the experiments by triplicate added a new factor to the DoE analysis (*Blocks*), but no relationship between this factor and both effects (*S/N* and *RES*) was found, which means that there was no significant experimental error related to the sample preparation affecting the quality of the results. Finally, all *F* influenced both the *S/N* and *RES* of the MALDI signal and therefore were considered for analysis, since $P<\alpha$ being α the commonly chosen 5%. Note that in the case of the study of the *RES* by modifying the addition of NaTFA, P=5.5%, which is not far from α. Concerning the interaction of NaTFA and proportion analyte/matrix, P=0.111, which indicates that its interpretation was not necessarily trustable and thus was not considered.

Table 2

Main effects plots (*MEP*) and Interaction plots (*IP*) are intuitive tools very useful to estimate the influence of factors though the different levels, both in general or in combination, respectively, to the considered *E*. The study of these plots was assessed as follows: **Figure 4** and **Figure 5** show the Main Effects Plot (*MEP*) for both *S/N* and *RES*, respectively. The overall influence of all factors on the quality parameters chosen for the assessment of the signal could be drawn from these plots. The horizontal line crossing these graphs is the so-called *grand mean*, which is the averaged sum of all data for a specific effect (*S/N* or *RES*). Values above this line can be considered significant, whereas values below demonstrate the little influence of a specific factor on the *E* under study. In order to numerically interpret their results, **Table 3** shows the changes among levels for a determined factor which were calculated for the data means of each effect $\overline{E(\Delta L|_F)}(\%)$ by the following expression:

$$\overline{E(\Delta L|_F)}(\%) = \left.\frac{\overline{E(L)}-\overline{E(L_{REF})}}{\overline{E(L_{REF})}}\right|_F \times 100 \qquad (3)$$

Figure 4,5-Table 3

The selection of the reference level (L_{REF}) for each factor is arbitrary and for convenience the choices were stated as follows: *s-DHB* for the factor *Matrix*, *1/5* for the factor *Proportion* and *NO* for the factor *NaTFA*. The following conclusions were drawn:

Firstly, experiments with dithranol provided the lowest *S/N* values. On the other hand, despite the *S/N* was generally higher when using HABA instead of s-DHB (+9.5%), the loss of *RES* was dramatically high (-81.6%), and therefore the use of s-DHB seemed to be more adequate. Furthermore, the influence of the analyte/matrix proportion was opposite for both effects; while increasing the amount of matrix generally increased the *S/N* (+30.1% from 1/5 to 1/20), the *RES* was contrarily negatively affected (-45.0% for the same conditions). In this case, since the overall variations were quite similar, the decision of which proportion was satisfactory for the analysis will be tackled later, when studying the Interaction Plots, specifically for the chosen matrix. Finally, the addition of NaTFA generally favoured the *RES* means (+4.5%), but the high reduction in the *S/N* means (-54.5%) advised not to use cationization agent in this study.

Further interpretation of the Interaction Plots for both *S/N* and *RES* shown at **Figure 6** and **Figure 7** respectively, offered more information regarding the synergetic influence of a couple of factors at all studied *L* for a specific *F*. For better understanding, discussion was focused on sub-plots *a* and *c* in both figures, in order to evaluate the interaction matrix/proportion, specifically for the use of s-DHB. Similar interpretation could be drawn in case the joint influence of two *F* was intended for the rest of matrices. By means of applying Eq. (3) for the variation of proportion when s-DHB was used, increasing it from 1/5 to 1/20 lead to an increase of 60.6 % in *S/N* means, accompanied by a decrease of 61.1% in *RES* means. The dilemma therefore was to settle on which of both effects was more decisive for the analysis. **Figure 8** shows the MALDI spectra of PLA analyzed with s-DHB, for better correlation between the DoE results and the quality of the signal. As pointed out by DoE, the addition of NaTFA strongly reduced the *S/N*. Likewise, the higher amount of matrix was used for the sample preparation, the less accurate could the peaks be isolated from the rest, because of a reduction in resolution. In addition, it was noticeable that even when the signal-to-noise ratio was enhanced when more amount of matrix was used, the relative intensity peaks were contrarily decreased. In conclusion, the chosen criterion was taken according to the parameters aimed in this analysis: first, identification (improved by the resolution) and afterwards, semi-quantification (enhanced by the signal-to-noise ratio) of oligomers. Therefore, selecting *RES* as the key parameter in this case, it was concluded that the application of a DoE for the improvement of the MALDI measure of PLA would be superior with the following combination of factors and levels: matrix (s-DHB), proportion analyte/matrix (1/5, V/V), and use of NaTFA (NO). The following sections thus show the MALDI analysis of the degradation subjected by both accelerated thermo-oxidative ageing and multiple reprocessing cycles.

Figures 6, 7, 8

3.3. Identification of oligomeric species

Figure 9 shows the MALDI spectra for virgin PLA corresponding to 22 LA units, as an example for identification of cyclic and linear oligomeric species, indicated for VPLA, and summarized at **Table 4**. Note that species appear at more *m/z* than those presented, but only values for n=22 are shown for clarity. Full symbols were used for Na^+ adducts and hollow symbols for H^+ adducts. Predominant species found for virgin PLA were, in order of abundance: (i) cyclic **$[LA_C]_n$**, mainly present as Na^+ adducts ($[M + Na]^+$: *m/z* 1608.3) and in less quantities, H^+ adducts ($[M + H]^+$: 1585.3 *m/z*); (ii) linear **$H\text{-}[LA_L]_n\text{-}OH$**, which bears a hydroxyl and a carbonyl end groups ($(M + Na]^+$: *m/z* 1626.3 and $[M + H]^+$: *m/z* 1604.3); and, (iii) linear **$H\text{-}[LA_L]_n\text{-}O\text{-}CH_3$**, which has a methoxyl group and a hydroxyl group bearing ($[M + Na]^+$: *m/z* 1640.3 and $[M + H]^+$: *m/z* 1618.3), commonly found at PLAs obtained by ring opening polymerization (ROP) due to the use of alkoxydic initiators, which remain in the terminal units [28]. Other low predominant species found that might be formed as by-products at the processing temperature were : (iv) linear **$CH_3\text{-}O\text{-}[LA_L]_n\text{-}CH_3$**, bearing two methoxyl groups ($[M + Na]^+$: *m/z* 1654.4 and $[M + H]^+$: *m/z* 1632.4); and (v) linear **$CH_3\text{-}CO\text{-}O\text{-}[LA_L]_n\text{-}H$** ,which has a hydroxyl and a carboxylic methyl ester groups as ending units ($[M + Na]^+$: *m/z* 1668.4 and $[M + H]^+$: *m/z* 1646.4), that might appear due to esterification reactions from different chain capping routes during polymerization. In addition, the MALDI spectra show that both even-membered and odd-membered oligomeric species appeared for virgin PLA. Since the presence of odd-membered oligomers may not be explained by reason of PLA ROP, it may be admitted that intermolecular transesterifications took place in parallel to the polymerization process, thus causing a random cleavage of the polylactide backbone, as suggested by *Montaudo et al*. [28].

Table 4 - Figure 9

3.4. Degradation studies

Figure 10 shows the proposed degradation mechanisms map for polylactide degradation, which involves the reactions of formation and disappearance of the aforementioned main oligomeric species. Low abundant species (linear **CH_3-O-$[LA_L]_n$-CH_3**, and linear **CH_3-CO-O-$[LA_L]_n$-H)** were not included since their abundance was not significant during the process and their reactivity can be explained under the same conditions as the methoxyl estered species **CH_3-O-$[LA_L]_n$-H**. Thermo-oxidative ageing and thermo-mechanical degradation influenced the oligomeric distribution of polylactide differently and therefore the discussion is stated in the next paragraphs according to the studied type of degradation.

The reactions of PLA degradation generally followed the postulated mechanistic routes of polyesters which are shown as follows for the case of PLA[1]: Route I: Hydrolysis, which leads to the formation of hydroxyl and carboxyl linear oligomers with shorter chain length. Route II: Esterification. Route III: Intramolecular transesterification, *a*: from the end of the chain (backbiting); *b*: in the middle of the chain, which leads to the formation of cyclic oligomers and linear species with shorter length. Route IV: Intermolecular transesterifications, which interchange ester units between different chains, leading to an increase in the heterogeneity of the polymer. Routes V: Chain scission reactions, acyl-O and alkyl-O β-C initiated homolytic chain-scissions at temperatures above melting; and radical reactions induced by oxygen, which may produce random chain cleavage, leading to the formation of mainly linear hydroxyl and carboxyl terminated species.

