Migration of oligomers from a food contact biopolymer based on polylactic acid (PLA) and polyester.

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Abstract

Polylactic acid (PLA) is a biopolymer commonly used in food packaging due to its good characteristics, similar to PET. To evaluate the safety of this material, the analysis of the non-intentionally added substances (NIAS) is required. Oligomers are NIAS and their behavior needs a deep study, especially if they migrate to the food. In this work, the analysis of the polymer and the migration to food simulants were carried out. A total dissolution/precipitation procedure was applied to PLA pellets and films, using dichloromethane and ethanol as solvent and antisolvent system respectively. The migration tests were carried out in three liquid simulants to mimic any kind of food. Since oligomers are not present in the positive list of the Directive 10/2011/EC, their concentration must be below the 0.01 mg/kg of food. UPLC-QTOF-MS, with and without ion mobility (IM) was used for the analysis. 39 different PLA oligomers made of repeated monomer units of [LA] ($C_3H_4O_2$) and with different structures were identified. They corresponded to cyclic oligomers with $[LA]_n$ structure and two groups of linear oligomers, one with an hydroxyl group, OH-$[LA]_n$-H, and the other one with an ethoxy group, CH_3-CH_2-O-$[LA]_n$-H. . Cyclic oligomers only appeared in the material and were not present in migration solutions. Linear oligomers HO-$[LA]_n$-H were already present in the pellets/film and they migrated in a higher extension to aqueous food

simulants (EtOH 10% and AcH 3%). However, linear oligomers CH_3-CH_2-O-[LA]$_n$-H were not present initially in the pellets/film, but were detected in migration to simulants with ethanol content, EtOH 95% and EtOH 10%. Furthermore, 5 cyclic polyester oligomers were identified in migration. Ethanol 95% and ethanol 10 % migration solutions were also analyzed by scanning electron microscopy (SEM) and the presence of microstructures that could be attributed to the oligomers migration was found. They could be seen as microplastics.

Keywords: *non-intentionally added substances (NIAS), biopolymer, polylactic acid (PLA), oligomers, biopolymer, UPLC-IMS-QTOF-MS, microplastics*

1. Introduction

Biopolymers have been regarded as alternative materials to conventional plastics made from petroleum because they are biodegradable, renewable and abundant and its use would significantly decrease our dependence on fossil resources.

Biopolymers encompass two kinds of polymers: biobased polymers and biodegradable and/or compostable polymers. A polymer is considered biobased when it comes from renewable sources (biomass) [1,2]. A polymer is considered biodegradable when is broken down by the action of microorganisms in their natural environment since is considered compostable when at least 90% of the polymer is degraded within 6 months in an industrial composting plant [3]. They can thus be divided into three main categories depending on its origin and degradability: biobased and biodegradable; biobased and non-biodegradable; and fossil-based and biodegradable. Polylactic acid (PLA) is considered a biopolymer because it derives from biomass sources and, in addition, it is compostable at industrial composting plants.

PLA is aliphatic polyester whose monomer, lactic acid, derives from carbohydrates from agricultural crops such as corn, potato, and cassava [5] by bacterial fermentation and it is one of the most commonly used biopolymer produced nowadays at industrial scale.

The most frequent route used for PLA manufacturing is the ring-opening polymerization of lactide. Another route is the direct condensation of lactic acid monomers. In 1992, PLA was approved by FDA as food contact material and in 2010, PLA reached the second highest consumption volume of any bioplastic in the world [6,7]. PLA is a thermoplastic biopolymer and easy to process with standard equipment. This fact, together with its good mechanical and barrier properties, similar to PET [8,9], makes it suitable and more attractive for the manufacturing of food packaging, such as bottles, food containers and wrappers [10,11]. Usually, PLA is blended to a polyester to improve its mechanical properties (flexibility, viscosity, etc.) and therefore to increase its applications. It has been also found blended to starch-based biopolymers [12]. Biodegradable aliphatic polyesters have been used in many areas during the last years due to the environmental contamination issues.

As in all polymers, biopolymers can contain oligomers [13-18], defined as molecules consisting of a few monomer units. They are often included under the group of non-intentionally added substances (NIAS) [19-22]. When PLA is used as food contact material, the oligomers can be transferred into food and consequently pose a risk to the consumer, thus, migration processes of these compounds must be evaluated [23]. Migration of these oligomers could be seen as microplastics coming from plastic food contact materials [24-26]. For this reason is very important to identify the kind of oligomers and to quantify them.

Thermal processing as well as moisture or other external phenomena can affect PLA stability and can lead to the formation of PLA oligomers during their manufacturing or storage. In addition, the contact with aqueous food can affect its composition, since water can hydrolytically degrade PLA leading to the formation of new oligomers [10,27-29] as well as other NIAS. It is important to study these oligomers in detail and develop new methodologies for PLA analysis that allow its detection [30]. Several works related to the degradation processes of PLA by hydrolysis and how this process affects its physico-chemical properties as well as the formation of new oligomers have been described in the literature [6,7,27,29,31-32]. For the determination of the oligomers content, different strategies have been followed. One of the most common strategies has been to transform

all the oligomers in lactic acid through an alkaline hydrolysis reaction and measure the differences in the lactic content before and after the PLA hydrolysis process [6,27,29]. The lactic acid was quantified by LC-UV as well as by LC-MS. Other works have directly analysed PLA extracts by ESI-MS with no chromatographic separation, and masses related to sodiated cyclic oligomers [23 + 72n] [6] as well as linear, in the case of aqueous solutions, [23 + 18+ 72n] [32] were observed. In the study performed by Dopico et al. [27], the surface of a PLA polymer was directly analysed by MALDI-TOF, detecting cyclic and linear oligomers in a mass range from m/z 700 to 5000 (n = 15 - 35). The linear oligomers increased when PLA was submitted to an ageing process. The study of PLA migration by LC-MS has been performed by some authors, such as Mutsuga et al., where oligomers were measured by ESI in negative mode [29] but as far as the authors know, only in the work carried out by Martinez Bueno et al [33], migration extracts were analyzed by LC coupled to high resolution mass spectrometry but oligomers were not detected.

The European Regulation 10/2011 on plastic materials intended to come into contact with food established specific rules for plastic materials but oligomers are not specifically regulated. Lactic acid is included in the list of authorized monomer without restrictions. However, cyclic lactide and linear or cyclic oligomers are not included in this list. For this reason, their migration should not exceed a level of 0.01 mg/kg food [34]. The lack of analytical standards and spectral database of these substances and the low migration values are an important issue that requires a great effort to assure food safety and quality. Thus, the analysis of this type of compounds needs high resolution mass spectrometry in order to perform its structural elucidation.

In this work, a dissolution/precipitation procedure with dichloromethane/ethanol was used for the analysis of the oligomer profile in PLA pellets and film samples. Migration tests were also carried out to determine the interactions of PLA oligomers when the biopolymer is in contact with different food simulants. The analyses were performed by UPLC-QTOF-MS-.

2. Material and methods

2.1 Reagents

A cyclic ester oligomer AA-DEG-IPA-DEG (95% w) formed by diethylene glycol (DEG), adipic acid (AA) and isophtalic acid (IPA), was used as standard for oligomers determination. It was provided by an adhesive producer. Its structure and purity were confirmed by NMR at the University of Zaragoza. Methanol (MeOH), ethanol (EtOH) and dichloromethane (DCM) were purchased from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q Ultramatric Wasserlab GR 216071 (Madrid, Spain). Acetic acid (CAS 64-19-7) was from Sigma-Aldrich Química S.A. (Madrid, Spain). Methanol and water for UPLC analysis (ultra LC–MS quality) were supplied by Baker (Deventer, The Netherlands).

2.2 Samples

Two different biopolymer samples based on PLA were supplied by 2 different packaging companies, PLA 1 and PLA 2. PLA 1 was blended to a biodegradable polyester. They were delivered in pellets as well as in films. For confidential reasons, additional information about the samples is not provided.

2.3. Total dissolution

Total dissolution was performed following the optimized protocol of PLA [30]. In this protocol,an amount of 0.25 g of PLA pellets or films was weighed in a 20 mL glass vial and 3 mL of DCM were added as solvent. The vial was closed with a screw cap and introduced in ultrasonic bath for 1 hour in order to guarantee a deep contact between solvent and polymer. After this time, PLA solution was taken and 6 mL of ethanol were added as antisolvent. The mixture was centrifuged at 500 rpm for 15 minutes and the supernatant was extracted. Subsequently, the residual precipitated polymer was washed with 3 mL of ethanol and the liquid phases were combined. Thus, the extract was kept at 4ºC for 1 hour to facilitate the polymer precipitation. Finally, the solution was filtered, evaporated under a nitrogen current and the residue was redissolved with methanol/water (1:1).

