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Abstract

The sample preparation procedure for MALDI-TOF MS of polymers is addressed in this study by the application of a statistical Design of Experiments (DoE). Industrial poly (ethylene terephthalate) (PET) was chosen as model polymer. Different experimental settings (levels) for matrixes, analyte/matrix proportions and concentrations of cationization agent were considered. The quality parameters used for the analysis were Signal-to-noise ratio and Resolution. A closer inspection of the statistical results provided the study not only with the best combination of factors for the MALDI sample preparation, but also with 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 measure of PET stated that the best combination of factors and levels was the following: matrix (dithranol), proportion analyte/matrix/cationization agent (1/15/1 V/V/V), and concentration of cationization agent ($2g \cdot L^{-1}$). In a second part, multiple processing by means of successive injection cycles was used to simulate the thermo-mechanical degradation effects on the oligomeric distribution of PET under mechanical recycling. The application of MALDI-TOF-MS showed that thermo-mechanical degradation primarily affected initially predominant cyclic species. Several degradation mechanisms were proposed, remarking intramolecular transesterification and hydrolysis. The ether links of the glycol unit in PET were shown to act as potential reaction sites, driving the main reactions of degradation.

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Keywords: MALDI-TOF, Design of Experiments (DoE), poly (ethylene terephthalate) (PET), recycling, thermo-mechanical degradation.

1. Introduction

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 [1]. Characteristics and main applications of MALDI can be found in several reviews [2-3]. Poly(ethylene terephthalate) (PET) has been extensively used over the last two decades, mainly in the food packaging sector, due to its excellent mechanical, chemical, processing and thermal properties [4], and therefore was taken as model polymer in this work.

The difficulty of performing good-quality and reliable MALDI measurements depend on many factors such as the molar mass of the polymer, the choice of solvent, the choice of matrix, the ratio analyte/matrix/cationization agent, the laser energy or the mode of detection (linear or reflector), among others [5]. Especially for the case of PET, the importance of the solubility in the sample preparation process has been discussed, and it was demonstrated that the use of the azeotropic mixture of dichloromethane (CH_2Cl_2) and 1,1,1,3,3,3-hexafluoro-2-propanol (commonly referred to as HFIP) 70:30 (V/V) could dissolve PET at room temperature as well as increase the solubility of different matrixes [6], which would enhance the homogeneity of the MALDI sample. Other studies have addressed the complexity of analyzing polymers with high molar mass [7-8] due to factors such as: the uncertainty in the choice of the optimal matrix to perform the measurement; the selection of the proportion analyte/matrix; or the suitability of adding a cationization agent. The decision usually is stated as a result of a trial and error empirical procedure [5, 9-11]. Despite some studies report issues such as the selection of the best matrix [6] or the correlation of the laser energy with the obtained MALDI response [12], novel fast and cost-effective methodologies should be implemented, in order to optimize the performance of the MALDI measurement.

Design of Experiments (DoE) [13] stands as a useful, reliable and immediate procedure that can provide us not only with the best combination of factors for the preparation of the MALDI sample, as shown in literature [14-16], but also help understand the influence of each factor individually or in combination on the quality of the response.

MALDI studies of polyesters are of great interest [17-19], especially the studies of poly (ethylene terephthalate) (PET) under different degradation environments [7-8, 20-25]. Degradation is usually explained as the formation and disappearance of linear and cyclic oligomeric species $[GT_L]_n$ and $[GT_C]_n$, where G is a glycol unit, T a terephthalate unit, n the number of repeating units forming the oligomers, and L and C stand for linear and cyclic, respectively. Degradation of PET under hydrolytic [7] conditions revealed the apparition of chain-scission processes generating carboxyl terminated oligomers (H-$[GT_L]_m$-OH and T-$[GT_L]_m$-OH) and ethylene glycol terminated oligomers H-$[GT_L]_m$-GH (m: number of repeating units being m<n). Plasma-oxidative and chemical treatment [21] induced ester scission processes that yield H-$[GT_L]_n$-OH species, decreased the presence of $[GT_C]_n$ and showed an increase of $[GT_C]_n$-G species. Thermal degradation, at the common processing temperature close to 280 ºC [22] showed the formation of cyclic and linear anhydride oligomers, which auto-catalyzed hydrolytic reactions with the consequent increase of carboxyl terminated polyester chains H-$[GT_L]_n$-OH. When this degradation was experienced in oxidizing atmosphere [24-25], the study of the discoloration mechanisms was achieved, also demonstrating that formation of cyclic and carboxyl terminated species formed via chain scission in the ether link of PET are indicative of degradation. Most of the aforementioned studies [17-25] were applied to PET samples synthesized at the labs, and not to industrially obtained specimens. In addition, these studies were performed after dissolution-concentration-separation-dissolution or to filtered extracts after PET dissolution [8, 25] which can offer good spectra, but also inadvertently discard the apparition of species which might be important for the elucidation of the degradation mechanisms. However, studies performed directly on polymeric pellets have been scarcely reported [24]. In contrast, the present study was performed on neat PET which is totally dissolved along with the matrix and a cationization agent and directly spotted on the MALDI plate, being the sample preparation procedure widely discussed.

The amount of post-consumer PET (pc-PET) present in urban solid waste is high and has to be managed. During its life cycle (synthesis, processing, use, discarding, cleaning, recycling), PET is subjected to the interaction of degradation agents such as oxygen, light, mechanical stresses, temperature or water. Individually or synergically, these agents may provoke the breakage of the macromolecules and diminish the final

properties and productivity during processing [26-29]. Major applications of recycled pc-PET are in the textile industry, but new mechanical recycling technologies such as superclean® and bottle-to-bottle® point out the legal possibility of using certain fraction of pc-PET in new food-in-contact packages [30-31]. Therefore, the influence of recycling on the PET structure and the consequent presence of low molecular weight compounds (LMWC) must be assessed in order to prevent the consumer from the ingestion of hazardous substances. With the purpose of facing this problem, the application of protocols to simulate the degradation subjected during the recycling process by polymers has been successfully performed for commodities [32-38] such as polyethylene (PE) [32], polypropylene (PP) [32], polystyrene (PS) [33-34], poly (vinyl chloride) (PVC) [35] or PET [36-38]. However, the studies on the application of MALDI to characterize the effect of multiple processing on the PET structure are still few [8, 25], and based on the analysis of low-molecular weight PET oligomers. Latest publications study the influence of thermo-mechanical degradation applied by successive extrusion cycles on industrial PET samples. Authors propose an oligomer degradation model in which the most indicative fact is the loss of $[GT_C]_n$-G species for the formation of H-$[GT_L]_n$-OH and $[GT_C]_n$ species via degradation mainly occurring in the ether link of the dyethylene glycol (DEG) unit. In the present work, the influence of successive injection molding operations is addressed.

Summing up, this work is devoted to a double purpose: in the first part of the study, a statistical Design of Experiments (DoE) is used to find the appropriate settings for the sample preparation of MALDI for polymers, taking poly(ethylene terephthalate) (PET) as model; in the second part, a simulation of mechanical reprocessing was carried out by multiple injection molding cycles on commercial PET and the influence of thermo-mechanical degradation was monitored by the use of MALDI.

2. Statistical Design of Experiments (DoE)

Design of Experiments (DoE) can remarkably improve the characteristics of the study: if the variables really act in an additive way, DoE permits the estimation with higher precision; but if variables do not act additively, DoE is capable of detecting and estimating the interactions of variables and measuring this no-additivity [13]. DoE plays a fundamental role in the resolution of scientific and industrial problems [39] which involve the study of the influence (Effect, *E*) of multiple input variables (Factors, *F*) on the experimental outcome, i.e. the response. *F* can be either quantitative (categorical variable) or qualitative (based on a continuous variable but only use a few controlled values in the experiment). Each factor must have two or more settings (Levels, *L*) so that the *E* of change in *L* can be assessed on the response. Any combination of factors and levels (*F/L*) corresponds to a run in practical experimentation. Factorial design concerns the selection and arrangement of *F/L* combination, where investigators select *F* to systematically vary along different *L* during an experiment in order to determine their *E* on the response. After screening the *F* to determine which are important for explaining process variation, DoE is useful to understand how *F* interact and drive the measurement and consequently helps select the *F/L* that produce optimal process performance.

DoE is performed by the use of the General Linear Model (GLM) [13], which comprises the univariate analysis of variance with balanced and unbalanced designs, analysis of covariance, and regression, for each response variable. Interpretation of results is drawn from main effects plots (*MEP*) and interaction plots (*IP*). *MEP* show the direct *E* of each *F* on the response, evaluated along the different *L* considered. *IP* are useful for the study of interactions between *F* by means of the comparison of the relative strength of the *E* across *F*. An interaction between *F* occurs when the change in response from the low-*L* to the high-*L* of one *F* is not the same as the change in response at the same two *L* of a second *F*. That is, the *E* of one *F* is dependent upon a second *F* [13]. It is also worth mentioning that the reproducibility of the experiments must be carefully taken into account. Repeated and replicated measurements are both multiple response measurements taken at the same *F/L* combination; but repeated measurements are taken during the same experimental run or consecutive runs, while replicated measurements are taken during identical but distinct experimental runs, which are often randomized. Whether repeats or

replicates are used, it depends on the sources of variability interesting to explore and the resource constraints of the experiment. Designs with both repeats and replicates enable to examine multiple sources of variability from experimental handling of tests. Further details can be found in literature [13].