Figure 10

It is known that the physical and chemical features of polylactide are directly connected to its stereochemistry. Indeed, the Molar Mass Distribution (MMD) of PLA would vary if the D/L ratio was different [45]. However, The PLA used in this work is eminently amorphous (>95 % L-lactide), and its morphological configuration remains nearly equal along the degradation processes [46], without showing a hint of crystallinity that might influence the MALDI signal due to different interactions with the matrix or condition the degradation mechanisms. According to *Montaudo et al.* [5], the linear mode is essentially used for the determination of the MMD of polymers, whereas the reflector mode allows

---

[1] Schemes available at the on-line additional information.

for the identification of oligomers or side-products, as well as the characterization of end-groups. Since this work was focused on the effects of reprocessing and thermo-oxidative aging on PLA structure and thus the apparition of new oligomers, and the correlation between the MMD of polymers drawn by MALDI and other techniques, i.e. Size Exclusion Chromatography (SEC), is not clear yet [47], the reflector mode was preferred. Since degradation starts primarily at the amorphous part of PLA backbone, the nature of the newly formed oligomers would be essentially the same.

Thermo-oxidative ageing

Figure 11 shows the evolution of the relative ion abundances of the oligomeric species along the accelerated thermo-oxidative ageing carried out at 60 ºC. Note that both intensities and area under curves for both Na^+ and H^+ ions were considered for calculations. Ageing at temperatures above the glass transition temperature of PLA (ca. 55 ºC [48]) enhances the molecular mobility of the chains and therefore might increment the possibilities of reaction. The variation of ion abundances showed a two-stage tendency: during the first step, up to 3 weeks, there was an increasing tendency of formation of cyclic $\mathbf{[LA_C]_n}$, along with a decrease of linear $\mathbf{H\text{-}[LA_L]_n\text{-}OH}$, which suggested the dominance of middle-chain intramolecular transesterifications (Route III), aided by temperature. Besides, there was a first increase of linear $\mathbf{H\text{-}[LA_L]_n\text{-}O\text{-}CH_3}$, that might be mainly produced by esterification (Route II) of $\mathbf{H\text{-}[LA_L]_n\text{-}OH}$. In addition, minor species might rapidly react to very low proportions along the rest of the thermo-oxidative ageing.

In a second stage, from 3 weeks up to 12 weeks, the tendency described for the main oligomeric species changed. There was a strong reduction of cyclic $\mathbf{[LA_C]_n}$ species to proportions below the values present in neat VPLA. On the other hand, the increasing tendency of $\mathbf{H\text{-}[LA_L]_n\text{-}O\text{-}CH_3}$ species was slightly maintained , occurring along with a remarkable increment of $\mathbf{H\text{-}[LA_L]_n\text{-}OH}$ species, which may be explained by hydrolytic reactions mainly affecting the $\mathbf{[LA_C]_n}$ induced by present water and methanol traces (Route I) that could easily attack a more liable structure, excited by temperature. As well, the auto-oxidation reactions [49] may be promoted at long exposition times at this second stage, auto-catalyzed by radical species, which may induce homolytic reactions in the polymeric structure (Routes V). Likewise, the apparition of new hydroxyl and carboxyl terminated groups might auto-catalyze these reactions. Intermolecular transesterifications

(Route IV) would take place favouring ester-exchange through chain cleavage along the polymer chain, as drawn from the odd-membered species found in the MALDI spectra [28].

Figure 11

Thermo-mechanical degradation

Figure 12 shows the evolution of the relative ion abundances of polylactide oligomeric species along the multiple reprocessing steps. Thermo-mechanical degradation induced by means of multiple reprocessing underwent modifications in the oligomeric distribution of the PLA sample, in a different fashion than that shown by the study of the influence of the accelerated thermo-oxidative ageing. Thermo-mechanical degradation followed a single step, in which the apparition or disappearance of species occurred gradually. Degradation agents associated to reprocessing were more aggressive for the polymeric distribution, since chains were submitted to temperatures above PLA melting temperature, in which the reactions among end-groups are enhanced. As well, the susceptibility of recyclates to both O-acyl and O-alkyl homolysis via β-C scission [50-52] at higher temperatures might increase due to a remarked weakness of the structure throughout the reprocessing cycles. Even more, short melting and cooling stressing cycles applied during the injection procedure might increase the possibilities of changes in the morphology. Undesirable components such as presence of water moieties and methanol traces from the initiation process could also affect the materials, by subjecting PLA chains to scission reactions that may decrease the molecular weight of the polymer [46]. Although care was taken when processing the samples in order to avoid the presence of humidity prior to the process, PLA is very hygroscopic, and after some cycles, the efficiency of water removal could be diminished, thus enhancing the occurrence of hydrolytic reactions. Finally, shear mechanical forces taking place during the injection and the grinding processes induce degradation in a larger extent and therefore would, inherently to reprocessing, break the polymeric chains, leading to smaller species that may vary the oligomeric distribution of polylactide. Main differences were stated as follows:

On the one hand, cyclic **$[\mathrm{LA_C}]_n$** species showed a gradual decrease to abundance values close to 35 % up to the third recyclate and recovered its presence at the fifth recyclate. Likewise, linear **HO-[LA_L]$_n$-H** presented a similar behaviour, ranging its variation from 25 % to a 15%. The most remarkable changes were those occurring for linear **CH_3-O-[LA_L]$_n$-H** species, which presented a noticeable increase, being the most predominant species after three processing cycles, and achieving a proportion of up to 40 % for the fourth recyclate. This fact, together with the reduction of linear **HO-[LA_L]$_n$-H** and cyclic **[LA_C]$_n$** suggested that the prime reactions taking place during thermo-mechanical degradation of PLA followed a two-step behaviour: (i) hydrolysis (Route I) or intramolecular transesterification (Rote III) to mainly release **HO-[LA_L]$_n$-H**, and (ii) immediate esterification of **HO-[LA_L]$_n$-H** into **CH_3-O-[LA_L]$_n$-H** (Route II), due to the high reactivity of **HO-[LA_L]$_n$-H** at the processing conditions.

On the other hand, the abundance of **CH_3-O-[LA_L]$_n$-H** would be also generally aided by intermolecular transesterifications (Route IV) of ester-terminated species, such as low abundant species (linear **CH_3-CO-O-[LA_L]$_n$-H** and **CH_3-O-[LA_L]$_n$-CH_3**), which might be incorporated into the main oligomeric degradation cycle. Note that the ion abundances of these species were above 5% for all recyclates, in contrast to the abundances found for thermally-aged PLA. Their presence can be explained as side-products/impurities after the grinding-melting-injection process, which induced a more heterogeneous degradation effect than that presented by thermo-oxidative ageing, since more degrading agents were present and therefore a new oligomeric distribution appeared after each reprocessing step. Finally, the homolytic chain-scission reactions induced by reprocessing would randomly attack the polymeric backbones, releasing shorter chains with different ending units which contributed to increase the heterogeneity and polydispersion of the material [28].

4. Conclusions

The sample preparation procedure for the analysis of polylactide (PLA) samples by means of MALDI-TOF MS was assessed. A statistical Design of Experiments (DoE) taking into account the following factors (levels): choice of the correct matrix (s-DHB, dithranol, HABA), the proportion analyte/matrix (1/5, 1/10, 1/20 V/V), and the use of cationization agent (YES, NO) was considered. The study was performed by means of analyzing the effect of changing the aforementioned factors among their different levels on spectroscopic signals taking into account quality parameters such as Signal-to-Noise ratio (*S/N*) and Resolution (*RES*). Main effects plots and interaction plots permitted to understand the influence of each factor to the quality of the MALDI spectra. The application of DoE for the improvement of the MALDI analysis of PLA stated that the best spectra were obtained with mixtures prepared with s-DHB as matrix, in a proportion analyte/matrix (V/V 1/5), without cationization agent, considering that *RES* was the key effect for the analysis.

Multiple processing by means of successive injection cycles was used to simulate the thermo-mechanical degradation effects on the oligomeric distribution of PLA under mechanical recycling. Likewise, an accelerated thermo-oxidative ageing over the glass transition was executed in order to simulate service life. Degradation primarily affected the initially predominant cyclic $[LA_C]_n$ and linear $H\text{-}[LA_L]_n\text{-}OH$ species. Intramolecular and intermolecular transesterifications as well as hydrolytic reactions occurred during the formation and disappearance of oligomeric species. In both mechanisms induced by thermo-oxidative and thermo-mechanical degradation, the formation of $H\text{-}[LA_L]_n\text{-}O\text{-}CH_3$ was highlighted, although a different behaviour was observed. Thermo-oxidative ageing presented a two-stage performance, governed by intramolecular transesterifications during the first stage and chain-scission reactions during the second stage, when over-exposure to temperature conditions triggered the depolymerization of cyclic species. On the other hand, thermo-mechanical degradation seemed to occur mainly via hydrolytic, homolytic and intermolecular transesterifications, giving rise to a noticeable major abundance of $H\text{-}[LA_L]_n\text{-}O\text{-}CH_3$ groups, especially after the third recyclate.