Three replicates were prepared from each sample and several procedural blanks were also analyzed. They were injected into a UPLC-QTOF-MS system.

2.4. Migration tests

For the migration experiments, PLA films were introduced in three food simulants according to the rate of 6 dm^2 of packaging material per 1 kg of simulant, in accordance with European Regulation 10/2011 [34].

The materials were tested in ethanol 10% (simulant A) and acetic acid 3% (simulant B) as aqueous simulants and in ethanol 95% as fat simulant. The vials with films were maintained in an oven at 60 ºC for 10 days. Simulants and test conditions used for the migration assays were chosen according to the European Regulation 10/2011 [34]. The samples were analyzed by UPLC-QTOF-MS and also by UPLC-IMS- QTOF-MS. Three replicates of every test were analyzed.

2.5. Instrumental analysis

2.5.1 Ultra-performance liquid chromatography-mass spectrometry quadrupole time-of-flight (UPLC-QTOF-MS)

Chromatography was carried out in an Acquity™ system using an Acquity UPLC BEH C18 column (2.1 mm x 100 mm x 1.7 μm particle size), both from Waters (Milford, MA, USA). The solvents used as mobile phases for positive mode were water and methanol both with 0.1 % formic acid (solvents A_1 and B_1 respectively). For negative mode, water and methanol (solvents A_2 and B_2 respectively) were used. The column flow was 0.3 mL/min and the column temperature was 40 °C. The gradient elution (12 min) was performed with mobile phase A/B variating from 98/2 % to 0/100 % over 8 min and maintained for two minutes. After that, mobile phase A/B changes to 98/2 % again to condition the column. The volume of sample injected was 10 μL.

The detector was an API source (atmospheric pressure ionization) with ESI (electrospray ionization) coupled to a mass spectrometer (Xevo G2) consisting of a quadrupole, a collision cell and a time-of-flight (QTOF) detectors all supplied by Waters (Milford, MA, USA).

The electrospray probe was used in positive (ESI+) and negative (ESI−) modes as well as sensitivity analyzer mode. The mass range was from 50 to 1200 Da. The capillar voltage was 2.5 kV. The sampling cone voltage was 30 V and 70 V for positive mode and 30 V for negative mode. Source temperature was 120°C. Nitrogen was used as the desolvation gas: the flow rate was 450 L/h at 400 °C. The cone gas flow rate was 20 L/h.

Acquisition was performed in MS^E mode to allow using low and high collision energy (CE) in the collision cell during the same run. The mass spectrum at low energy (CE 4 V) provided information about the precursor ion (function 1) and the mass spectrum at high energy (CE ramp: from 15 to 30 V) information about fragment ions (function 2). The accuracy and reproducibility were guaranteed by the infusion of a LockSpray solution of leucine-enkephalin (2 ng/mL in water/acetonitrile with 0.1 % formic acid) at a flow rate of 5 µL/min. MassLynx version 4.1 (Waters, Milford, MA, USA) was used to analyze the samples.

2.5.2 Identification of oligomers of PLA detected by UPLC-QTOF-MS

High-resolution mass spectrometry with a tandem quadrupole-time of flight mass spectrometer was used for the identification of non-volatile compounds. This technique provides molecular fragmentation combined with mass accuracy in order to elucidate the molecular structure that could lead to the identification of the compounds. Conditions used for the acquisition were explained in section 2.5.1.

Function 1 corresponds to the acquisition without collision energy, where the molecular formula of the oligomers based on the molecular ion can be obtained through the measurement of its accurate mass and the isotopic ratios.

Finally, with the use of function 2, the fragmentation spectra of the oligomers were obtained and the proposed candidates were checked through MassFragment® software from Waters. This software enabled us to evaluate and confirm whether the product ions detected in the high collision energy spectrum could be linked to the fragments generated from the chemical structures of the candidates proposed. Since oligomers are not included in any chemical databases and there are not standards available, it was necessary to draw the molecules in ChemDraw Ultra 12.0 to obtain a *.mol* document for further confirmation of unknown oligomers.

2.5.3 Semi-quantification of oligomers of PLA detected by UPLC-QTOF-MS

PLA oligomers were semi-quantified by calibration curve of oligomer AA-DEG-IPA-DEG as standard. The working range in the instrument was 0.03-1.30 µg/g and the coefficient of determination (R^2) obtained in the calibration curve was 0.9999. The limit of detection (LOD) and quantification (LOQ) were 0.01 and 0.03 µg/g respectively. The LOD and LOQ were calculated as the minimum concentration whose signal was equal to 3 times and 9 times the baseline noise. Five independent replicates of solutions containing 0.01 and 0.03 µg/g of the standard were injected for their calculation.

2.5.4 Ultra-performance liquid chromatography-ion mobility mass spectrometry quadrupole time-of-flight (UPLC-IMS-QTOF-MS)

UPLC-IMS-QTOF-MS analysis was performed in a Vion IMS QTOF supplied by Waters (Milford, MA, USA). Chromatography was carried out following the same conditions described in 2.5.1. MS data were acquired in (HD)MS^E mode, in the range 50-1000 m/z. Low collision energy was set at 4 V and high collision energy at a ramp from 20 to 40 V. Source and desolvation conditions were the same as described in 2.5.1. IMS was used to measure the ions drift-time, that after a calibration process was transformed to collision cross-section values (CCS). Nitrogen was used as drift gas. Trap conditions were as follows: IMS wave velocity of 250 m/s, IMS pulse height 45 V, trap bias

40V, trap wave velocity 100 m/s, trap pulse height A 10V, trap pulse height B 5V and gate release 2 ms. Data were processed using UNIFI v1.8 software.

2.6. Analysis by scanning electron microscopy (SEM)

Analyses were performed with a JEOL JSM 6400 microscope, using a voltage of 15kV. In order to analyse the morphology of the samples a detector of secondary electrons was used. Some drops of the migration samples were placed on the wafer and left dry. Blanks of migration were also analysed.

3. Results and discussion

3.1 PLA oligomers identified in PLA-based biopolymer

Table 1 summarizes the oligomers identified in total dissolution extracts of PLA1 pellets and films and in migration simulants after the exposure. A total of 39 different oligomers, cyclic and linear, were identified. Among them, 24 were identified in total dissolution of pellets and film samples, 50% of them were cyclic and 50% linear. Ten out of 24 oligomers were also detected in migration together with 15 new oligomers formed in the reaction between PLA components and food simulants. All the oligomers detected in migration were linear oligomers. Most of them had been detected in previous studies, their exact mass was provided but no candidates were proposed [35]. These oligomers are also displayed in table 1 according to their retention time. In all cases, the ion detected was the sodium adduct [MNa^+]. All the oligomers detected contained the monomer unit [LA], that corresponded to the molecular formula $C_3H_4O_2$. They were classified in 2 groups based on their chemical structure: group 1 formed by cyclic oligomers, $[LA]_n$; and group 2 formed by linear oligomers, OH-$[LA]_n$-H and CH_3-CH_2-O-$[LA]_n$-H. The exact mass for n values ranging from n =1 to n =20 was calculated in all oligomer structures and extracted in the chromatograms, in order to check their presence in the samples. All these chemical structures or similar were previously described by Badía et al. [36]. Other similar structures described by Badía were searched in the

samples, such as CH_3-O-$[LA]_n$-H and CH_3-CO-O-$[LA]_n$-H but none of them was found in pellets or films.

The value of n ranged from n=5 to n=16 in $[LA]_n$, from n=3 to n=15 in OH-$[LA]_n$-H and from n=2 to n=15 in CH_3-CH_2-O-$[LA]_n$-H.

The order in the chromatogram for oligomers with the same number of monomers but different structures was as follows: firstly OH-$[LA]_n$-H, then $[LA]_n$ and finally, CH_3-CH_2-O-$[LA]_n$-H.