The application of DoE is therefore very valuable, not only for the determination of the optimal settings of an experiment, but also for the understanding of the effect of each factor at different levels, individually or in combination, to the final response.

3. Experimental procedure

3.1. Materials and reagents

Poly (ethylene terephthalate) (PET) SEDAPET SP04 was a bottle-grade PET obtained from Catalana de Polimers S.A. [40], Grup LaSeda (Barcelona, Spain) in the form of pellets. MALDI matrixes, namely 1,8,9-anthracenetriol (dithranol), 2-(4-hydroxyphenylazo) benzoic acid (HABA), trans-3-indoleacrylic acid (IAA), 2,4,6-trihydroxy acetophenone (THA), ferulic acid (FA), 2,5-dihydroxybenzoic acid (DHB), as well as the cationization agent, sodium trifluoroacetate (NaTFA), were purchased from Sigma-Aldrich (Stockholm, Sweden). 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and dichloromethane (CH_2Cl_2) were purchased from VWR (Sweden).

3.2. Reprocessing simulation

Prior to processing, virgin PET (VPET) pellets were dried during 5 h at 160 ºC in a dehumidifier Conair Micro-D FCO 1500/3 (UK), in order to remove as much humidity as possible from the PET flakes [26]. Afterwards, samples were processed by means of injection moulding employing 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 270, 275, 280, 285 and 280ºC. Moulds were set at 15 ºC. Cooling time residence was 40 s and total residence time ca. 60 s. Samples were dried before each processing cycle. After injection, a fraction of the samples was kept as test specimen and the rest was ground by means of a cutting mill Retsch SM2000 (UK), which provided pellets of size Φ < 20 mm to be fed back into the recirculation process. Up to five processing cycles were applied to obtain the different testing specimens of reprocessed PET (RPET-i, with i: 1-5).

3.3. 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 [41]. Individual solutions of *M* and *A* in HFIP/CH_2Cl_2 (30:70, V:V) were arranged at a concentration of 10 $g \cdot L^{-1}$. According to the Design of Experiments (see section 4.1), different volumetric proportions of *M*/*A* were applied. As well, the solutions of *C* at different concentrations were added to the *MAC* in the same volumetric amount as the *A*. More details are given in **Table 1**. The mixtures were then vortexed using a Vortex Genie (Scientific Industries, Bohemia, NY, USA). 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 by means of a Bruker UltraFlex MALDI-TOF mass spectrometer with a SCOUT-MTP ion source (Bruker Daltonics, USA) equipped with a nitrogen laser (337 nm), 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 1600–10000 Da in order to exclude high intensity signals arising from the low mass ions and to cover the whole PET mass spectrum. The laser intensity was set to the maximum value possible to acquire high resolution spectra, which were gathered by irradiating 40-50 different positions at the center area on the sample spot, with a total of 2500 shots per sample. Time-to-mass conversion of time-of-flight mass spectra was achieved using a self-calibration method [42]. All spectra were treated using FlexAnalysis 2.4 (Bruker Daltonics, USA) software. Interpretation of data was performed taking into account all decimals of the atomic masses composing the oligomers, but note that *m/z* values are given with only one decimal along the document. Statistical Design of Experiments was aided by Minitab® 15.1.0.0. software (Minitab Inc., USA).

4. Results and discussion

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

In order to optimize the MALDI measurement, the identification of the experimental factors (and levels) susceptible of being tailored is a critical step. Generally speaking, in a MALDI experiment, two main stages can be distinguished: Sample Preparation (*SP*) and Sample Analysis (*SA*). *Wetzel et al.* [14] considered experimental parameters in the *SA* stage for different mixtures of polystyrene with dithranol and retinoic acid focusing on those mixtures which gave better quality signal. On the other hand, *Brandt et al.* [16] investigated the effect of the molar mixing ratio for several synthetic polymers, stating that the optimal value depended on the studied combination of matrix, polymer and solvent used in the *SP* stage, setting in this case the experimental settings for the *SA.*

Given that each variable (factor *F*) is assessed at different settings (levels *L*), it is therefore a huge amount of *F/L* combinations and thus considering a Design of Experiments to study the interaction of all of them shall not be operative, since this fact would imply the necessity of performing a large number of runs [16]. Different variables were therefore identified, since a *SP* of good quality must consider the interaction matrix-analyte, which mainly means the choice of a suitable matrix, the selection of the best solvent for the mixture matrix/analyte/cationization agent (*MAC*), the proportion between all the components of the *MAC* and the role of adding salt. On the other hand, an appropriate *SA* takes into account parameters such as the power of the laser employed or the detection mode (linear-reflector, positive-negative). Preliminary experiments and some considerations were thus performed to decrease this amount of *F/L*.

Actions on the Sample Analysis stage are stated as follows: After a screening, the power of the laser was set to the maximum possible (70% according to technical settings), in order to avoid burning the *MAC* during the flight of ions and therefore reduce the appearance of high-intensity background peaks, which could decrease the signal-to-noise ratio and the resolution of the measurement [43]. According to *Montaudo et al.* [5], the

linear mode is essentially used for the determination of the molar mass distribution (MMD) of polymers, whereas the reflector mode allows 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 on PET 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 [44], the reflector mode was preferred. In addition, the MALDI measurements were performed in the <10000 Da mass range, in which the highest mass resolution and the best conditions for the formation of molecular ions were encountered. Polycondensation polymers usually present cyclic low molar mass oligomers that may affect the physical properties of polyesters, causing problems in their processing steps [25]. In this range, the study of the degradation reactions taking place during the processing of polymers can be achieved. Finally, the use of cationization agents preferably promotes positive ions to the flying adducts, so therefore the positive detection mode was more suitable.

On the other hand, actions taken to reduce the span of *F/L* at the Sample Preparation stage required special attention, due to the strong influence on the quality of the MALDI spectra [5]: Firstly, an ideal matrix should have the following properties: high electronic absorption at the employed wavelength, good vacuum stability, low vapor pressure, and good miscibility with the analyte in the solid-state [5]. Exhaustive list of useful matrices are available in the literature [45]. Dithranol, HABA, FA, IAA, THA and s-DHB have been successfully used in other studies with PET or polyesters [6], and therefore were considered for sample preparation. Secondly, a very important factor taken into account was the selection of an appropriate solvent that could dissolve PET at room temperature. For this reason, the use of the azeotropic mixture of dichloromethane (CH_2Cl_2) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (70:30, V:V) was chosen [6]. In this sense, when the choice of a suitable matrix was addressed, it was extremely important that the mixture behaved as one during the evaporation process of the solvent, avoiding therefore sample segregation, which could thus impoverish the quality of the signal [6]. When the aforementioned matrixes were tested at 10 $g \cdot L^{-1}$ in the azeotrope, only the three first resulted soluble. Finally, the ionization process of MALDI can be aided by the addition of a sodium (Na) or potassium (K) salt. Since polyesters are relatively polar polymers, Na^+ and K^+ charged species can be observed in the MALDI spectra, even if

they are not deliberately added to the matrix/analyte mixture [46-48]. 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 *MAC* sample [5]. The presence of specific functional groups in the polymer, such as carboxyl and hydroxyl is very important in the cationization process [49]. In addition, matrixes such as HABA and dithranol are particularly insensitive to impurities [2] and therefore the study of the addition of a cationization agent for this MALDI experiment of poly (ethylene terephthalate) is justified. Sodium Trifluoroacetate (NaTFA) was chosen as a source of ions. The quantity of NaTFA to be added to the *MAC* was also investigated, in order to explore its influence on the quality of the MALDI response.

4.2. Design of Experiments applied to MALDI spectra of PET

In the previous section, the span of MALDI key factors *F* was reduced to three: selection of a suitable matrix, proportion of analyte/matrix and the concentration of the cationization agent (NaTFA). A general factorial design [13] was performed, taking into account the levels *L* at which each *F* were analyzed summarized at **Table 1**.