Acknowledgements

The authors would like to acknowledge the Spanish Ministry of Science and Innovation for the financial support through the Research Project UPOVCE-3E-013. The Spanish Ministry for Education is acknowledged for the concession of a predoctoral research position to J.D. Badía by means of the FPU program. AIMPLAS is acknowledged for providing and processing the material, respectively. Royal Institute of Technology (KTH, Sweden) and Universitat Politècnica de València (UPV, Spain) are also thanked for additional economical support.

References

1. Mohanty A K, Misra M, Hinrichsen G. *Biofibres, biodegradable polymers and biocomposites: an overview.* Macromolecular Materials and Engineering 2000; 276/277: 1-24.

2. Auras R, Harte B, Selke S. *An overview of polylactides as packaging materials.* Macromolecular bioscience 2004; 4: 835-865.

3. Tsuji H, Doi, Y.. *Polyesters III. Applications and commercial Products. In Biopolymers* [ed.] A Steinbüchel. Weinheim : Wiley-VCH Verlag GMbh, 2002.

4. Awaja F, Pavel D. *Recycling of PET.* , European Polymer Journal 2005; 41: 1453-1477.

5. Finnveden G, Johansson J, Lind P, Moberg A. *Life cycle assessment of energy from soild waste-Part1: General methodology and results*. Journal of Cleaner Production 2005; 13(3): 213-229.

6. Karlsson S. *Recycled polyolefins. Material properties and means for quality determination.* Advances in Polymer Science 2004; 169: 201-230.

7. Ehrig, R J. *Plastics Recycling: Products and Processes.* New York : Hanser Publishers, 1992.

8. Klemchuck, P P. *Polymer Stabilization and Degradation.* Washington : ACS, 1985.

9. Strömberg E, Karlsson S. *The design of a test protocol to model the degradation of polyolefins during recycling and service life*. Journal of Applied Polymer Science 2009; 112: 1835-1844.

10. Vilaplana F, Ribes-Greus A, Karlsson S. *Degradation of recycled high-impact polyestyrene. Simulation by reprocessing and thermo-oxidation*. Polymer Degradation and Stability 2006; 91: 2163-2170.

11. Vilaplana F, Karlsson S, Ribes-Greus, A.*Changes in the micro-structure and morphology of high-impact polystyrene subjected to multiple processing and thermo-oxidative degradation.* European Polymer Journal 2007;43: 4371-4381.

12. Yarahmadi N, Jakubowicz I, Gevert T. *Effects of repeated extrusion on the properties and durability of rigid PVC scrap.* Polymer Degradation and Stability 2001; 73 (1): 93-99.

13. Badia J D, Vilaplana F, Karlsson S, Ribes-Greus A. *Thermal analysis as a quality tool for assessing the influence of thermo-mechanical degradation on recycled poly(ethylene terephthalate)*. Polymer Testing 2009;28: 169-175.

14. La-Mantia F, Vinci M. *Recycling poly(ethylene terephthalate)*. Polymer Degradation and Stability 1994; 45: 121-125.

15. Torres N, Robin J J, Boutevin B. *Study of thermal and mechanical properties of virgin and recycled poly(ethylene terephthalate) before and after injection molding.* European Polymer Journal 2000; 36: 2075-2080.

16. Badia J D, Strömberg E, Ribes-Greus A, Karlsson S. *A statistical design of experiments for optimizing the MALDI-TOF-MS sample preparation of polymers. An application in the assessment of the thermo-mechanical degradation mechanisms of poly (ethylene terephthalate)*. Analytica Chimica Acta. 2011. In press.

17. Polce, Michael J y Wesdemiotis, Chrys. Introduction to Mass Spectrometry of Polymers. In: [ed.] Giorgio Montaudo y Robert P Lattimer. *Mass Spectometry of Polymers.* Boca Raton, Florida : CRC Press LLC, 2002.

18. Montaudo G., Samperi F, Montaudo M-S. *Characterization of synthetic polymers by MALDI-MS.* , Progress in Polymer Science 2006; 31: 277-357.

19. Weidner, SM, Trimpin S. *Mass spectrometry of synthetic polymers.* Analytical Chemistry 2008; 80: 4349-4361.

20. Williams J B, Gusev A I, Hercules D M.*Characterization of polyesters by Matrix-Assisted Laser Desorption/Ionization Mass Spectometry*. Macromolecules 1996; 30: 3781.

21. Carroccio S, Rizzarelli P, Scaltro G, Puglisi C. *Comparative investigation of photo- and thermal-oxidation processes in poly(butylene terephthalate).* Polymer 2008; 49: 3371-3381.

22. Spassky N, Simic V, Montaudo M S, Hubert-Pfalzgraf L G. *Inter- and intramolecular ester exchange reactions in the ring-opening polymerization of (D,L)-lactide using lanthanide alkoxide initiators.* Macromolecular chemistry physics 2000; 201 (17): 2432-2440.

23. Shyamroy S, Garnaik B, Sivaram S. *Structure of poly(L-lactic cid)s prepared by the dehydropolycondensation of L-lactic acid with organotin catalyst*. Journal of Polymer science. Part A: Polymer Chemistry 2005; 43 (10): 2164-2177.

24. Jalabert M, Fraschini C, Prud'Homme R. *Synthesis and characterization of poly(L-lactide)s and poly(D-lactide)s of controlled Molecular Weight.* , Journal of Polymer Science: Part A: Polymer Chemistry 2007; 45: 1944-1955.

25. Nagahata R, Sano D, Suzuki H, Takeuchi K. *Microwave-Assited Single--Step Synthesis of Poly(lactic acid) by Direct Polycondensation of Lactic Acid.* Macromolecular Rapid Communications 2007; 28(4): 437-442.

26. Kowalski A, Libiszowski J, Duda A, Pencek S. *Polymerization of L,L-Dilactide initiated by Tin(II) butoxide.* Macromolecules 2000; 33(6): 1964-1971.

27. Takizawa K, Nulwala H, Hu J, Yoshinaga K, Hawker C J. *Molecularly defined (L)-lactic acid oligomers and polymers: Synthesis and Characterization.* Journal of Polymer Science: Part A: Polymer Chemistry 2008; 46 (18): 5977-5990.

28. Montaudo G, Montaudo M S, Puglisi C, Samperi F. *Evidence for Ester-Exchange reactions and cyclic oligomer formation in the Ring--Opening Polymerization of Lactide with Aluminium Complex Initiators.* Macromolecules 1996; 29(20): 6461-6465.

29. Waschen O, Reichert K H. *Thermal decomposition of biodegradable polyesters-III. Studies on the mechanisms of thermal degradation of oligo-L-lactide using SEC, LACCC and MALDI-TOF MS.* Polymer degradation and Stability 1997; 55: 225-231.

30. Montaudo G., Montaudo M. S., Samperi F. Matrix-Assisted Laser desorption Ionization/Mass Spectometry of Polymers (MALDI-MS). In: Lattimer R. P. Montaudo G. *Mass Spectometry of Polymers.* Boca Raton, Florida : CRC Press LLC, 2001, pág. 422.

31. Hoteling A J, Kawaoka K, Goodberlet M C, Yu W M, Owens K G. *Optimization of Matrix-Assissted Laser Desorption/Ionization Time-of-flight Collision-Induced Dissociation using Poly(ethylene glycol).* Rapid Communications in Mass Spectrometry 2003; 17: 1671-1676.

32. Meier M, Schubert U. *Evaluation of a new multilayer spotting technique for MALDI-TOF-MS for synthetic polymers.* Rapid Communications in Mass Spectrometry 2003; 17: 713-716.

33. Hotelling A J, Nichols W F, Giesen D J, Lenhard J R, Knochenmuss R. *Electron transfer reactions in Laser Desorption/Ionization and Matrix-Assisted Laser Desorption /Ionization: Factors Influencing Matrix and Analyte Ion Intensities.* European Journal in Mass Spectrometry 2006; 12: 345-358.