All PLA oligomers had common fragmentation spectra, which confirmed the similarity of their structures (Figure 1). Their common masses were 89.0600, 145.0503 and 217.0709 m/z, corresponding to the formula $C_3H_5O_3$, $C_6H_9O_4$ and $C_9H_{13}O_6$, respectively. Figure 1 shows the high collision energy spectra of the 3 different kinds of PLA oligomers for n=6: $[LA]_6$ (a), OH-$[LA]_6$-H (b) and CH_3-CH_2-O-$[LA]_6$-H (c). Fragments observed successfully matched with the proposed structures. These three masses were more intense in the cyclic oligomers than in the linear ones. In the cyclic oligomers, 217.0709 was the most intense while in the linear oligomers its intensity was similar to 145.0491.

Lactide ($C_6H_8O_4$), the monomer of PLA, was not detected in the analysis. This was due to its difficult ionization in the mass spectrometer. As the purpose of this paper was the determination of oligomers, the initial monomer was not finally included in the study.

3.2 Comparison of PLA oligomers content in pellets and films

The next aim was to compare the PLA oligomers profile in pellets and film in order to determine if the extrusion process had induced any change in PLA´s composition. The results showed that the areas of the oligomers identified in the total dissolution analysis of pellets and films were very similar. Figure 2a shows the areas of cyclic and linear oligomers in pellets vs films, and a good correlation can be observed (Pearson correlation value of 0.998). Therefore, no changes in the oligomer profile due to the manufacturing process are expected .These results are interesting as they demonstrate that applying high temperatures as those used in the film manufacturing from the

pellets does not affect the composition of the material. Thus both pellets or films can be used for determining the oligomers profile of a PLA sample.

Figure 2b shows the distribution (ng/g) of the oligomers in the film. Only the cyclic oligomers from n=5 to 11 and the linear oligomers with HO-$[LA]_n$-H structure from n=5 to 8 were detected in the raw material, while CH_3-CH_2-O-$[LA]_n$-H were not present.. The results show that the concentration values were much higher (5 to 20 times) for the cyclic oligomers than for the linear ones. This means that the cyclation of the oligomeric molecules is easier than the linear reaction between different oligomers, what results in a higher concentration of the cyclic ones. The cyclic structure of the oligomers will probably affect also their migration. The oligomer with the highest values was $[LA]_7$ followed by n= 6 and 8.

3.3 Migration of PLA oligomers

Figure 3 shows the distribution (ng/g) of the two kinds of PLA oligomers, HO-$[LA]_n$-H (a) and CH_3-CH_2-O-$[LA]_n$-H (b), detected in migration from films to three different food simulants (EtOH 10%, AcH 3% and EtOH 95%). The values of n ranged from 3 to 16. Cyclic oligomers, which have been previously detected in total dissolution of films, were not present in any migration test.

Oligomers with HO-$[LA]_n$-H structure were already present in the film. They migrated in a higher extension to aqueous food simulants (AcH 3% and Et10%) than to fatty simulants (ethanol 95%). Chromatogram of these linear oligomers from n=3 to n=10 in 3% acetic acid follow a Gaussian profile and the distribution was very similar in the three simulants, where n=6-7 showed the highest values (Supplementary Material 1).

CH_3-CH_2-O-$[LA]_n$-H was not initially present in the film but it was detected in migration to simulants with ethanol content, EtOH 95% and EtOH 10%. This chemical structure was selected based on the previous studies performed by Badía et al [36], where CH_3-O-$[LA]_n$-CH3 oligomer was formed when PLA was in contact with methanol. In this case, the use of ethanol as simulant in migration test suggests that a structure CH_3-CH_2-O-$[LA]_n$-H would be more feasible. Both of them

matched with the fragments found in the high collision energy mass spectra.

As it has been described, cyclic oligomers were not present in food simulants. This fact could be attributed either because they did not migrate or because they reacted with the simulants and thus the cycle was opened, forming new linear compounds. To elucidate this question, a new assay was performed. A solution of cyclic oligomers was exposed to different food simulants and stored in an oven at 60ºC for 10 days. Since there were not standards for these oligomers, an aliquot of a total PLA dissolution in MeOH/H_20 was taken and diluted five times with MeOH/H_20, EtOH 95%, EtOH 10% or AcH 3%. Figure 4 shows the areas of $[LA]_n$, OH-$[LA]_n$-H and CH_3-CH_2-O-$[LA]_n$-H at the initial time and after the storage time in the three food simulants. Oligomers with n values from 4 to 9 were selected for this study since they showed the highest areas. Similar profiles were observed for oligomers with different n value. The results are showed in figure 4. Figure 4a shows that the cyclic oligomers, $[LA]_n$, barely decreased when it was in contact with ethanol 95%, (Figure 4a), demostrating that they were quite stable in this simulant. In contrast, they clearly decreased in those simulants with a high water content such as EtOH 10% and AcH 3%, observing in parallel an increase of the linear oligomers in these simulants. The oligomers with OH-$[LA]_n$-H structure increased in contact with aqueous simulants (Figure 4b) while the oligomers with CH_3-CH_2-O-$[LA]_n$-H structure increased with ethanolic simulants, reaching the highest values for the simulant with the highest ethanol content, EtOH 95% (Figure 4c).

Thus, since migration of cyclic oligomers should mainly take place in ethanol 95%, and the results showed its stability in this simulant, the absence of cyclic oligomers in migration was not because they disappeared due to reaction processes between them and food simulants, but because they did not migrate. The presence of linear oligomers in migration was probably due to the interaction between the simulants and PLA, that provided with preference these oligomers due hydrolysis processes.

Migration tests were also performed from film PLA 2. But in this case, the material was clearly damaged after the study. According to EU/10/2011 [34], if the material presents physical changes

after the migration test, this material cannot be used under the tested conditions. For this reason, the concentration of oligomers is not shown in this manuscript. It is important to highlight that the concentration of oligomers was much higher than expected, although similar oligomer profiles were observed.

3.4 Collision cross section values of PLA oligomers

A good correlation was observed between the CCS values of the 3 kinds of oligomers versus their molecular weight (Supplementary Material 2). The correlation equations for each type of oligomers were:

- $[LA]_n$: $y = 0.1763x + 107.69$ ($R^2 = 0.9910$)
- OH-$[LA]_n$-H: $y = 0.1817x + 105.90$ ($R^2 = 0.9976$)
- CH_3-CH_2-O-$[LA]_n$-H: $y = 0.1841x + 108.62$ ($R^2 = 0.9983$)

According to the theory of ion mobility, the CCS values of cyclic and linear oligomers should be very different. As it can be observed, the three kinds of oligomers showed similar correlation equations, having the one corresponding to the cyclic oligomers a slope slightly lower. Bigger differences in CCS values were expected between linear and cyclic oligomers with a similar molecular weight, as CCS depends on the tridimensional molecule structure and linear molecules are expected to have higher CCS values. Probably, the linear molecules stayed folded in the original position, maintaining their CCS similar to those of the cyclic ones and this could be the explanation of the similar behavior between the cyclic and linear oligomers.

3.5 Other oligomers identified in migration

In PLA 1 migration, 5 cyclic polyesters oligomers were also identified. They were composed by adipic acid (AA), phthalic acid (PA) and butanediol (BD). The structures were $[AA]_2$-$[BD]_2$ (7.21_401.2183), PA-AA-$[BD]_2$ (7.71_443.1676), $[AA]_3$-$[BD]_3$ (7.84_601.3229), PA-$[AA]_2$-$[BD]_3$ (8.20_643.2741) and PA-$[AA]_3$-$[BD]_4$ (8.45_843.3785). All masses had been detected in a previous

work performed in our laboratory [30] and their structure elucidated (supplementary material 3), except for 8.45_843.3785, which has been elucidated in this work by the first time. Figure 5 shows the concentration of these oligomers in three different simulants. Unlike PLA oligomers, cyclic oligomers from polyester migrated to food simulants and no hydrolysis to linear oligomers was observed. Migration values were especially high in the fatty food simulant rather than in aqueous simulants.

3.6 Analysis of oligomers migration by SEM

Figure 6 shows the images at 2 different gains, x1500 and x8000 for a migration solution of ethanol 95% and ethanol 10%. Images show structures bigger that 1 μm, which could imply the presence of microplastics in the migration solutions [25]. No structures were observed in blanks of migration (data not showed). The observed structures were different in ethanol 95% and ethanol 10%, what makes sense, since the oligomers that migrated to these two simulants were different. While cyclic oligomers from polyester migrated in a major extension to ethanol 95%, linear PLA oligomers were the main migrants to ethanol 10%.