Table 1

In order to perform the Design of Experiments, a significant step is the correct choice of parameters to be considered as reliable effects *E*. Some authors considered the Signal-to-Noise ratio *S/N* for assessing the quality of MALDI in DoE studies [14-15]. Others also used the Resolution *RES* as suitable quality indicator [16]. Both responses were chosen in this study as Effects for the DoE. After a first screening, the adducts which provided with the signal of highest intensity were the sodium adducts corresponding to cyclic oligomers $[GT_C]_n$ (see structure in **Table 3**), and thus were chosen for the analysis. *Wetzel et al.* [14] studied the *S/N* of three selected peaks in the low, center and high *m/z* range. *Brandt et al.* [16] applied their study focused on the peak at the maximum of the distribution. With the aim of improving the validity of this analysis, a span of 1500 *m/z* between 2000 and 3500 *m/z* in which up to seven $([GT_C]_n\ Na)^+$ peaks (2136.8, 2329.0, 2521,2, 2713.2, 2905.5, 3097.6, 3289.8 and 3482.0 *m/z*), with a separation of a [GT] repeating unit (192.168 m/z) was chosen for characterization. This region was preferred

in order to avoid the interaction with the decomposition range of the matrix at lower *m/z*, covering the *m/z* range where the MALDI signal of PET is significant. Absolute and relative *E* (being *E= S/N* or *RES*) were analyzed as follows:

$$E = E_{max} \cdot I_i \quad (1)$$

$$I_i = E_{max} \cdot I_{max} \quad (2)$$

where *I* is the intensity of the peak, *Imax* the maximum intensity, *Emax* the maximum 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) [13] applied for the evaluation of the quality of the DoE. The statistic *P* measures the significance of a change in level of a factor, individual or in combination with other factors (interaction), to the effect. If *P* is lower than or equal to a confidence value (α), then the factor or the interaction is significant, and its assessment is worth. It can be seen how for the absolute effects (*S/N* and *RES*), all factors and interactions have to be considered, since $P<\alpha$ (the common α =5% has been chosen). The use of relative effects (S/N_{rel} and RES_{rel}) might reduce the number of experimental runs to study, since some factors and interactions seem to be not significant ($P>\alpha$). It is also important to state that the experimental procedure followed for the sample preparation was reproduced under the same conditions to assure the good performance of the DoE [13]. In this sense, the fact of carrying out the experiments by triplicate added a new factor to the DoE analysis ("*Blocks*" in Minitab), but no relationship between this factor and both effects (*S/N* and *RES*) was found, which confirms that there was no significant experimental error related to the sample preparation affecting the quality of the results. Apart from *P*, 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 effect to characterize the experiment under consideration. Therefore, absolute effects were chosen, since the relative parameters offered a confidence value far below 95%. All experiments for the combination of the three factors at all levels were therefore applied

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 effect. **Figure 1** and **Figure 2** show the *MEP* of the DoE results for both *S/N* and *RES* effects respectively, in which the comparison of the changes in the level means provided information concerning the factors that may influence the response the most. The horizontal line crossing these graphs is the *grand mean*, which is the average of all data for a specific effect (*S/N* or *RES*), and is a valuable reference for the data interpretation. The following interpretations can be drawn: Firstly, it is clearly seen how, considering all experiments, those performed with dithranol provided a MALDI signal with higher *S/N* and higher RES, far from those given by HABA and FA. In addition, regarding the influence of the proportion analyte/matrix, the variation was different for both effects: whereas an increase of the percentage of matrix increased the *S/N* of the MALDI spectra, it came together with a reduction of the *RES*. Finally, an increase of the concentration of NaTFA enhanced the *S/N* of the signals, being the results in the case of the *RES* higher in this fashion: 0.0>2.0>0.5>1.0 $g \cdot L^{-1}$, being the *RES* when 2 $g \cdot L^{-1}$ representative, since it is close to the *grand mean*.

Figures 1,2

Interaction plots (*IP*) will help elucidate the best conditions for the analysis. **Figure 3** and **Figure 4** show the *IP* for the three factors to *S/N* and *RES*, respectively. These plots are useful to observe the general influence of all factors in combinations. The following facts were stressed: Initially, it can be observed how for the three matrixes, increasing the proportion of analyte/matrix in the MALDI sample represented a general increase in the *S/N* (Fig 3a and Fig 3c) and a decrease in the *RES* (Fig 4a and Fig 4c), being this effect more appreciable for dithranol>FA>HABA, and the latter completely insensitive. On the other hand, an increase in the concentration of the cationization agent was not relevant for the *S/N* results in the case of using HABA and FA, whereas it was remarkably positive when using dithranol (Fig 3b and Fig 3e), as previously suggested. However, for the examination of the influence of the salt on the *RES*, it is seen how it was negatively affected when HABA and FA were considered, while for the case of employing dithranol, the *RES* increased to higher values as more salt was added to the MALDI sample (Fig 4b and Fig 4e). Finally, the interaction between proportion of analyte/matrix used and concentration of NaTFA showed a general increase in the *S/N*

when both factors were increased (Fig 3d and Fig 3f), whereas no clear tendency was found for the study of the *RES*, as it is shown at Fig 4d and Fig 4f.

Figures 3,4

Discussion was stated in association with the MALDI spectra. For a better comparison, a selection of the MALDI spectra is shown at **Figure 5**, focused on a unique adduct centered at 2329.0 m/z, corresponding to the cyclic predominant adduct $([GT_C]_n\ Na)^+$, for 12 repeating units, which intensity for all spectra was remarkable. The following conclusions were remarked: Firstly, the effect of the matrix used was the key quality factor for the analysis, since both *S/N* and *RES* considerably increased with the use of dithranol, to the detriment of HABA and FA, even though FA behaved better than HABA. This fact can be checked by the observation of the spectra depicted at Fig 5 d-e-f. Furthermore, the discussion about which proportion resulted best for the analysis had to be decided in terms of which parameter is more decisive for the analysis. It is shown that the higher amount of matrix was used, the better *S/N* was obtained, but the lower *RES* was achieved. In terms of spectra, the analysis of Fig 5 d-g-j clearly shows what was hypothesized by the DoE analysis. Especially for the case of using dithranol, it can be seen how the increase of *S/N* was more substantial than the small decrease experienced by the *RES*. Even more, the statistical analysis of the response (**Table 2**) gave a higher regression value, which strengthens the choice of the *S/N* as key parameter in this study. Thus, it was decided to prepare MALDI samples with the analyte/matrix proportion 1/15 V/V, which in the *MAC* means 1/15/1 V/V/V. Finally, the study of the influence of the cationization agent was considered. The main effects plot (**Figure 1**) stated that the higher the addition of salt was employed, the higher the *S/N* obtained was. Concerning the *RES* (**Figure 2**), it was higher in this fashion: 0.0>2.0>0.5>1.0 $g \cdot L^{-1}$, being the *RES* representative when 2 $g \cdot L^{-1}$ were used, since it was close to the *grand mean*. Interaction plots can be used as reduced DoE for a specific factor. In other terms, once decided dithranol as established matrix, the observation of the influence of the other variables could be followed by the *IP*. Fig 3b and Fig 3e showed that the higher the amount of NaTFA was used, the higher the *S/N* value was obtained for dithranol, which was clear up to now. The influence of NaTFA on the *RES* (Fig 4b and Fig 4e) was surprisingly remarkable, because only in the case of applying dithranol, the increment in concentration of NaTFA increased the *RES*, unlikely pointed out by *MEP*. Deep observation of Fig 5

a-b-c-d clearly showed that the best spectrum was given for the highest concentration of salt.

Taking into account all these considerations, the application of the DOE for the improvement of the MALDI measure of PET stated that the best combination of levels and factors was the following: matrix (dithranol), proportion (1/15/1), and concentration of cationization agent (2 $g \cdot L^{-1}$).

Figure 5

4.3. Study of PET thermo-mechanical degradation

The second part of the study was devoted to the characterization of the thermo-mechanical degradation mechanisms of PET subjected to multiple injection molding cycles, as a good example for the application of the previous analysis. PET degradation reactions generally follow the postulated mechanistic routes of polyesters: 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, both from the end of the chain (backbiting) or in the middle of the chain, which lead to the formation of cyclic oligomers and linear species with shorter length. Route IV: Intermolecular transesterifications, which interchange ester units between different chains, which lead to an increase in the heterogeneity of the polymer.Route V: Chain scissions inherent to thermo-mechanical reactions may produce random chain cleavage, leading to the formation of mainly linear hydroxyl and carboxyl terminated species. Route VI: Oxidation processes, giving rise to glycol-aldehyde terminated units. In addition, MALDI experiments in which the use of NaTFA is used as catalyzer, shows acetylation reactions, which are considered as Route VII.