34. Sroka-Bartnika A, Olejniczak S, Sochaki M, Biela T, Potrzebowski M J. *Solid-State NMR Spectroscopy as a Tool supporting Optimization of MALDI-TOF MS analysis of Polylactides.* Journal of the American Society of Mass Spectrometry 2009; 20: 67-72.

35. Mukerjee R, Jeff-Wu C F. *A modern theory of factorial design.* New York : Springer Science+Bussines Media Inc., 2006.

36. Box G E, Hunter J S, Hunter W G. *Statistics for experimenters. Design, innovation and discovery.* New Jersey : John Wiley & sons, 2005.

37. *ISO 291:1997. Plastics - standard athmospheres for conditioning and testing.*

38. Montaudo G. *Mass spectrometry of synthetic polymers: mere advances or revolution?.* Trens in Polymer Science 1996; 4(3): 81-86

39. Rader H I, Schrepp W. *MALDI-TOF mass spectrometry in the Analysis of Synthetic Polymer* Acta Polymerica 1998; 49: 272.

40. Hoteling A J, Mourey T H, Owens K G. *Importance of solubility in the Sample Preparation of Poly(ethylene terephthalate) for MALDI-TOF-MS.* Analytical Chemistry 2005; 77: 750-756.

41. Danis P O, Karr D E. *A facile sample preparation for the analysis of synthetic organic polymers by Matrix-Assisted Laser Desorption/Ionization.* Organic Mass Spectrometry 1993;28: 923.

42. Lloyd P M, Scrivener E, Maloney D R, Haddleton D M, Derrick P J. *Cation Attachment to Synthetic Polymers in Matrix-assisted Laser Desorption/Ionization Mass Spectrometry.* Polymer preprint 1996; 37(1): 847-848.

43. King R C, Goldschmidt R, Xiong Y, Owens K G. *Mechanistic studies of the Cationization of Synthetic Polymers by Alkali Metals in the Matrix-Assisted Laser Desorption/Ionization experiment.* 43rd ASMS Conference in Mass Spectrometry: 1237. Atlanta, GA; 1995.

44. Xu N, Huang Z W, Watson J T, Dage D A. *Mercaptobenzothiazoles: a new class of matrices for Laser Desorption Ionization Mass Spectrometry*. Journal of the American Society of Mass Spectrometry 1997; 8: 116.

45. Södergard A, Stolt M. *Properties of lactic acid based polymers and their correlation with composition*. Progress in Polymer Science 2002; 27 (6):1123-1163

46. Badia J D, Strömberg E, Karlsson S, Ribes-Greus A. *Influence of mechanical recycling to the structural, morphological, mechanical, thermal and viscoelastic properties of polylactide.* Manuscript in preparation.

47. Montaudo G, Montaudo MS. *Polymer Characterization methods.* In: Montaudo G, Lattimer RP. *Mass Spectrometry of Polymers*. Boca Raton : CRC Press LLC, 2002.

48. Santonja-Blasco L, Moriana R, Badía J D, Ribes-Greus A. *Thermal analysis applied to the characterization of degradation in soil of polylactide: I. Calorimetric and viscoelastic analyses.* Polymer Degradation and Stability 2010; 95: 2185-2191.

49. Bolland J L, Gee G. *Kinetic studies in the chemistry of rubber and related materials. II. The kinetics of oxidation of unconjugated olefins.* Transactions in the Faraday Society 1946; 42: 236-243

50. Kopinke F D, Mackencie K. *Mechanistic aspects of the thermal degradation of poly(lactic acid) and poly(beta-hydroxybutyric acid).* Journal of Analytical and Applied Pyrolysis 1997; 40: 43-53.

51. Kopinke F D, Remmler M, Mackenzie K, Möder M, Wachsen O. *Thermal decomposition of biodegradable polyesters - II: Poly(lactic acid)*. Polymer Degradation and Stability 1996; 53: 329-342.

52. Mc Neill I. C., Leiper H. A. *Degradation studies of some polyesters and polycarbonates - 2: Polylactide: Degradation under isothermal conditions, thermal degradation mechanisms and photolysis of the polymer*. Polymer Degradation and Stability 1985; 11: 309-326.

CAPTIONS TO TABLES

Table 1. Summary of factors, levels and general characteristics of the Design of Experiments applied in this study.

Table 2. Results of Analysis of Variance after application of the Design of Experiments to MALDI-TOF MS spectra of virgin PLA.

Table 3. Relative variations in *S/N* and *RES* means taking as reference run (s-DHB, 1/5, NO)

Table 4. Oligomeric species found for PLA by means of MALDI analysis

Table 1. Summary of factors, levels and general characteristics of the Design of Experiments applied in this study.

Factor	Type	Number of levels	Levels			Effects	
Matrix	Qualitative	3	s-DHB	dithranol	HABA	S/N	S/N_{rel}
Proportion of analyte/matrix (V/V)	Quantitative	3	1/5	1/10	1/20	RES	RES_{rel}
NaTFA	Qualitative	2	YES	NO			

Replicates	3	**Total blocks**	3
Base runs	18 ($3^2 \cdot 2^1$)	**Total runs**	54

Table 2. Results of Analysis of Variance after application of the Design of Experiments to MALDI-TOF MS spectra of virgin PLA.

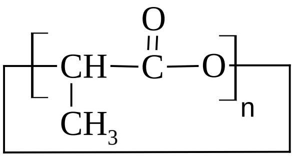
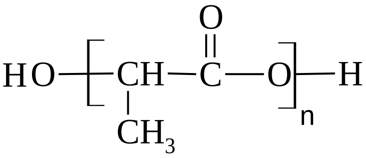
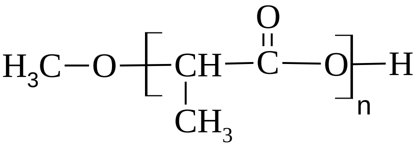
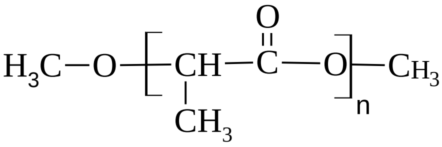
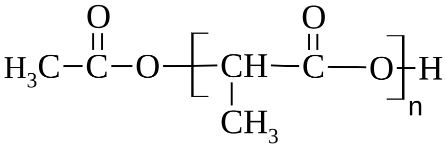
			EFFECT			
			S/N	**RES**	**S/N_{rel}**	**RES_{rel}**
			p-value (α=0.05)			
FACTORS	**Blocks**		0.779	0.315	0.064	0.210
	Main	**Matrix**	0	0	0.024	0.016
		Proportion	0	0	0.937	0.575
		NaTFA	0	0.055	0	0
	Interactions	**Matrix & Proportion**	0	0	0	0
		Matrix & NaTFA	0	0	0	0
		Proportion & NaTFA	0.111	0	0.319	0.048
		Matrix & Proportion & NaTFA	0	0	0	0
R^2 (%)			99.00	98.56	86.48	90.87

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

Table 3. Relative variations in *S/N* and *RES* means taking as reference run (s-DHB, 1/5, NO)

MAIN EFFECTS					
	Matrix		**Proportion**		**Salt**
	Dithranol	HABA	1/10	1/15	YES
S/N	-32.0 %	9.5 %	7.6 %	30.1 %	-54.52 %
RES	-20.1 %	-81.6 %	-23.0 %	-45.0 %	4.5 %

Table 4. Oligomeric species found for PLA by means of MALDI analysis

Species	Structures	m / z $[M + H]^+$		m /z $[M + Na]^+$	
$[LA_C]_n$	 $\left[\overset{}{CH}(CH_3)-\overset{O}{\overset{\|\|}{C}}-O \right]_n$	□	1586,4	■	1608,4
$HO\text{-}[LA_L]_n\text{-}H$	 $HO-\left[CH(CH_3)-\overset{O}{\overset{\|\|}{C}}-O \right]_n-H$	△	1604,4	▲	1626,4
$CH_3\text{-}O\text{-}[LA_L]_n\text{-}H$	 $H_3C-O-\left[CH(CH_3)-\overset{O}{\overset{\|\|}{C}}-O \right]_n-H$	○	1618,4	●	1640,4
$CH_3\text{-}O\text{-}[LA_L]_n\text{-}CH_3$	 $H_3C-O-\left[CH(CH_3)-\overset{O}{\overset{\|\|}{C}}-O \right]_n-CH_3$	▽	1632,4	▼	1654,4
$CH_3\text{-}CO\text{-}O\text{-}[LA_L]_n\text{-}H$	 $H_3C-\overset{O}{\overset{\|\|}{C}}-O-\left[CH(CH_3)-\overset{O}{\overset{\|\|}{C}}-O \right]_n-H$	◇	1646,4	◆	1668,4

CAPTIONS TO FIGURES

Figure 1. Experimental procedure followed for the simulation of degradation induced by PLA due to mechanical recycling and service life.