The wafer with the samples of ethanol 95 % was afterwards placed in the oven at 260ºC for 1 hour in order to see the effect of heat on these structures. Images after this period showed that they seemed to have melted, which could be attributed to a plastic nature of the structures. In any case, future studies in this research line should be done in order to confirm this hypothesis.

4. Conclusions

Identification and quantification of oligomers in food contact materials is an important and difficult task due to the little knowledge about them. Oligomers are neither included in libraries nor in chemical databases. There are not commercial standards and none knowledge of the toxicological properties of oligomers. However, oligomers migrate from the polymers in a quite wide extension and dissolve in the food or beverages, where they could be seen as microplastics.

Total dissolution/precipitation of PLA pellets and film has resulted to be a good sample treatment to determine the oligomers composition of PLA-base materials, mainly composed by cyclic oligomers, $[LA]_n$, and linear ones such as HO-$[LA]_n$-H. Migration tests confirmed the presence of some of these oligomers in food simulants as well as new neo-formed oligomers such as, due to the reaction processes between PLA components and food simulants.. What this work shows is the importance of the evaluation of migration, since the compounds present in food will depend on a great extent on the chemical reactions between packaging and food or food simulants and these neo-formed compounds could have toxic effects on consumers health. Production processes should be optimized in order to reduce and minimize as much as possible the presence of these oligomers in PLA-based materials since its possible transference to food has been demonstrated. It should be also considered that migration test were performed at temperatures close to the PLA glass transition temperature (50-60ºC) and this fact could affect to its stability. New experiments at lower migration temperatures could demonstrate a better applicability of these materials to food packaging under refrigerated storage conditions.

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6. Compliance with ethical standards

The authors declare that they do not have potential conflicts of interest. No human participants or animals are involved in the research.

7. References

1. Jabeen N, Majid I, Nayik GA (2015) Bioplastics and food packaging: A review. Cogent Food & Agriculture 1 (1):1117749. doi:10.1080/23311932.2015.1117749

2. Geueke B (2014) Dossier – Bioplastics as food contact materials. Food Packaging Forum. doi:10.5281/zenodo.33517

3. AENOR (2001) UNE-EN 13432:2001. Requirements for packaging recoverable through composting and biodegradation. Test scheme and evaluation criteria for the final acceptance of packaging.

4. Othman SH (2014) Bio-nanocomposite Materials for Food Packaging Applications: Types of Biopolymer and Nano-sized Filler. Agriculture and Agricultural Science Procedia:296-303. doi:10.1016/j.aaspro.2014.11.042

5. Jiang L, Zhang J (2011) Biodegradable and Biobased Polymers. Applied Plastics Engineering Handbook: Processing, Materials, and Applications. doi:10.1016/b978-1-4377-3514-7.10009-1

6. Bor Y, Alin J, Hakkarainen M (2012) Electrospray Ionization-Mass Spectrometry Analysis Reveals Migration of Cyclic Lactide Oligomers from Polylactide Packaging in Contact with Ethanolic Food Simulant. Packag Technol Sci 25 (7):427-433. doi:10.1002/pts.990

7. Lazzari S, Codari F, Storti G, Morbidelli M, Moscatelli D (2014) Modeling the pH-dependent PLA oligomer degradation kinetics. Polym Degrad Stab 110:80-90. doi:https://doi.org/10.1016/j.polymdegradstab.2014.08.012

8. Auras R, Harte B, Selke S (2004) An Overview of Polylactides as Packaging Materials. Macromol Biosci 4 (9):835-864. doi:10.1002/mabi.200400043

9. Gandini MNBaA (2008) Monomers, Polymers and composites from Renewable Resources. ElSevier:433-450

10. Salazar R, Domenek S, Plessis C, Ducruet V (2017) Quantitative determination of volatile organic compounds formed during Polylactide processing by MHS-SPME. Polym Degrad Stab 136:80-88. doi:10.1016/j.polymdegradstab.2016.12.010

11. Jamshidian M, Tehrany EA, Imran M, Jacquot M, Desobry S (2010) Poly-Lactic Acid: Production, Applications, Nanocomposites, and Release Studies. Comprehensive Reviews in Food Science and Food Safety 9 (5):552-571. doi:10.1111/j.1541-4337.2010.00126.x

12. Osorio J, Aznar, M., Nerín, C. (2019) Identification of key odorant compounds in starch-based polymers intended for food contact materials. Food Chem. doi:10.1016/j.foodchem.2019.01.157

13. Úbeda S, Aznar M, Vera P, Nerín C, Henríquez L, Taborda L, Restrepo C (2017) Overall and specific migration from multilayer high barrier food contact materials – kinetic study of cyclic polyester oligomers migration. Food Addit Contam A 34 (10):1784-1794. doi:10.1080/19440049.2017.1346390

14. Hoppe M, de Voogt P, Franz R (2016) Identification and quantification of oligomers as potential migrants in plastics food contact materials with a focus in polycondensates – A review. Trends Food Sci Technol 50:118-130. doi:10.1016/j.tifs.2016.01.018

15. Heimrich M, Nickl H, Bönsch M, Simat TJ (2015) Migration of Cyclic Monomer and Oligomers from Polyamide 6 and 66 Food Contact Materials into Food and Food Simulants: Direct Food Contact. Packag Technol Sci 28 (2):123-139. doi:10.1002/pts.2094

16. Ubeda S, Aznar M, Nerín C (2018) Determination of oligomers in virgin and recycled polyethylene terephthalate (PET) samples by UPLC-MS-QTOF. Anal Bioanal Chem. doi:10.1007/s00216-018-0902-4

17. Gómez Ramos MJ, Lozano A, Fernández-Alba AR (2019) High-resolution mass spectrometry with data independent acquisition for the comprehensive non-targeted analysis of migrating chemicals coming from multilayer plastic packaging materials used for fruit purée and juice. Talanta 191:180-192. doi:10.1016/j.talanta.2018.08.023

18. Omer E, Cariou R, Remaud G, Guitton Y, Germon H, Hill P, Dervilly-Pinel G, Le Bizec B (2018) Elucidation of non-intentionally added substances migrating from polyester-polyurethane lacquers using automated LC-HRMS data processing. Anal Bioanal Chem 410 (22):5391-5403. doi:10.1007/s00216-018-0968-z

19. Nerin C, Alfaro P, Aznar M, Domeño C (2013) The challenge of identifying non-intentionally added substances from food packaging materials: A review. Anal Chim Acta 775:14-24. doi:10.1016/j.aca.2013.02.028

20. Vera P, Canellas E, Nerín C (2018) Identification of non volatile migrant compounds and NIAS in polypropylene films used as food packaging characterized by UPLC-MS/QTOF. Talanta 188:750-762. doi:10.1016/j.talanta.2018.06.022

21. Tian L, Lin L, Bayen S (2019) Optimization of the post-acquisition data processing for the non-targeted screening of trace leachable residues from reusable plastic bottles by high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry. Talanta 193:70-76. doi:10.1016/j.talanta.2018.09.070

22. Canellas E, Vera P, Nerín C (2015) UPLC-ESI-Q-TOF-MS(E) and GC-MS identification and quantification of non-intentionally added substances coming from biodegradable food packaging. Anal Bioanal Chem 407 (22):6781-6790. doi:10.1007/s00216-015-8848-2

23. Catalá R, Gavara R (2002) Migración de componentes y residuos de envases en contacto con alimentos. Instituto de Agroquímica y Tecnología de Alimentos (CSIC)

24. Bouwmeester H, Hollman PCH, Peters RJB (2015) Potential Health Impact of Environmentally Released Micro- and Nanoplastics in the Human Food Production Chain: Experiences from Nanotoxicology. Environ Sci Technol 49 (15):8932-8947. doi:10.1021/acs.est.5b01090

25. Gigault J, Halle At, Baudrimont M, Pascal P-Y, Gauffre F, Phi T-L, El Hadri H, Grassl B, Reynaud S (2018) Current opinion: What is a nanoplastic? Environ Pollut 235:1030-1034. doi:10.1016/j.envpol.2018.01.024

26. Silva AB, Bastos AS, Justino CIL, da Costa JP, Duarte AC, Rocha-Santos TAP (2018) Microplastics in the environment: Challenges in analytical chemistry - A review. Anal Chim Acta 1017:1-19. doi:10.1016/j.aca.2018.02.043