The MALDI spectrum of VPET is shown in **Figure 6**. In the inset, the zoomed area corresponding to two repeating $[GT]_n$ units (n=10, 11), in the *m/z* 1900-2300 range is given as an example for identification of main cyclic and linear oligomeric species, which structures are depicted at **Table 3** and **Table 4**, respectively. Note that species appeared at more *m/z* than those presented, but only values for n=10,11 are presented for

better visualization. The predominant oligomeric species are cyclic oligomers, **$[GT_C]_n$,** mainly detected as Na^+ adducts ($[M + Na]^+$: *m/z* 1944.7, 2136.9), as well as cyclic oligomers with an extra glycol linkage in the backbone **$[GT_C]_n$-G**, found as $[M + Na]^+$: *m/z* 1988.8, 2180.9. Linear oligomers present in VPET were assigned as those bearing two glycol units **H-$[GT_L]_n$-GH** (found at $[M + Na]^+$: *m/z* 2006.8, 2198.9), and those having glycol and carboxyl end groups **H-$[GT_L]_n$-OH** (found at $[M + Na]^+$: *m/z* 1962.7, 2154.9).

Figure 6

Thermo-mechanical degradation subjected by multiple reprocessing induced modifications in the oligomeric distribution of the PET sample. **Figure 7** shows the influence of degradation on the mass spectra corresponding to each reprocessed material, in comparison with the virgin one, where the *m/z* range has been reduced to one repeating unit $[GT]_n$ (n=10) for better visualization. New cyclic and linear oligomeric species were detected, as shown in **Table 3** and **Table 4**, respectively. Even though it is known that ion abundances in MALDI experiments can considerably vary, the increase of oligomers gained by the reprocessed materials, in comparison with the virgin PET, was prominent and reproducible.

Tables 3,4-Figure 7

Low abundant ions of the linear oligomers found were **H-$[GT_L]_n$-G_2H** (or **HG-$[GT_L]_n$-GH**), which bear a glycol and a diethylene glycol (DEG) unit ($[M + Na]^+$: *m/z* 2050.8); **HT-$[GT_L]_n$-OH**, having two terephthalate units ($[M + Na]^+$: *m/z* 2210.8); **H-$[GT_L]_n$-GA**, bearing a glycol and a glycol-aldehyde unit ($[M + Na]^+$: *m/z* 2034.7); and **TFA-$[GT_L]_n$-OH**, which has carboxyl and trifluoroacetate (TFA) end groups ($[M + Na]^+$: *m/z* 2058.7). Minor cyclic oligomeric adducts were **T-$[GT_C]_n$**, which presents an extra terephthalate unit in the backbone, found as $[M + Na]^+$: *m/z* 2092.8; and **$[GT_C]_n$-G_2** ($[M + Na]^+$: *m/z* 2032.8), which has an extra DEG unit in the backbone. The evolution of their relative ion abundances, taking into account intensities and areas for calculations, is depicted at **Figure 8**.

Figure 8

Figure 9 shows a proposal of mechanisms of polymer degradation. In order to follow the discussion, the nomenclature $\mathbf{R^{I}_{a}R^{II}_{b}}$ has been used, where R^{I} stands for *Reaction* and **a** for the specific reaction shown at the degradation map in **Figure 9,** and R^{II} stands for *Route*, being **b**, the likely mechanism for this reaction, according to the aforementioned general chemical routes. The most remarkable changes were those experienced by the cyclic species $\mathbf{[GT_C]_n}$ and $\mathbf{[GT_C]_n}$**-G** and, with less presence, by linear species **H-**$\mathbf{[GT_L]_n}$**-GH** and **H-**$\mathbf{[GT_L]_n}$**-OH.** The relative abundance of the $\mathbf{[GT_C]_n}$ oligomers increased with further reprocessing cycles, which can be explained by intramolecular cyclization reactions of $\mathbf{[GT_C]_n}$**-G** species (R_1R_{III} (m<n)). Intermolecular reactions between esters and ethers groups [50] (R_2R_{IV} (≠n)) or by linear oligomer cyclization reactions, from **H-**$\mathbf{[GT_L]_n}$**-GH** and **H-**$\mathbf{[GT_L]_n}$**-OH** ($R_{3,4}R_{III}$ (m<n)), releasing ethylene glycol and water as by-products [50], can also occur , but general increasing tendency of these latter species suggests that it was the loss of ethylene glycol from cyclic $\mathbf{[GT_C]_n}$**-G** the main reaction after the first reprocessing. Actually, the strong decrease of relative abundance of $\mathbf{[GT_C]_n}$**-G** throughout the entire reprocessing cycles reveals these were the most reactive species. Both mainly produced **H-**$\mathbf{[GT_L]_n}$**-OH** by cleavage of the ester bond, giving rise to a vinyl-ester terminated PET which hydrolyzed yielding a vynil alcohol which rearranged into acetaldehyde, thus leaving carboxyl end groups (R_5R_I) [21-22,51]; and with minor intensity, **H-**$\mathbf{[GT_L]_n}$**-GH**, by hydrolysis (R_6R_I) and by intermolecular reactions involving a water molecule [50] (R_6R_{IV} (≠n)). These last species could interact between them by interchanging glycol and water units, being the production of **H-**$\mathbf{[GT_L]_n}$**-OH** more remarkable (R_7R_{IV} (≠n)). In addition, alternative **H-**$\mathbf{[GT_L]_m}$**-OH** (m=n-1) could be formed as a result of chain scission reactions of the ester groups of **H-**$\mathbf{[GT_L]_n}$**-GH** (R_7R_I, (m<n)), by the loss of the transformed vinyl-ester group [21], which might be enhanced by the shear induced by thermo-mechanical processing. Likewise, the occurrence of lighter **H-**$\mathbf{[GT_L]_m}$**-OH** groups is reported as an effect of intramolecular cyclization reactions [21] , releasing a sub-product of R_3R_{III}, with m<n. The carboxyl end groups are mainly attributed to thermal degradation reactions during reprocessing [52] and therefore are a good indicator of chain scission processes. These results are in agreement with those reported in other studies, where the presence of carboxyl groups was monitored by techniques such as titration or Fourier Transform Infrared Spectrometry (FTIR) [29]. It is worth mentioning that, besides indicating the

degradation already undergone by the polymer, the role of carboxyl end groups is indicative of further degradation, since they can act as hydrolysis catalyzers [26-28].

Low abundant species slightly interacted in the general oligomers degradation mechanism, since its presence was maintained at low abundance levels during the thermo-mechanical degradation. **TFA-[GT$_L$]$_n$-OH** is formed after an acetylating reaction between the TFA and the hydroxyl end groups [25] (R_8R_{VII}). Species such as **HG-[GT$_L$]$_n$-GH** and **[GT$_C$]$_n$-G$_2$**, which formation/elimination is driven by intramolecular transesterifications and hydrolytic processes ($R_9R_{I,III}$ (m<n)), played a role in the formation of **[GT$_C$]$_n$** ($R_{10}R_{I,III}$ (m<n)) , **[GT$_C$]$_n$-G** ($R_{11}R_{I,III}$ (m<n)), **H-[GT$_L$]$_n$-GH** and **H-[GT$_L$]$_n$-OH** (($R_{12}R_{I,III}$(m<n), $R_{13,14}R_{I,III}$(m<n)$_{,IV}$ ($\neq$n)) **,** by means of the mechanisms explained above, involving ethylene glycol units. Other minor species such as **HT-[GT$_L$]$_n$-OH** and **T-[GT$_C$]$_n$**, which equilibrium might be guided by hydrolytic or intramolecular reactions [50]. ($R_{15}R_{I,III}$ (m<n)), interacted with the formation of **[GT$_C$]$_n$,** with the loss of terephthalic units ($R_{16}R_{III}$) . Thermo-mechanical degradation inducing chain scission to PET can be explained through reactions $R_{17\text{-}20}R_V$ (m<n), which gives rise to similar species to the original but with less repeating units in its backbone. Chain scissions leading to species different to the original might also occur, but were not considered in the oligomers degradation mechanism for the sake of clarity. It can be seen how the formation of **HT-[GT$_L$]$_n$-OH** through reactions $R_{21}R_I$ and $R_{22}R_{I,III}$(m<n)$_{,IV}$($\neq$n), was more remarkable along the reprocessing cycles, in agreement with other studies [25]. This oligomer could also be produced as a result of β-scission reactions from decomposition of **[GT$_C$]$_n$** at high temperatures $R_{21}R_{V(m<n)}$ [50]. Likewise, oxidation processes led to the formation of **H-[GT$_L$]$_n$-GA**, as suggested by other authors [25] ($R_{23\text{-}25}R_{VI}$). Finally, intermolecular transesterifications have to be also considered to occur during the whole processing, leading to a more heterogeneous chain distribution which may affect the polidispersity index of PET [53].

Figure 9

5. Conclusions

The sample preparation procedure for the analysis of poly(ethylene terephthalate) (PET) 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 (dithranol, HABA, FA), the proportion analyte/matrix (1/5, 1/10, 1/15 V/V), and the amount of cationization agent (C_{NaTFA} = 0, 0.5, 1, 2 $g \cdot L^{-1}$ in the same volumetric amount as the analyte) were considered. The study was performed analyzing the effect of changing the aforementioned factors among their different levels on quality parameters such as signal-to-noise ratio (*S/N*) and resolution (*RES*), both in absolute and relative terms. 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 measure of commercial PET stated that the best combination of levels and factors was the following: matrix (dithranol), proportion analyte/matrix (1/15 V/V), and concentration of NaTFA (2 $g \cdot L^{-1}$).