Figure 2. Chemical structures of the matrixes used for the MALDI analysis.

Figure 3. Schematic summary of the purpose of combining DOE with MALDI analysis.

Figure 4. Main effects plot for the analysis of the signal-to-noise ratio (*S/N*)

Figure 5. Main effects plot for the analysis of the Resolution (*RES*)

Figure 6. Interaction plot for the analysis of the signal-to-noise ratio (*S/N*)

Figure 7. Interaction plot for the analysis of the Resolution (*RES*).

Figure 8. MALDI-TOF MS spectra of PLA at different MALDI sample preparations to check the applicability of the Design of Experiments applied

Figure 9. Identification of oligomeric species that compose PLA (see Table 4 for symbology)

Figure 10. Proposed degradation mechanism map for polylactide.

Figure 11. Influence of thermo-oxidative ageing on the relative abundances of PLA oligomeric species.

Figure 12. Influence of thermo-mechanical cycles on the relative abundances of PLA oligomeric species

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

FIGURE 1

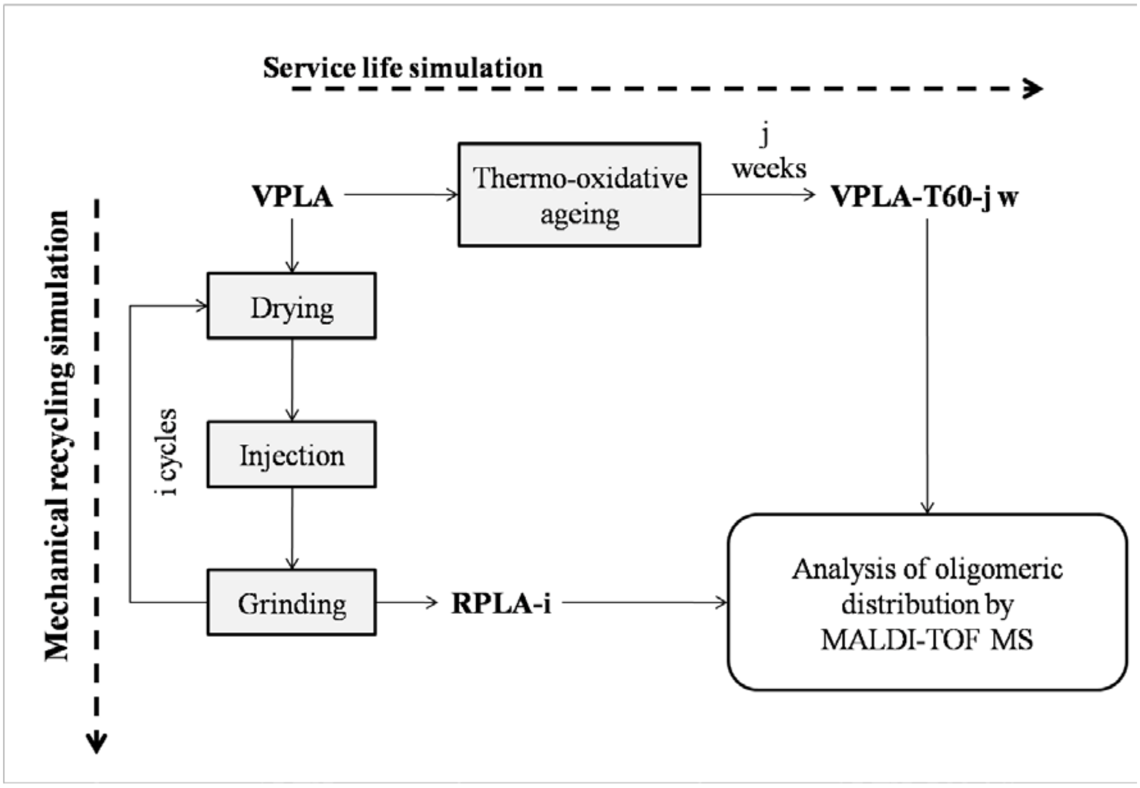
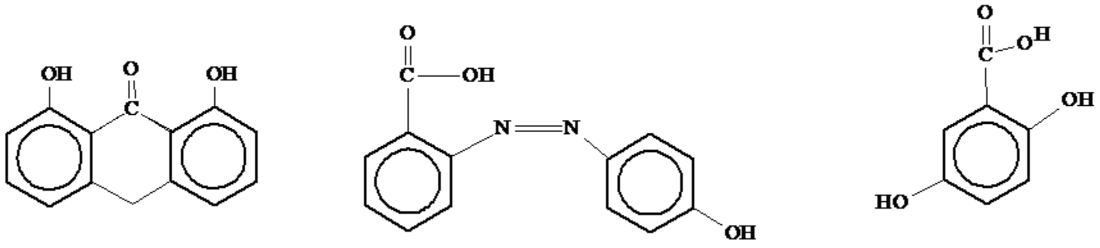


FIGURE 2



dithranol **HABA** **s--DHB**

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

FIGURE 3

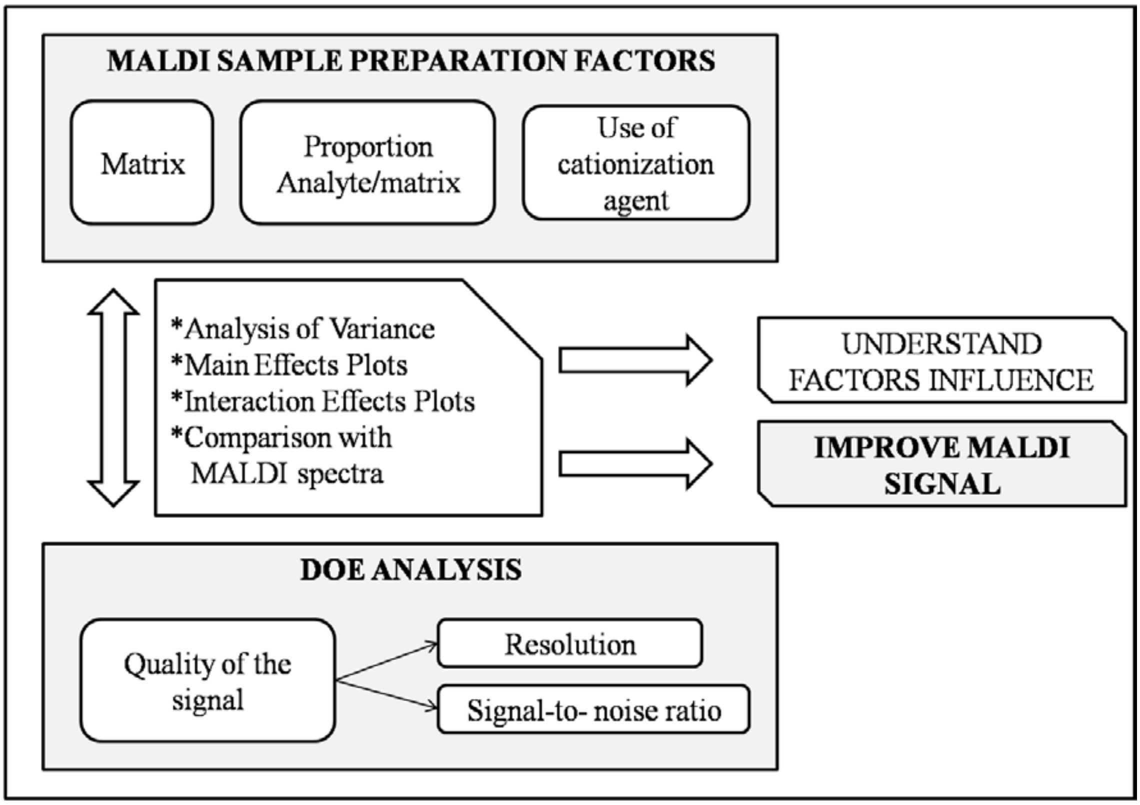
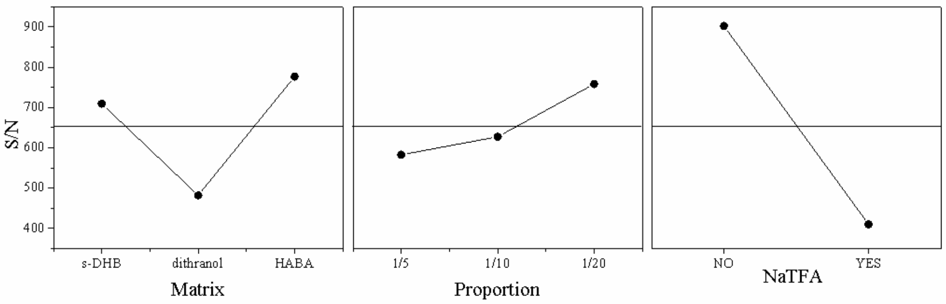