27. Dopico-García S, Ares-Pernas A, Otero-Canabal J, Castro-López M, López-Vilariño JM, González-Rodríguez V, Abad-López MJ (2013) Insight into industrial PLA aging process by complementary use of rheology, HPLC, and MALDI. Polym Adv Technol 24 (8):723-731. doi:10.1002/pat.3136

28. Osaka I, Yoshimoto A, Watanabe M, Takama M, Murakami M, Kawasaki H, Arakawa R (2008) Quantitative determination of cyclic polylactic acid oligomers in serum by direct injection liquid chromatography tandem mass spectrometry. J Chromatogr B 870 (2):247-250. doi:10.1016/j.jchromb.2008.06.035

29. Mutsuga M, Kawamura Y, Tanamoto K (2008) Migration of lactic acid, lactide and oligomers from polylactide food-contact materials. Food Addit Contam A 25 (10):1283-1290. doi:10.1080/02652030802017529

30. Aznar M, Ubeda S, Dreolin N, Nerín C (2018) Determination of non-volatile components of a biodegradable food packaging material based on polyester and polylactic acid (PLA) and its migration to food simulants. J Chromatogr A. doi:10.1016/j.chroma.2018.10.055

31. Inkinen S, Hakkarainen M, Albertsson A-C, Södergård A (2011) From Lactic Acid to Poly(lactic acid) (PLA): Characterization and Analysis of PLA and Its Precursors. Biomacromolecules 12 (3):523-532. doi:10.1021/bm101302t

32. Andersson SR, Hakkarainen M, Inkinen S, Södergård A, Albertsson A-C (2010) Polylactide Stereocomplexation Leads to Higher Hydrolytic Stability but More Acidic Hydrolysis Product Pattern. Biomacromolecules 11 (4):1067-1073. doi:10.1021/bm100029t

33. Martínez-Bueno MJ, Hernando MD, Uclés S, Rajski L, Cimmino S, Fernández-Alba AR (2017) Identification of non-intentionally added substances in food packaging nano films by gas and liquid chromatography coupled to orbitrap mass spectrometry. Talanta 172:68-77. doi:https://doi.org/10.1016/j.talanta.2017.05.023

34. EC (2011) Commission regulation (EU) no 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Official Journal of the European Union (L12:1 89)

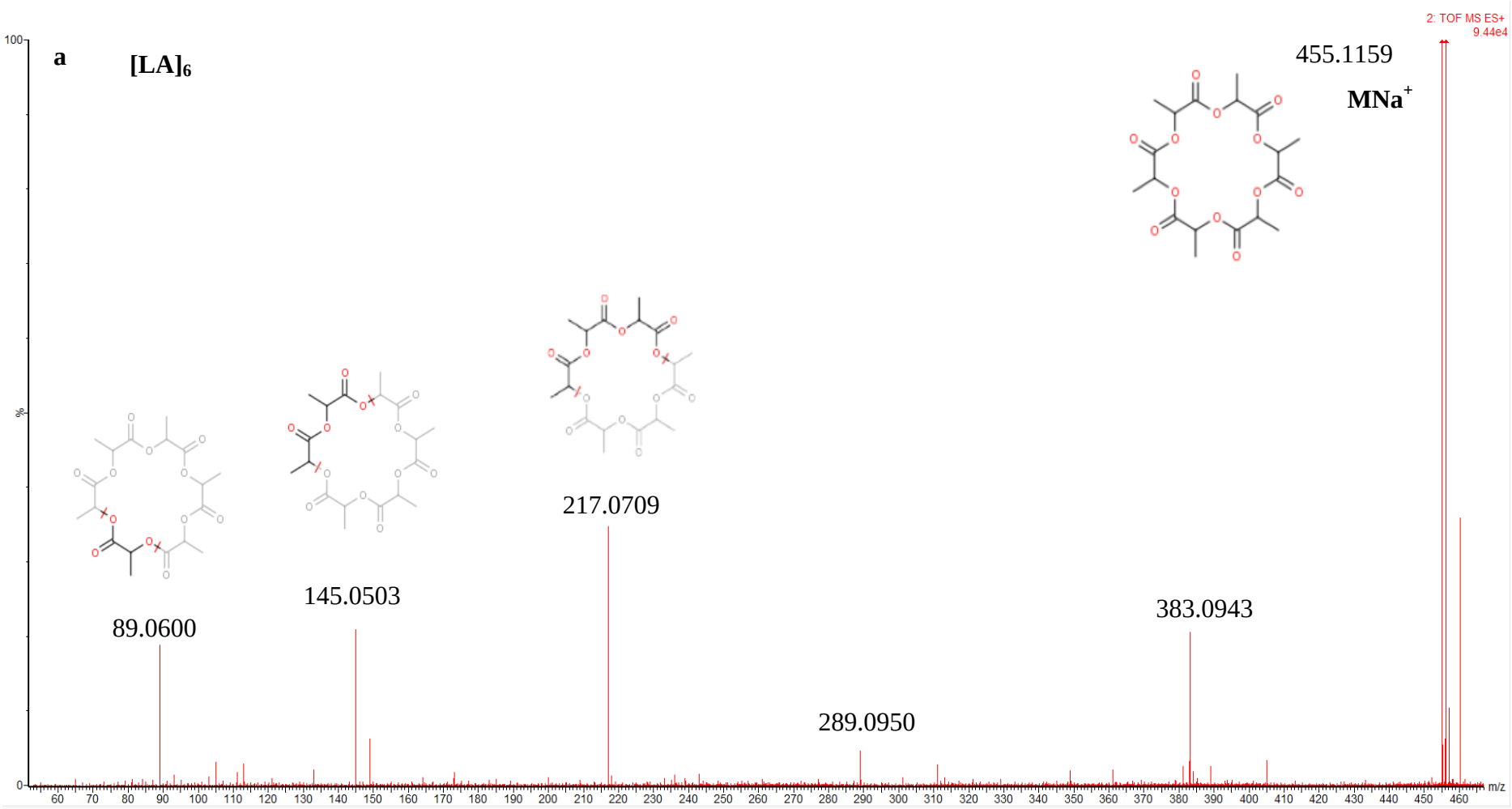
35. Bradley EL (2010) Biobased materials used in food contact applications: an assessment of the migration potential. The Food and Environment Research Agency:201