Multiple processing by means of successive injection cycles was used to simulate the thermo-mechanical degradation effects on the oligomeric distribution of PET under mechanical recycling. Several degradation mechanistic routes were proposed. Degradation primarily affected to initially predominant cyclic species, by dramatically decreasing the abundance of $[GT_C]_n$-G, which was mainly transformed into $[GT_C]_n$, H-$[GT_L]_n$-GH and H-$[GT_L]_n$-OH, among other low abundant oligomeric species, that played an important role in the formation-disappearance reactions occurred due to multiple reprocessing. Such is the case of cyclic oligomers like T-$[GT_C]_n$ and $[GT_C]_n$-G_2; or linear oligomers like HG-$[GT_L]_n$-GH, HT-$[GT_L]_n$-OH, H-$[GT_L]_n$-GA, or TFA-$[GT_L]_n$-OH.

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CAPTIONS TO FIGURES

Figure 1. Main effects plot for the analysis of the signal-to-noise (S/N) ratio.

Figure 2. Main effects plot for the analysis of the Resolution (RES).

Figure 3. Interaction plot for the analysis of the signal-to-noise (S/N) ratio.

Figure 4. Interaction plot for the analysis of the Resolution (RES).

Figure 5. MALDI-TOF MS spectra in the m/z 2300-2400 range to check the applicability of the Design of Experiments applied (note that scales have been modified for better visualization).

Figure 6. MALDI-TOF MS spectrum of virgin PET. Inset: zoomed area in the m/z range 1900-2300 for identification of predominant oligomers.

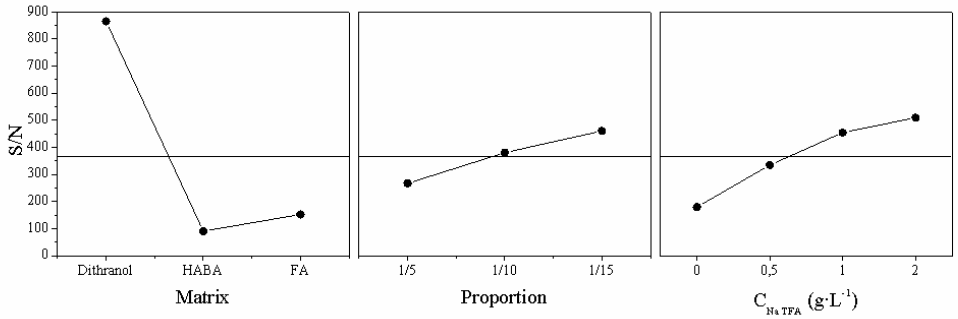
Figure 7. MALDI-TOF MS spectra in the m/z 1900-2100 range to characterize the appearance of oligomeric species due to thermo-mechanical degradation. Zoomed areas represent low abundant oligomeric species.

Figure 8. Normalized ion abundances of PET oligomeric species through the multiple reprocessing cycle

Figure 9. Proposed mechanisms of thermo-mechanical degradation of PET.

Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S (2011). 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*, *692*(1-2), 85-95.

FIGURE 1



Badia, J. D., Strömberg, E., Ribes-Greus, A., & Karlsson, S (2011). 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*, *692*(1-2), 85-95.

FIGURE 2

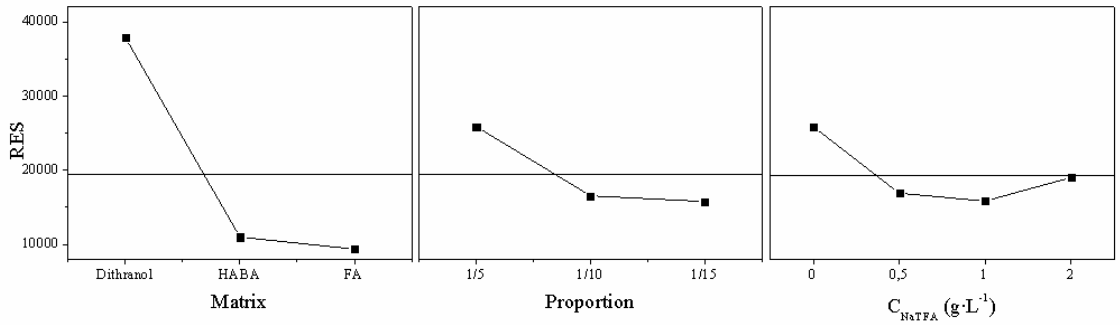


FIGURE 3

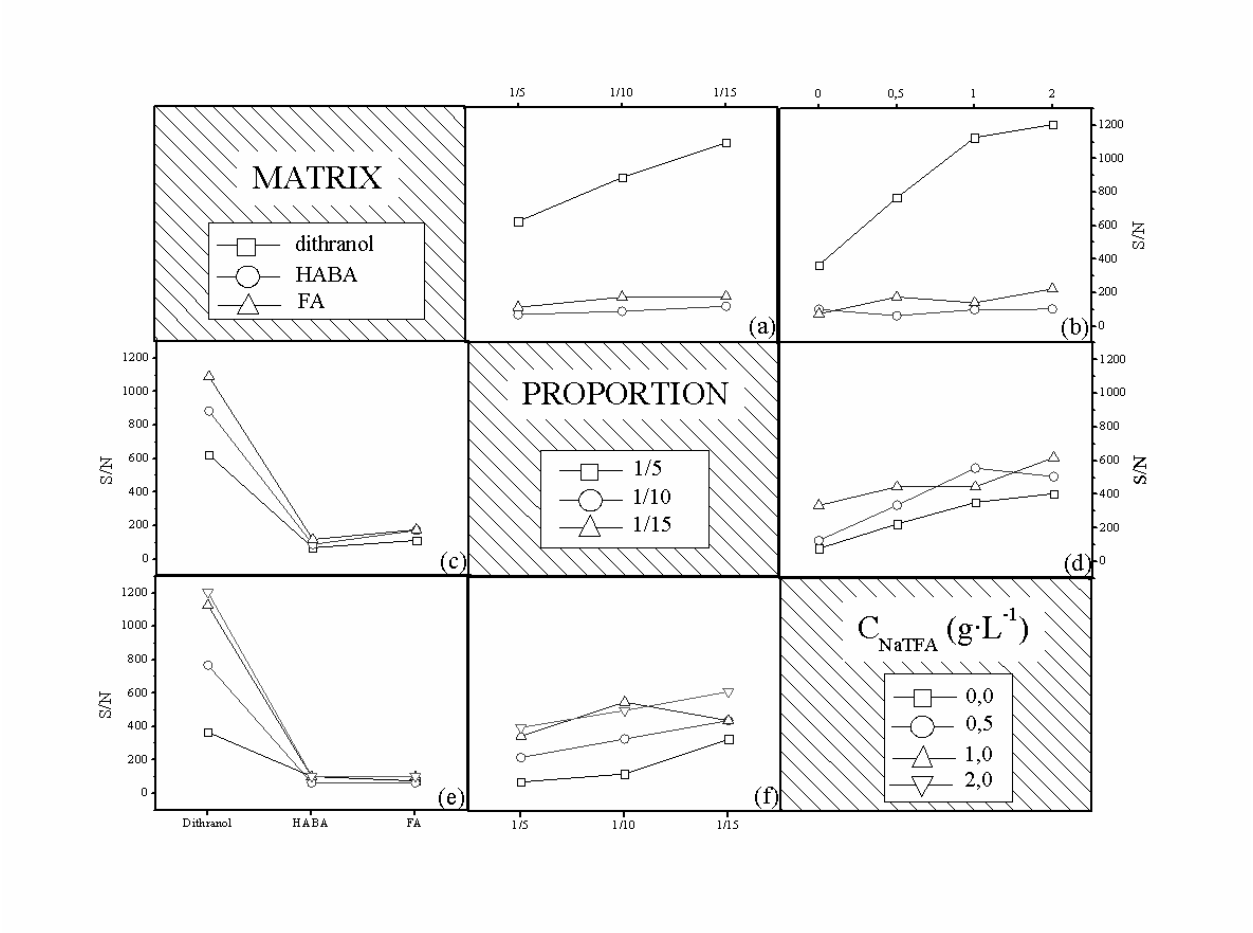

MATRIX
dithranol
HABA
FA
PROPORTION
1/5
1/10
1/15
C_{NaTFA} (g·L^{-1})
0,0
0,5
1,0
2,0
S/N
(a)
(b)
(c)
(d)
(e)
(f)
Dithranol
HABA
FA