FIGURE 4


S/N
900
800
700
600
500
400
s-DHB
dithranol
HABA
Matrix
1/5
1/10
1/20
Proportion
NO
YES
NaTFA

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

FIGURE 5

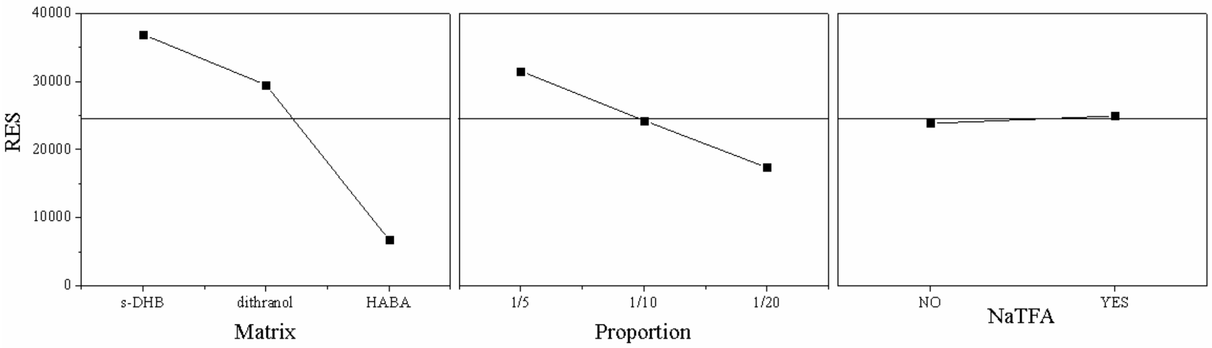
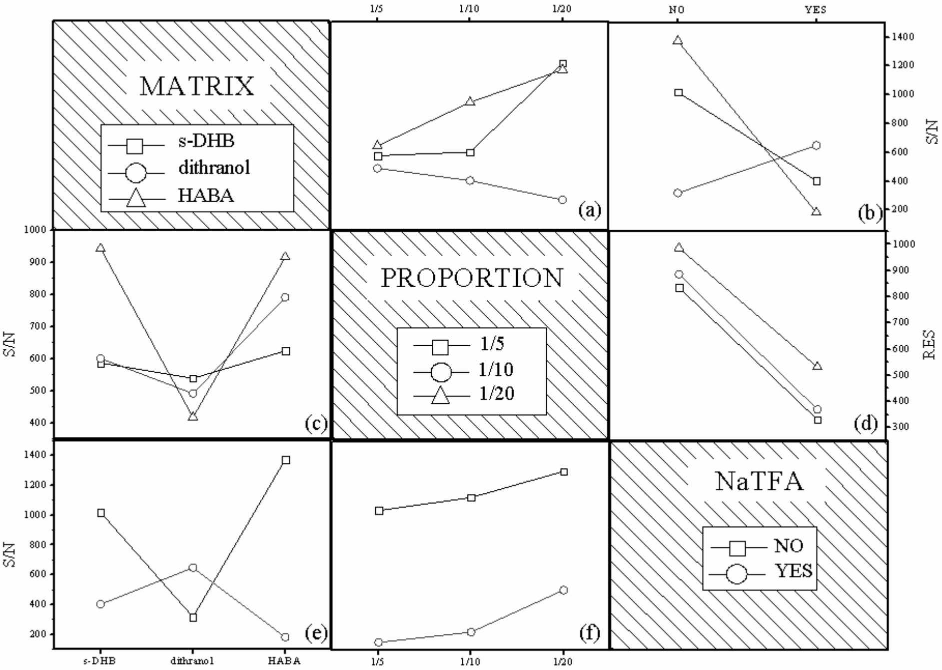


FIGURE 6


MATRIX
s-DHB
dithranol
HABA
PROPORTION
1/5
1/10
1/20
NaTFA
NO
YES
(a)
(b)
(c)
(d)
(e)
(f)
1/5
1/10
1/20
NO
YES
1400
1200
1000
800
600
400
200
S/N
1000
900
800
700
600
500
400
300
RES
1000
900
800
700
600
500
400
S/N
1400
1200
1000
800
600
400
200
S/N
s-DHB
dithranol
HABA
1/5
1/10
1/20

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

FIGURE 7

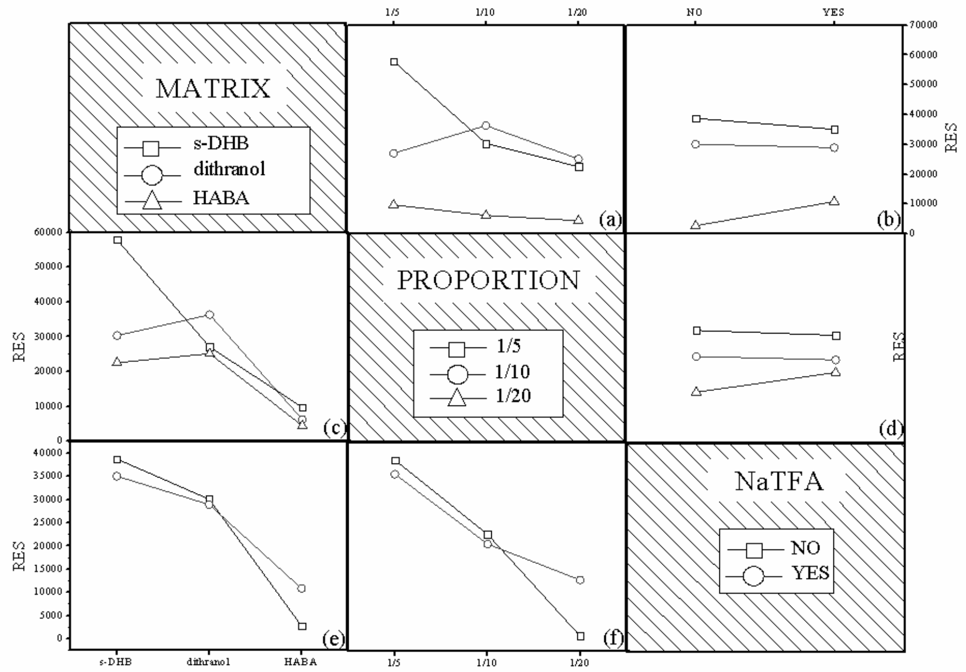


FIGURE 8

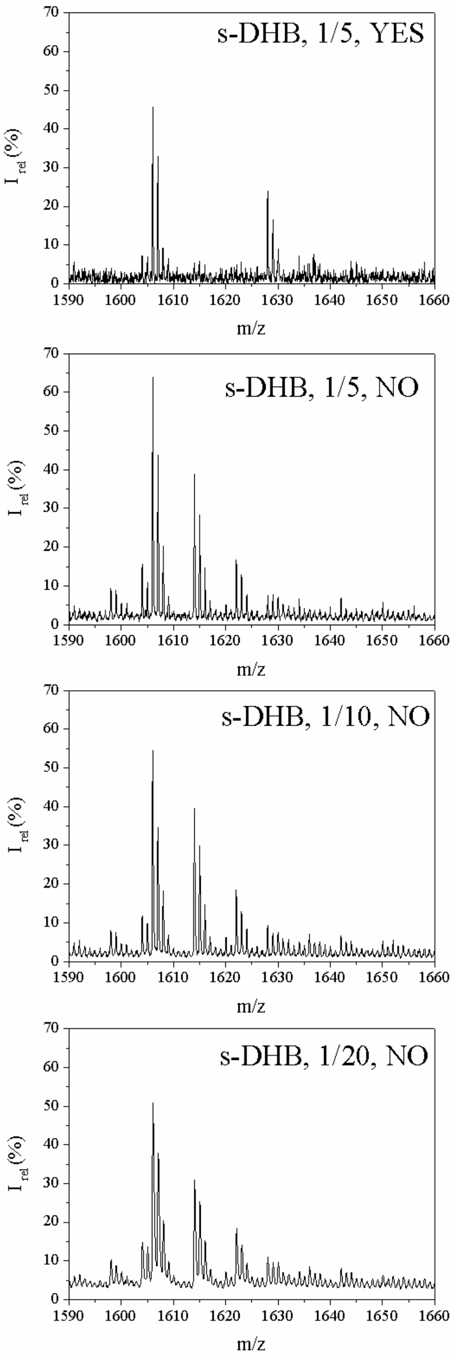

s-DHB, 1/5, YES
s-DHB, 1/5, NO
s-DHB, 1/10, NO
s-DHB, 1/20, NO
I_{rel} (%)
0
10
20
30
40
50
60
70
1590
1600
1610
1620
1630
1640
1650
1660
m/z