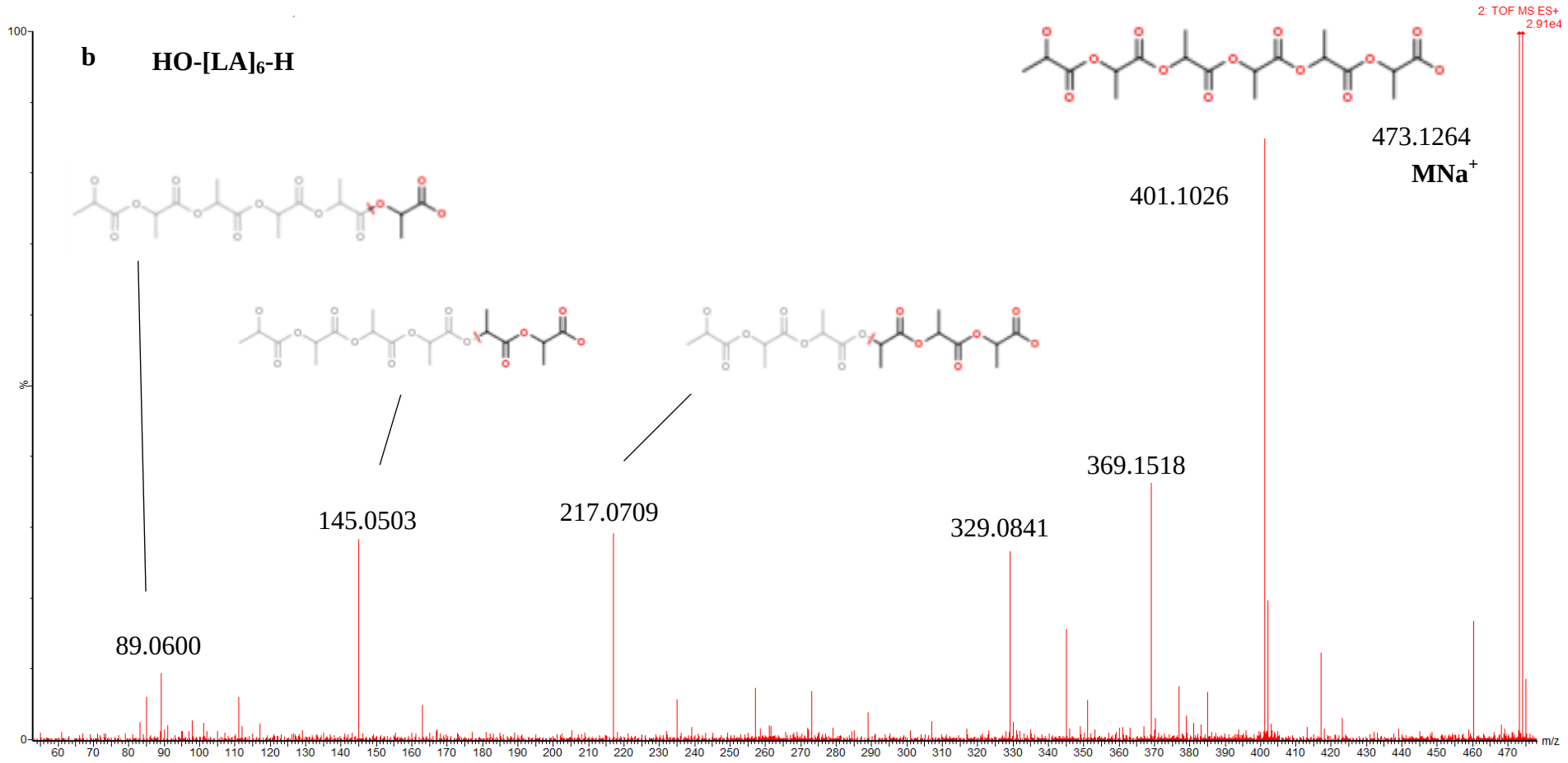
36. Badía JD, Strömberg E, Ribes-Greus A, Karlsson S (2011) Assessing the MALDI-TOF MS sample preparation procedure to analyze the influence of thermo-oxidative ageing and thermo-mechanical degradation on poly (Lactide). Eur Polym J 47 (7):1416-1428. doi:10.1016/j.eurpolymj.2011.05.001

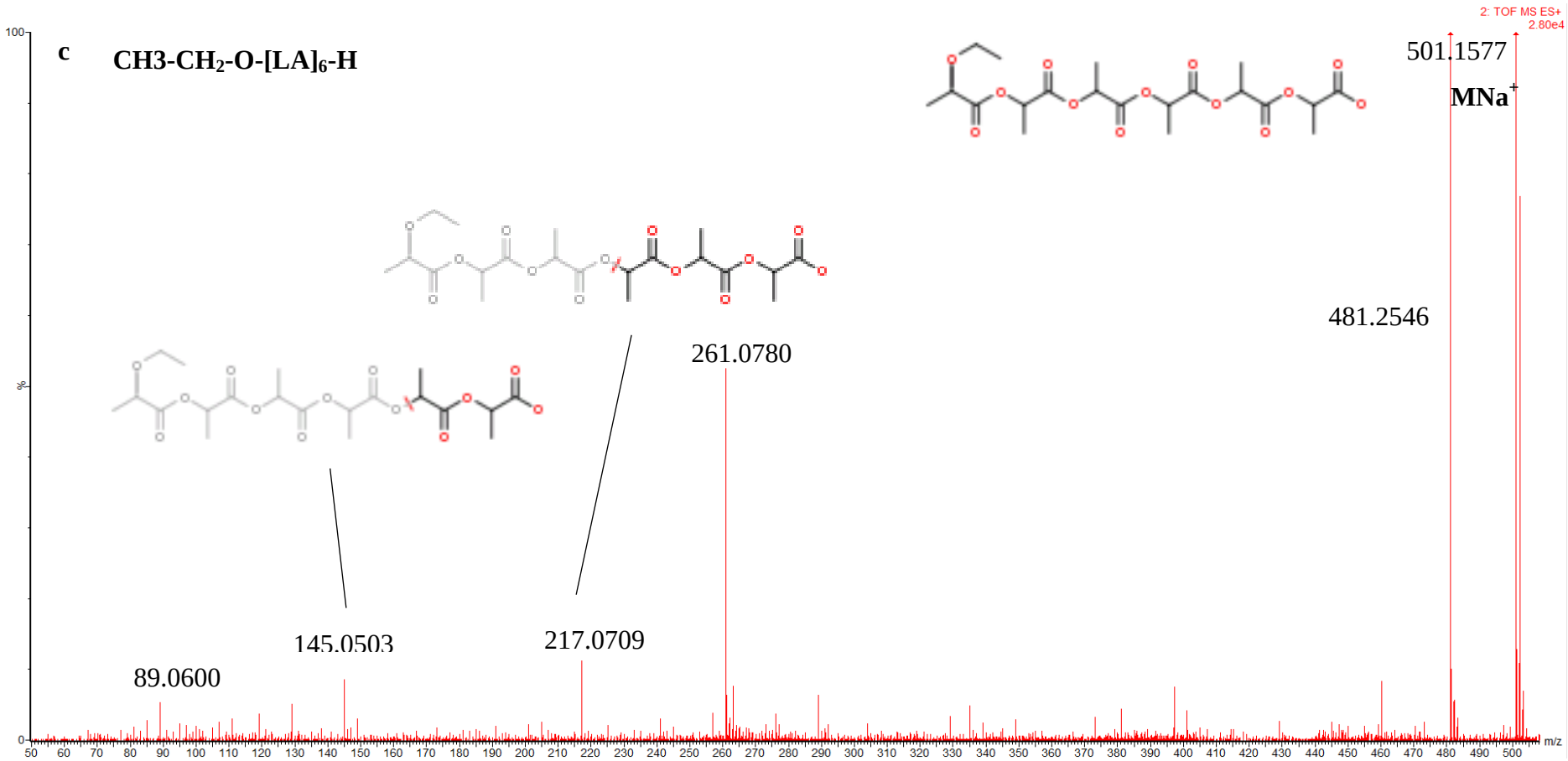
Table 1. Oligomers identified in total dissolution of PLA materials (pellets and film) and in migration to 3 food simulants: ethanol 95%, ethanol 10% and acetic acid 3%. Retention time (rt), measured mass (mass), molecular formula (MF) and number of times that monomer of PLA is repeated (n).