FIGURE 4

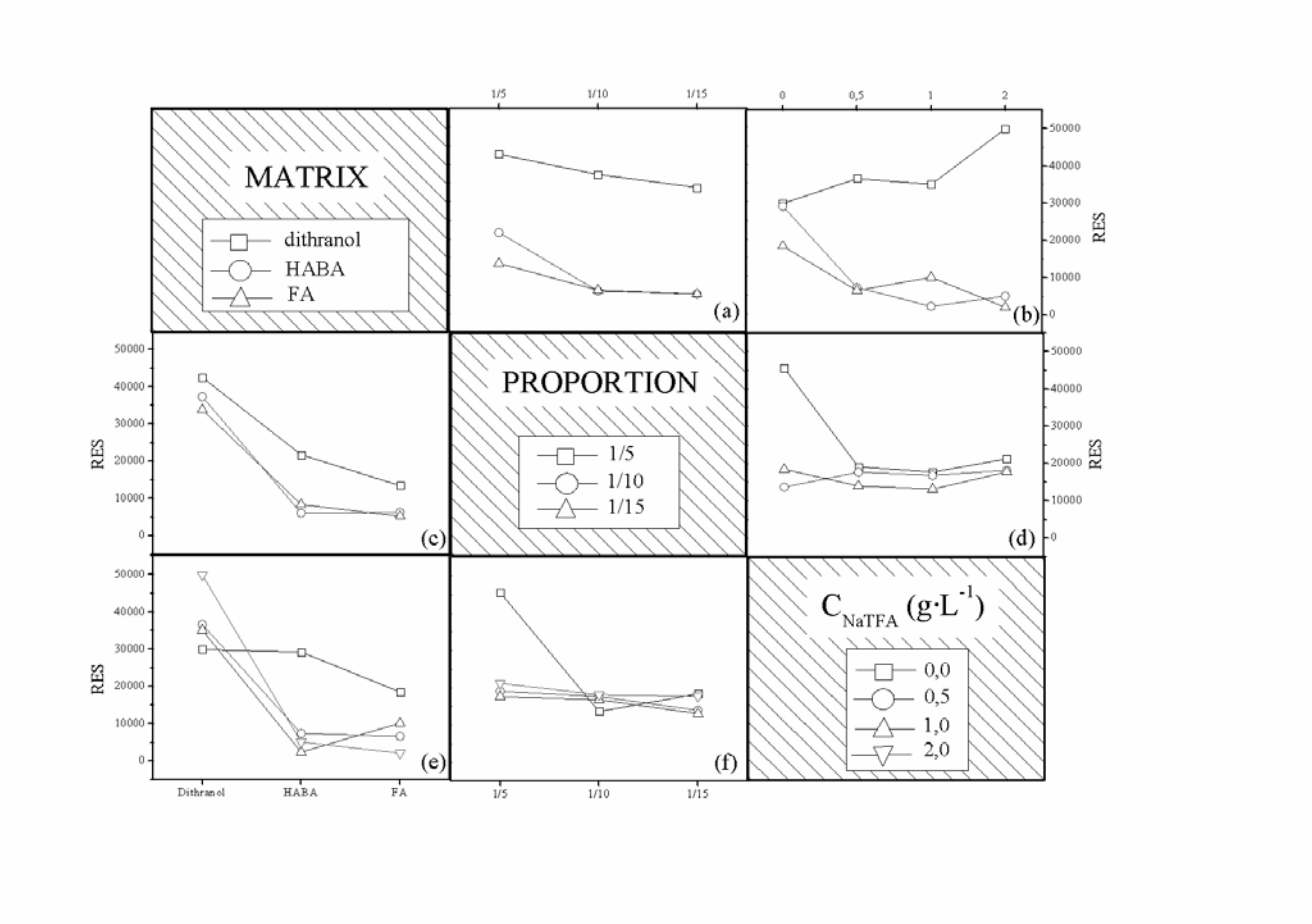

MATRIX
dithranol
HABA
FA
PROPORTION
1/5
1/10
1/15
C_{NaTFA} (g·L^{-1})
0,0
0,5
1,0
2,0
RES
(a)
(b)
(c)
(d)
(e)
(f)
Dithranol
HABA
FA