FIGURE 9

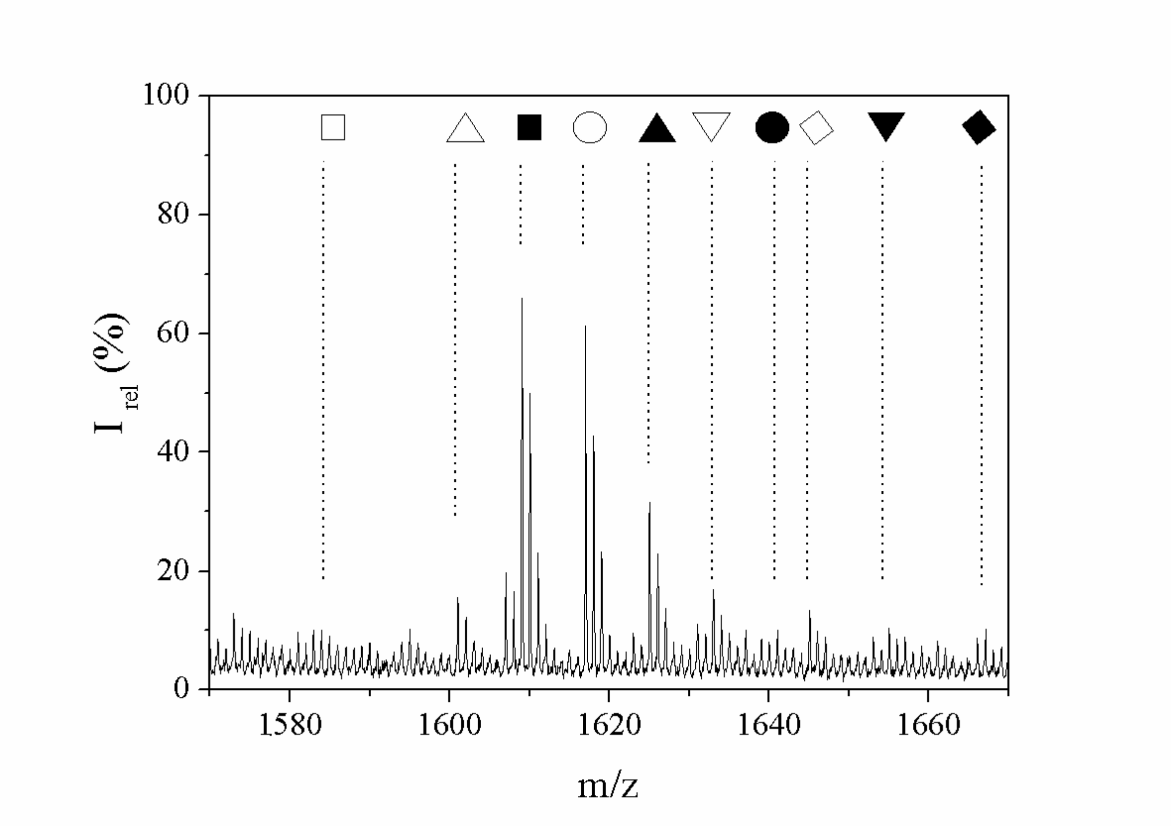
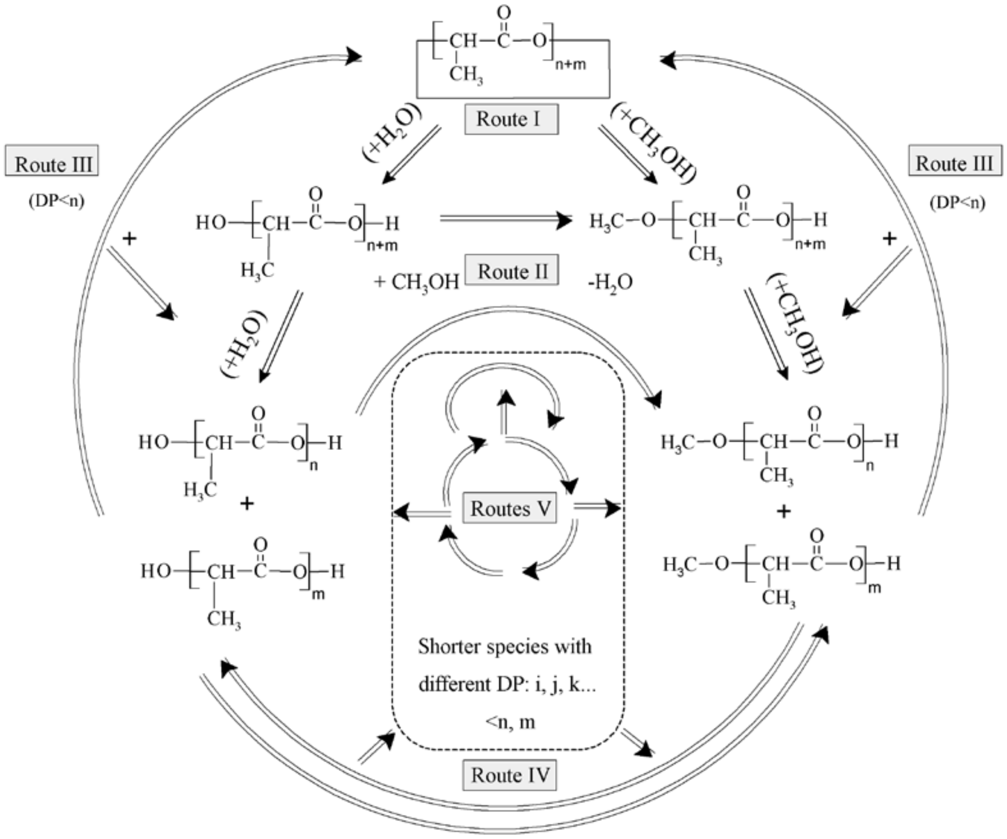


FIGURE 10


Route I
(+H2O)
(+CH3OH)
Route III
(DP<n)
+
Route III
(DP<n)
+
+ CH3OH
Route II
-H2O
(+H2O)
(+CH3OH)
Routes V
+
+
Shorter species with different DP: i, j, k... <n, m
Route IV

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S. (2011). Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (lactide). *European Polymer Journal*, *47*(7), 1416-1428.