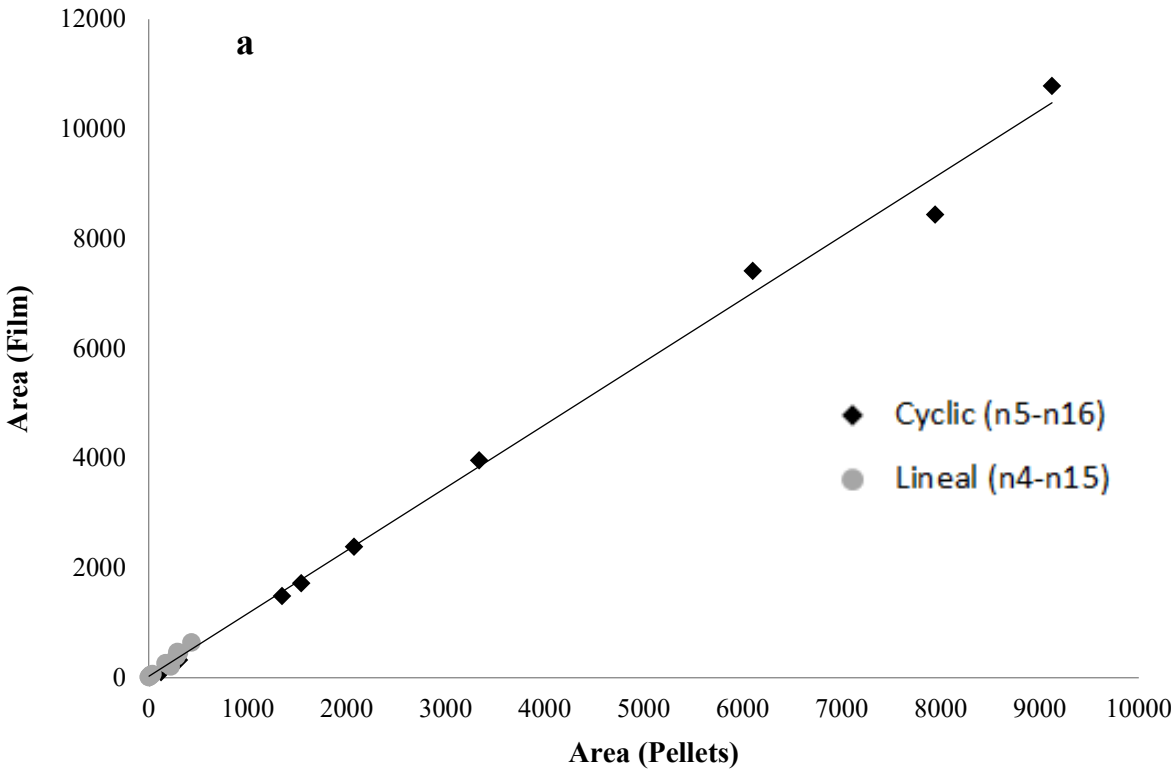
rt	Mass [MNa^+]	MF	Oligomers		PLA samples			
			Type	n	Pellets/films	Migration EtOH 95%	Migration EtOH 10%	Migration AcH 3%
4.50	257.0631	$C_9H_{14}O_7$	Linear HO-$[LA]_n$-H	3			x	x
5.09	213.0733	$C_8H_{14}O_5$	Linear CH_3-CH_2-O-$[LA]_n$-H	2		x	x	
5.31	329.0842	$C_{12}H_{18}O_9$	Linear HO-$[LA]_n$-H	4	x	x	x	x
5.77	285.0944	$C_{11}H_{18}O_7$	Linear CH_3-CH_2-O-$[LA]_n$-H	3		x	x	
5.87	401.1053	$C_{15}H_{22}O_{11}$	Linear HO-$[LA]_n$-H	5	x	x	x	x
6.26	357.1155	$C_{14}H_{22}O_9$	Linear CH_3-CH_2-O-$[LA]_n$-H	4		x	x	
6.27	473.1264	$C_{18}H_{26}O_{13}$	Linear HO-$[LA]_n$-H	6	x	x	x	x
6.41	383.0948	$C_{15}H_{20}O_{11}$	Cyclic $[LA]_n$	5	x			
6.58	545.1475	$C_{21}H_{30}O_{15}$	Linear HO-$[LA]_n$-H	7	x	x	x	x
6.61	429.1366	$C_{17}H_{26}O_{11}$	Linear CH_3-CH_2-O-$[LA]_n$-H	5		x	x	
6.80	455.1159	$C_{18}H_{24}O_{12}$	Cyclic $[LA]_n$	6	x			
6.82	617.1686	$C_{24}H_{34}O_{17}$	Linear HO-$[LA]_n$-H	8	x	x	x	x
6.94	501.1577	$C_{20}H_{30}O_{13}$	Linear CH_3-CH_2-O-$[LA]_n$-H	6		x	x	
7.02	689.1897	$C_{27}H_{38}O_{19}$	Linear HO-$[LA]_n$-H	9	x	x	x	x
7.07	527.1370	$C_{21}H_{28}O_{14}$	Cyclic $[LA]_n$	7	x			
7.18	761.2108	$C_{30}H_{42}O_{21}$	Linear HO-$[LA]_n$-H	10	x	x	x	x
7.30	599.1581	$C_{24}H_{32}O_{16}$	Cyclic $[LA]_n$	8	x			
7.31	573.1788	$C_{23}H_{34}O_{15}$	Linear CH_3-CH_2-O-$[LA]_n$-H	7		x	x	
7.32	833.2319	$C_{33}H_{44}O_{23}$	Linear HO-$[LA]_n$-H	11	x	x	x	x
7.32	645.1999	$C_{26}H_{38}O_{17}$	Linear CH_3-CH_2-O-$[LA]_n$-H	8		x	x	
7.44	671.1792	$C_{27}H_{36}O_{18}$	Cyclic $[LA]_n$	9	x			

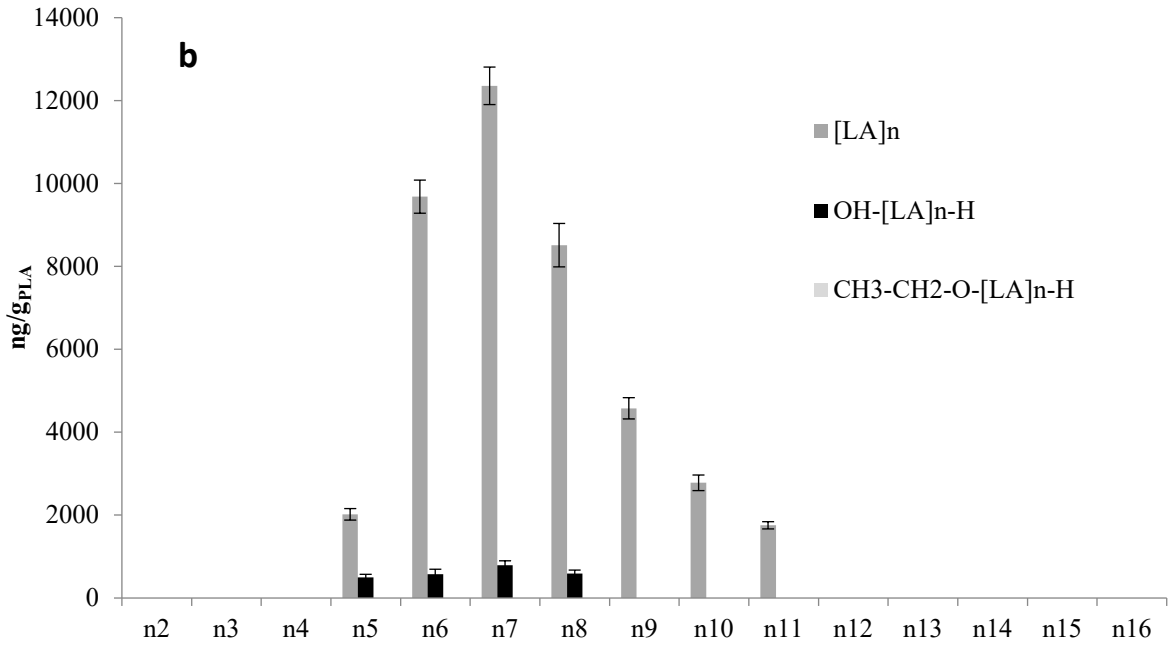
7.44	905.2530	$C_{36}H_{48}O_{25}$	Linear HO-$[LA]_n$-H	12	x	x	x	x
7.45	717.2210	$C_{29}H_{42}O_{19}$	Linear CH_3-CH_2-O-$[LA]_n$-H	9		x	x	
7.53	977.2741	$C_{39}H_{52}O_{27}$	Linear HO-$[LA]_n$-H	13	x	x	x	x
7.56	789.2421	$C_{32}H_{46}O_{21}$	Linear CH_3-CH_2-O-$[LA]_n$-H	10		x		
7.58	743.2003	$C_{30}H_{40}O_{20}$	Cyclic $[LA]_n$	10	x			
7.60	1049.2952	$C_{42}H_{56}O_{29}$	Linear HO-$[LA]_n$-H	14	x			
7.64	861.2632	$C_{35}H_{48}O_{23}$	Linear CH_3-CH_2-O-$[LA]_n$-H	11		x		
7.72	1121.3163	$C_{45}H_{60}O_{31}$	Linear HO-$[LA]_n$-H	15	x			
7.73	933.2843	$C_{38}H_{52}O_{25}$	Linear CH_3-CH_2-O-$[LA]_n$-H	12		x		
7.75	815.2214	$C_{33}H_{44}O_{22}$	Cyclic $[LA]_n$	11	x			
7.80	1005.3054	$C_{41}H_{56}O_{27}$	Linear CH_3-CH_2-O-$[LA]_n$-H	13		x		
7.84	887.2425	$C_{36}H_{48}O_{24}$	Cyclic $[LA]_n$	12	x			
7.88	1077.3265	$C_{44}H_{60}O_{29}$	Linear CH_3-CH_2-O-$[LA]_n$-H	14		x		
7.93	1149.3476	$C_{47}H_{64}O_{31}$	Linear CH_3-CH_2-O-$[LA]_n$-H	15		x		
7.98	959.2635	$C_{39}H_{52}O_{26}$	Cyclic $[LA]_n$	13	x			
8.05	1031.2846	$C_{42}H_{56}O_{28}$	Cyclic $[LA]_n$	14	x			
8.12	1103.3057	$C_{45}H_{60}O_{30}$	Cyclic $[LA]_n$	15	x			
8.16	1175.3268	$C_{48}H_{64}O_{32}$	Cyclic $[LA]_n$	16	x			