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FIGURE 5

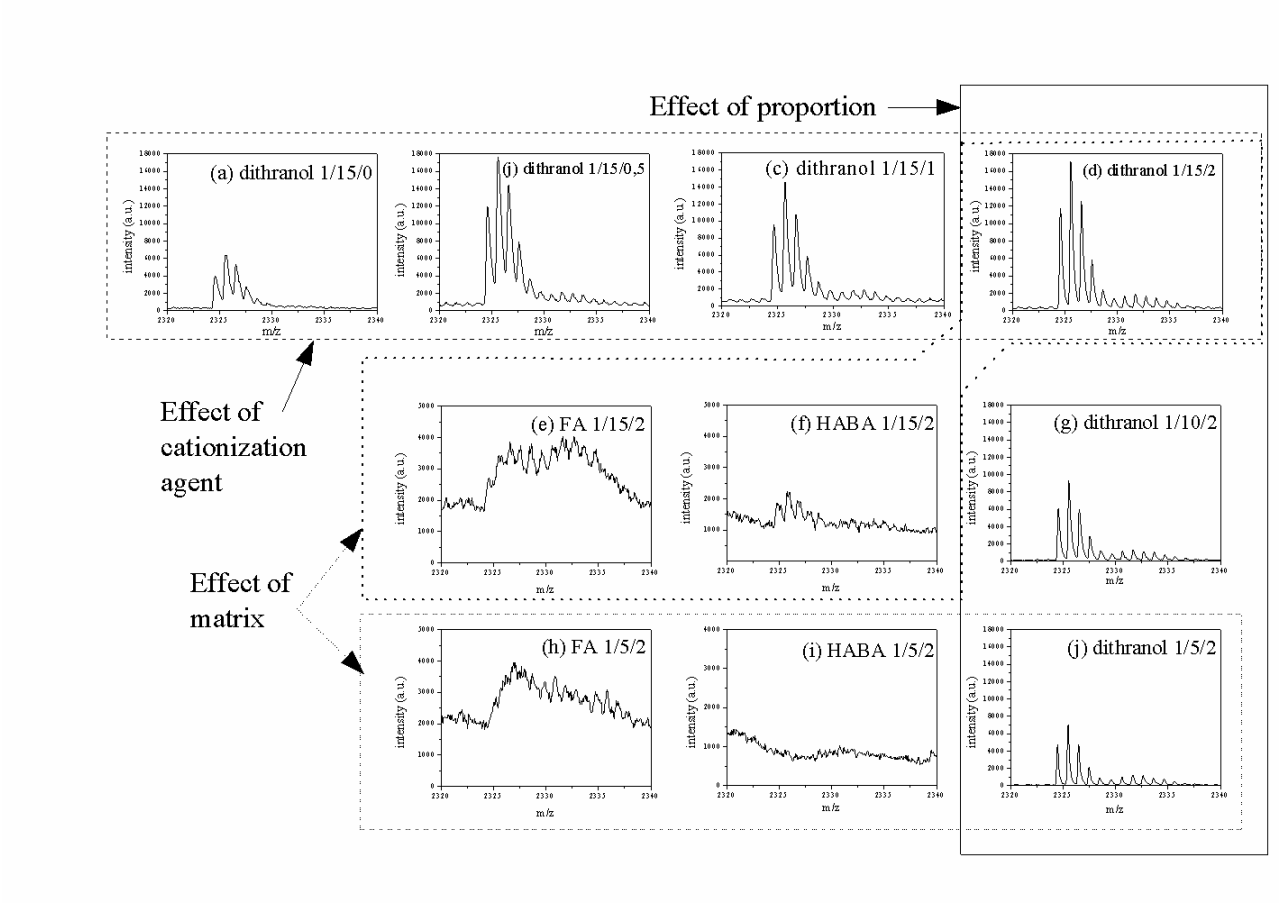

FIGURE 6

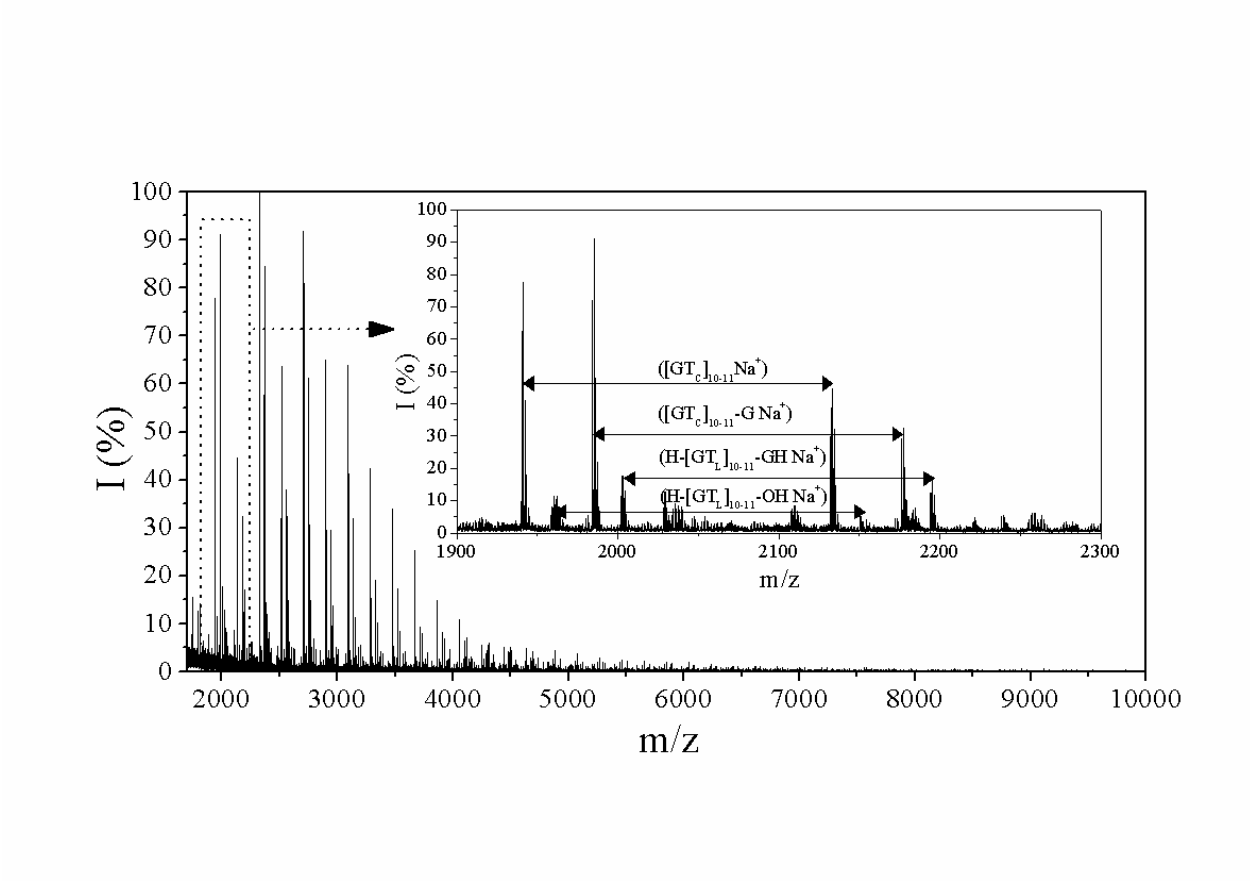

FIGURE 7

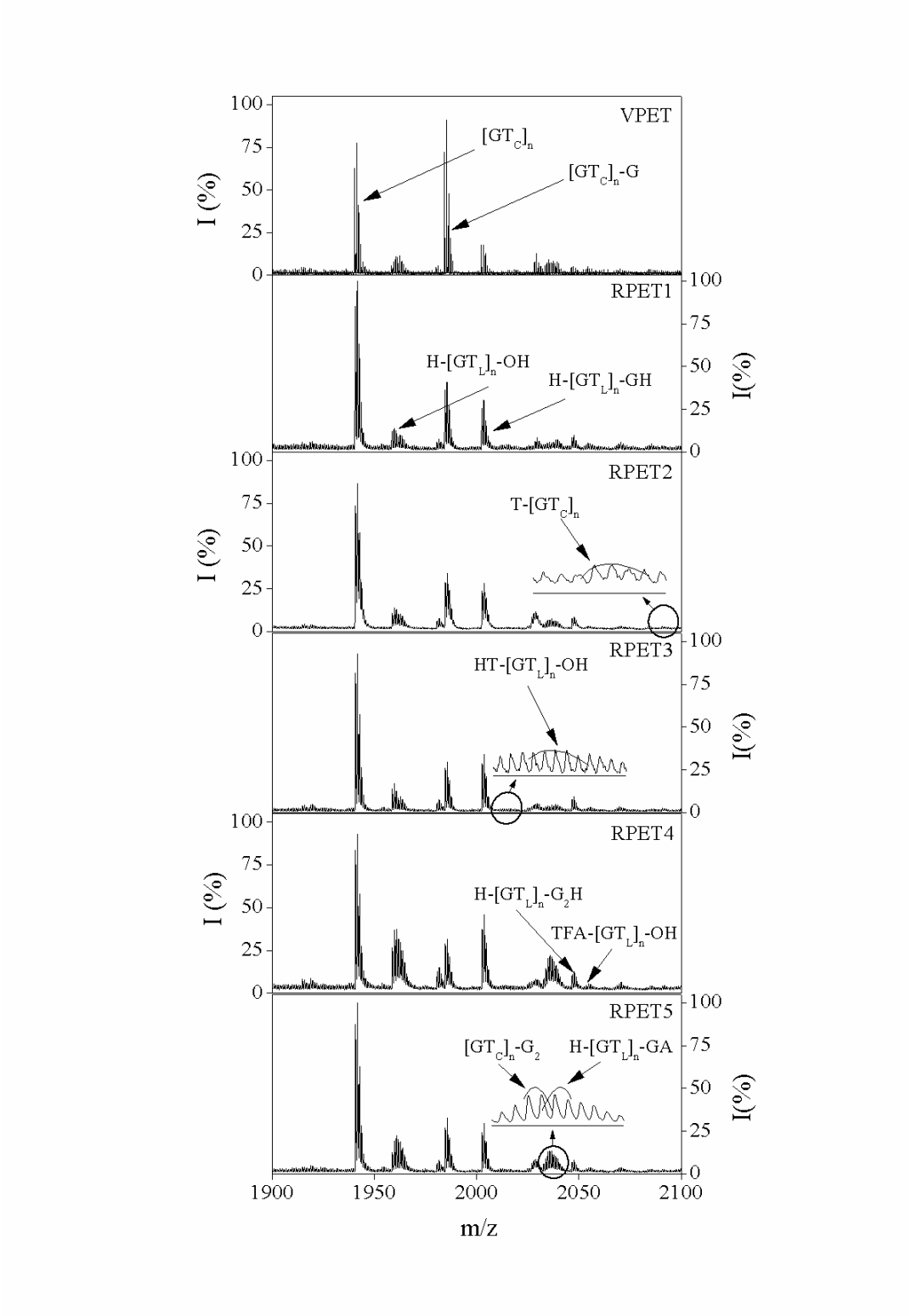
VPET
[GTC]n
[GTC]n-G
RPET1
H-[GTL]n-OH
H-[GTL]n-GH
RPET2
T-[GTC]n
RPET3
HT-[GTL]n-OH
RPET4
H-[GTL]n-G2H
TFA-[GTL]n-OH
RPET5
[GTC]n-G2
H-[GTL]n-GA
I (%)
I(%)
m/z
1900
1950
2000
2050
2100