FIGURE 11

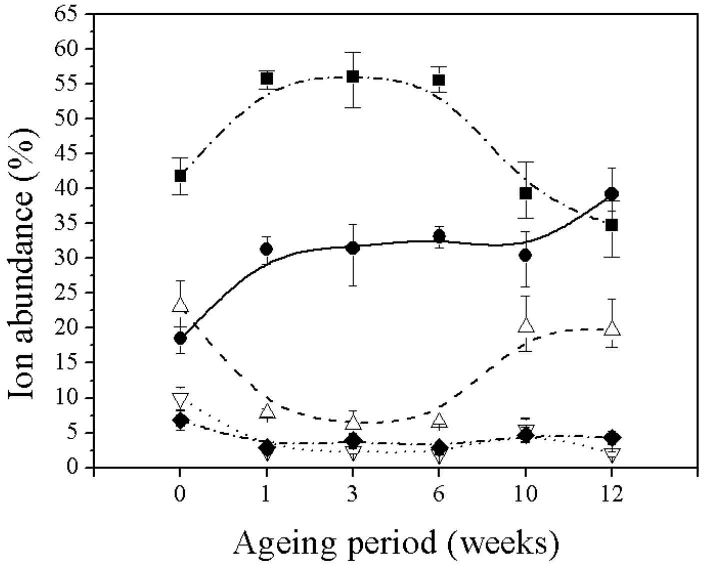
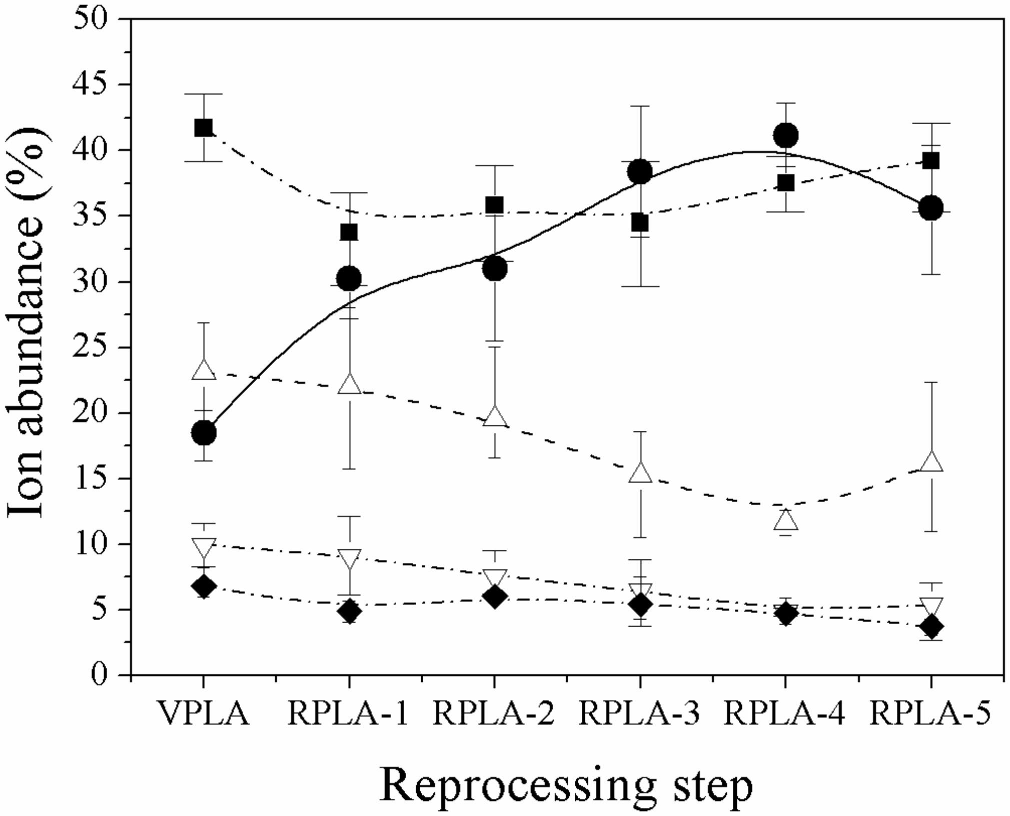


FIGURE 12


Ion abundance (%)
0
5
10
15
20
25
30
35
40
45
50
VPLA
RPLA-1
RPLA-2
RPLA-3
RPLA-4
RPLA-5
Reprocessing step

ASSESSING THE MALDI-TOF MS SAMPLE PREPARATION PROCEDURE TO ANALYZE THE INFLUENCE OF THERMO-OXIDATIVE AGEING AND THERMO-MECHANICAL DEGRADATION ON POLY (LACTIDE)

J.D. Badía[1], E. Strömberg[2], , A. Ribes-Greus[1] S. Karlsson[2,*]

CAPTIONS TO SCHEMES

Scheme 1. Route I: Hydrolysis

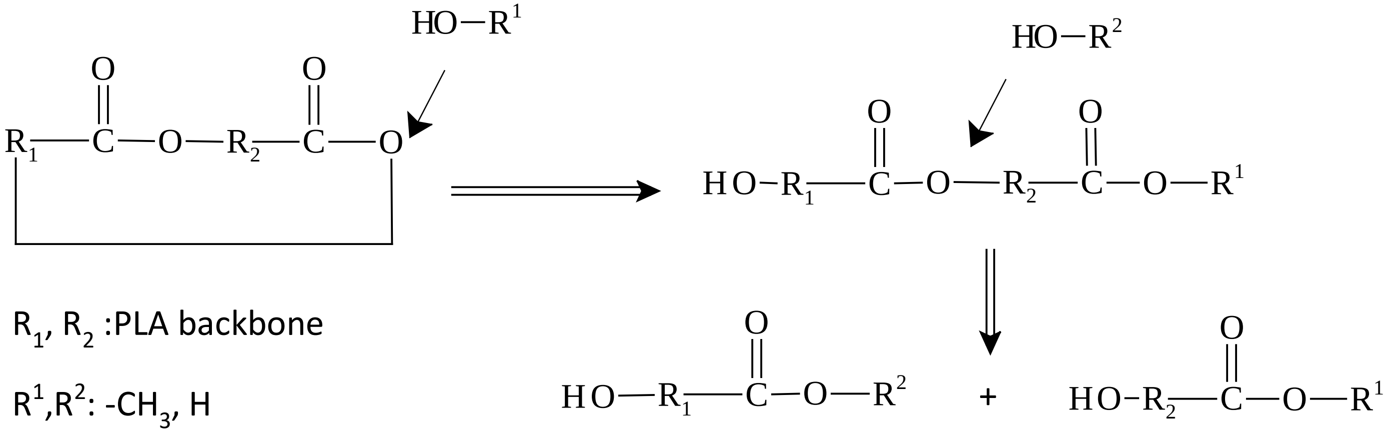
Scheme 2. Route II: Esterification

Scheme 3. Route III: Intramolecular transesterification (a: back-biting; b: middle-chain)

Scheme 4. Route IV: Intermolecular transesterification

Scheme 5. Route V: Chain scissions

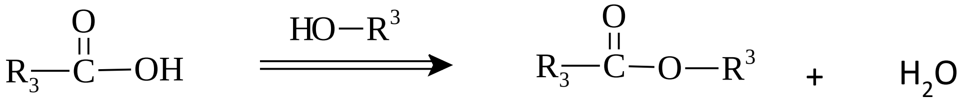
Scheme 1. Route I: Hydrolysis



R_1, R_2 :PLA backbone

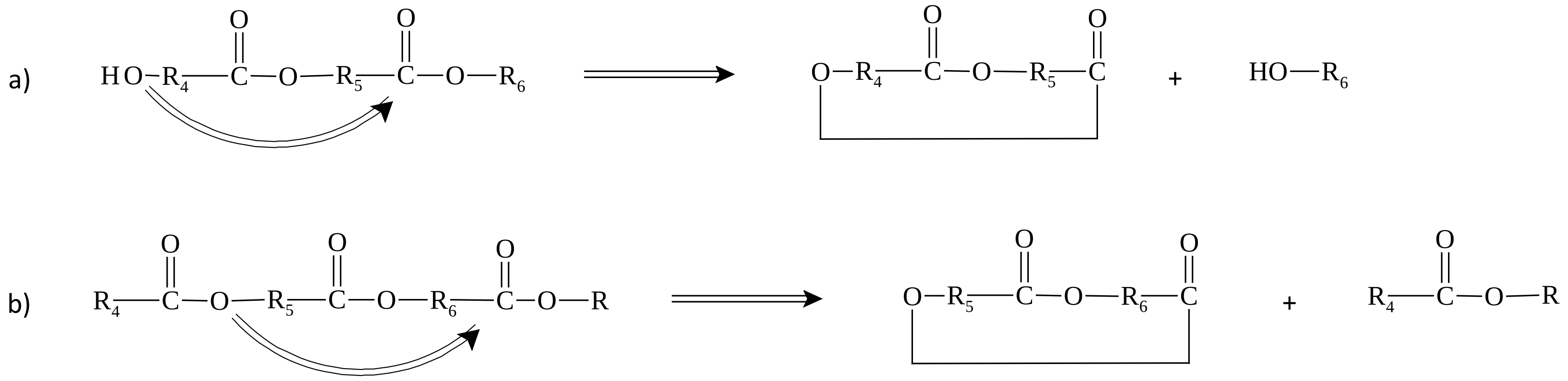
R^1,R^2: -CH_3, H

Scheme 2. Route II: Esterification



$$R_3-\overset{O}{\overset{\|}{C}}-OH \xrightarrow{HO-R^3} R_3-\overset{O}{\overset{\|}{C}}-O-R^3 + H_2O$$

Scheme 3. Route III: Intramolecular transesterification (a: back-biting; b: middle-chain)



Scheme 4. Route IV: Intermolecular transesterification

$$R_1-\overset{\overset{\displaystyle O}{\|}}{C}-O-R_2 + R_3-\overset{\overset{\displaystyle O}{\|}}{C}-O-R_4 \Longrightarrow R_1-\overset{\overset{\displaystyle O}{\|}}{C}-O-R_4 + R_3-\overset{\overset{\displaystyle O}{\|}}{C}-O-R_2$$

Scheme 5. Route V: Chain scissions