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FIGURE 8

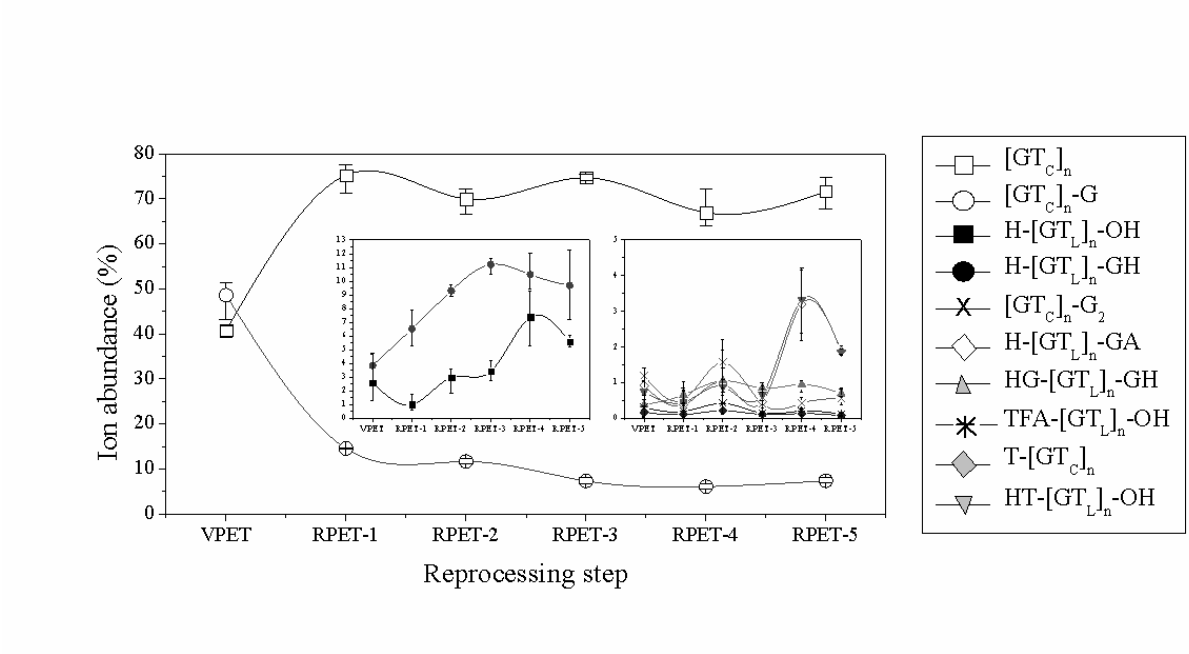

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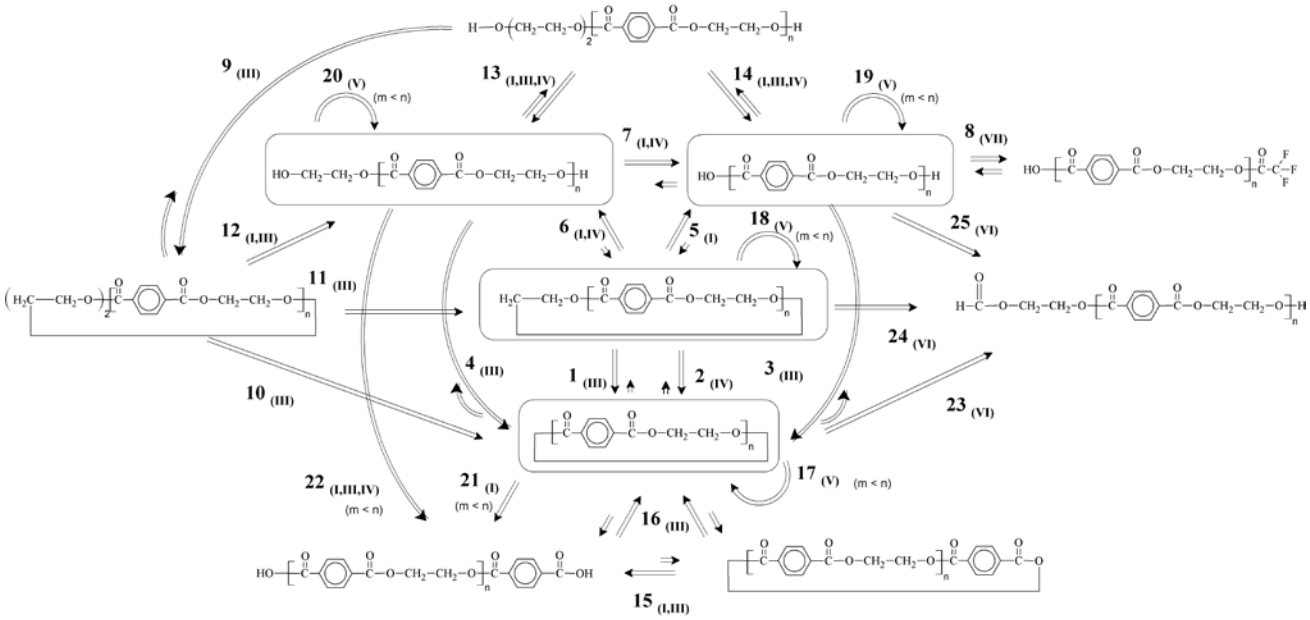
FIGURE 9



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 PET.

Table 3. Structures and m/z values for cyclic oligomeric species assigned in the MALDI-TOF MS spectra of virgin and reprocessed PET (for n=10, 11).

Table 4. Structures and m/z values for linear oligomeric species assigned in the MALDI-TOF MS spectra of virgin and reprocessed PET (for n=10, 11).

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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	Dithranol	HABA	FA		S/N	S/N_{rel}
Proportion of analyte/matrix (V:V)	Quantitative	3	1/5	1/10	1/15		RES	RES_{rel}
C_{NaTFA} ($g \cdot L^{-1}$)	Quantitative	4	0	0.5	1	2		
Replicates	3	**Total blocks**	3					
Base runs	36 ($3^2 \cdot 4^1$)	**Total runs**	108					

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

			EFFECT			
			S/N	RES	S/N_{rel}	RES_{rel}
			p-value (α=0.05)			
FACTORS	Blocks		0.414	0.009	0.357	0.183
	Main	Matrix	0	0	0	0
		Proportion	0	0	0.021	0.059
		C_{NaFTA}	0	0	0.067	0.107
	Interactions	Matrix & Proportion	0	0	0.014	0.013
		Matrix & C_{NaFTA}	0	0	0	0
		Proportion & C_{NaFTA}	0	0	0.005	0.033
		Matrix & Proportion & C_{NaFTA}	0	0	0.129	0.001
R^2 (%)			96.21	91.31	69.88	76.67

Table 3. Structures and m/z values for cyclic oligomeric species assigned in the MALDI-TOF MS spectra of virgin and reprocessed PET (for n=10, 11)

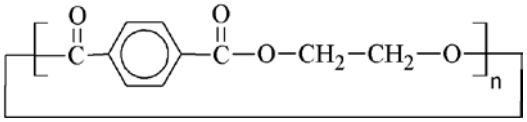
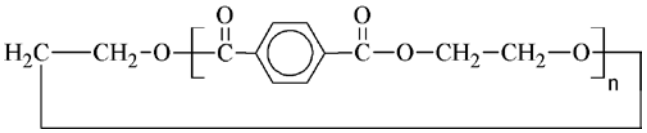
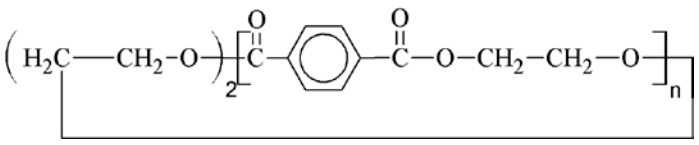
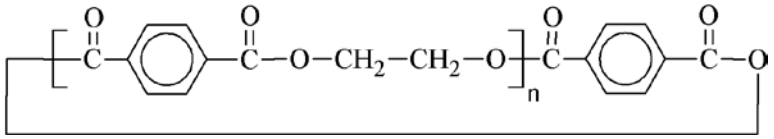
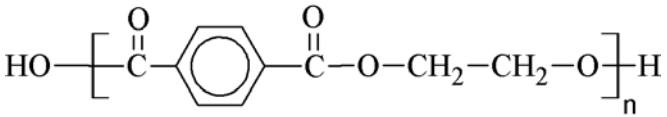
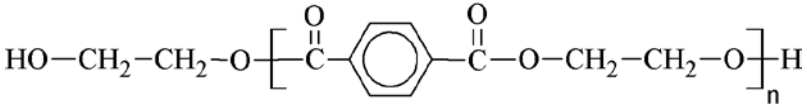
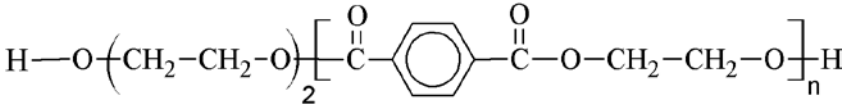
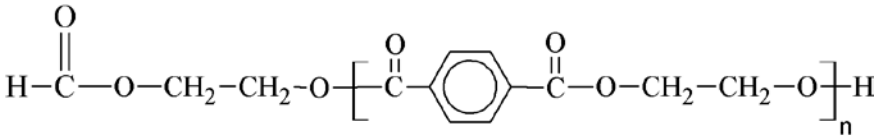
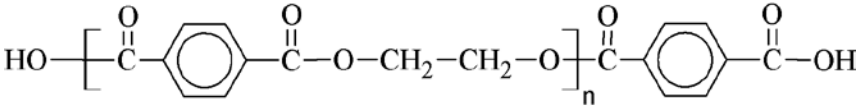
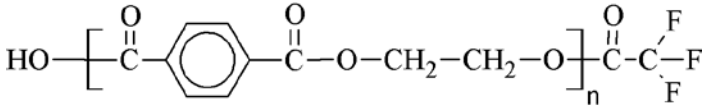
Species	Structures	$[M + Na]^+$
$[GT_C]_n$		1944.7/2136.9
$[GT_C]_n$-G		1988.8/2180.9
$[GT_C]_n$-G_2		2032.8/2225.0
T-$[GT_C]_n$		2092.8/2285.0

Table 4. Structures and m/z values for linear oligomeric species assigned in the MALDI-TOF MS spectra of virgin and reprocessed PET (for n=10, 11)

Species	Structures	$[M + Na]^+$
H-[GT$_L$]$_n$-OH		1962.7/2154.9
H-[GT$_L$]$_n$-GH		2006.8/2198.9
H-[GT$_L$]$_n$-G$_2$H		2050.8/2242.9
H-[GT$_L$]$_n$-GA		2034.75/2226.9

		
HT-[GT$_L$]$_n$-OH		2210.8/2303.0
TFA-[GT$_L$]$_n$-OH		2058.7/2250.9

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