a
[LA]6
455.1159
MNa+
217.0709
145.0503
89.0600
383.0943
289.0950
2: TOF MS ES+
9.44e4
100
%
0
m/z
60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 460


b
HO-[LA]6-H
2: TOF MS ES+
2.91e4
100
%
0
473.1264
MNa+
401.1026
369.1518
329.0841
217.0709
145.0503
89.0600
m/z
60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 460 470


c
CH3-CH2-O-[LA]6-H
2: TOF MS ES+
2.80e4
501.1577
MNa+
481.2546
261.0780
217.0709
145.0503
89.0600
100
%
0
m/z
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 460 470 480 490 500


a
Area (Film)
Area (Pellets)
Cyclic (n5-n16)
Lineal (n4-n15)
0
2000
4000
6000
8000
10000
12000
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000


b
ng/g PLA
[LA]n
OH-[LA]n-H
CH3-CH2-O-[LA]n-H
0
2000
4000
6000
8000
10000
12000
14000
n2
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n5
n6
n7
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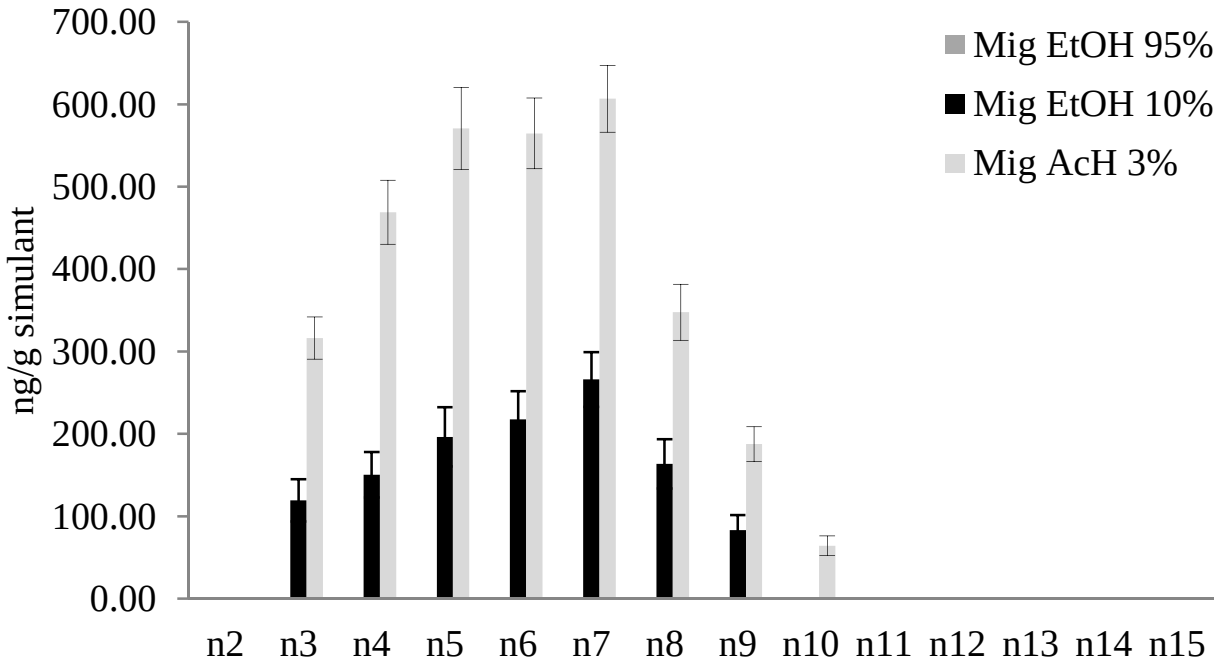
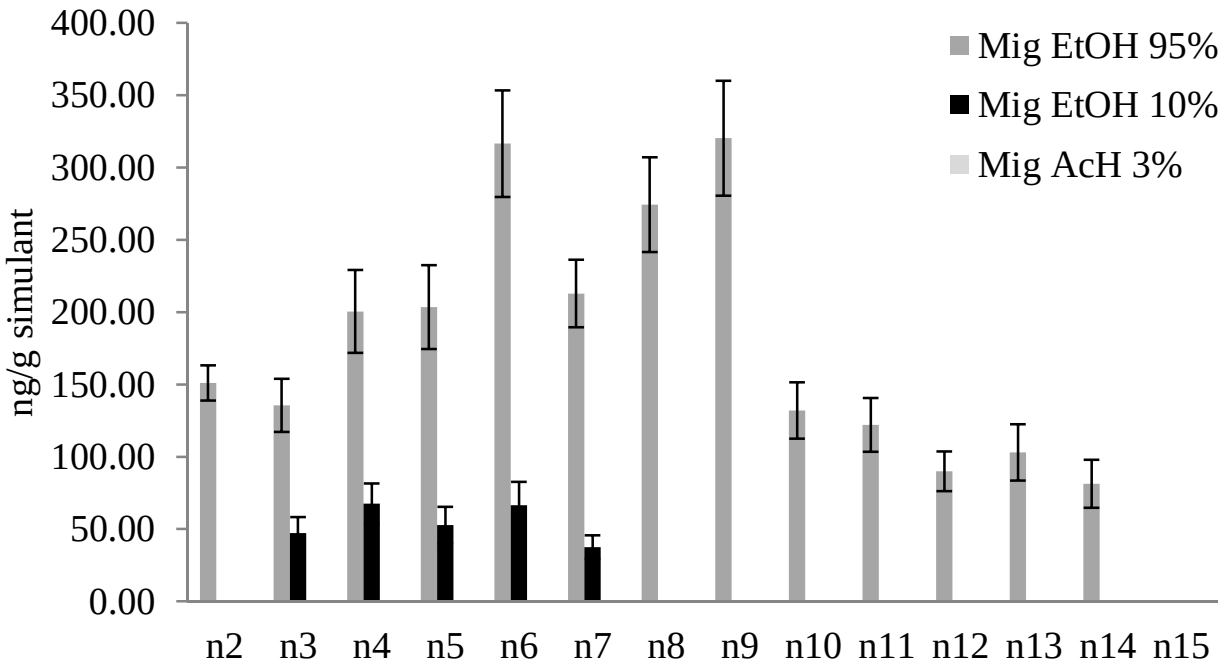
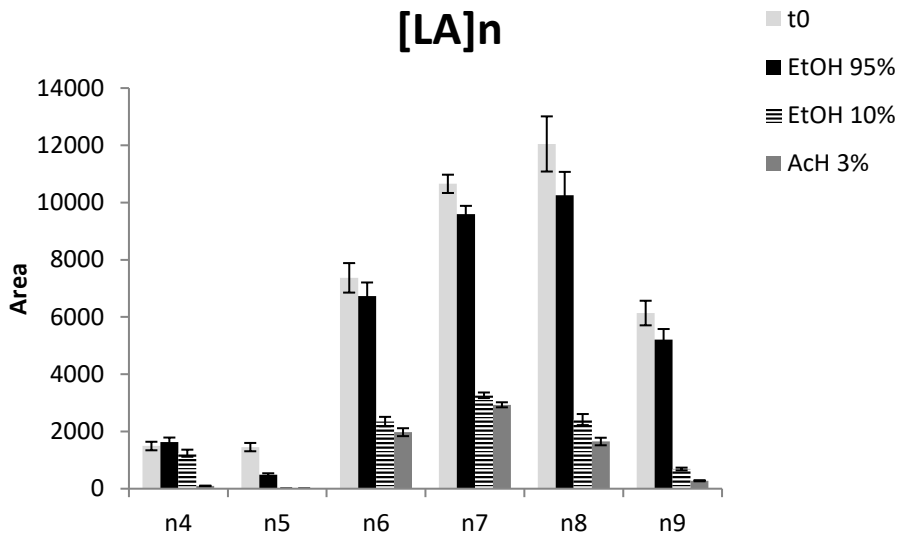
n9
n10
n11
n12
n13
n14
n15
n16

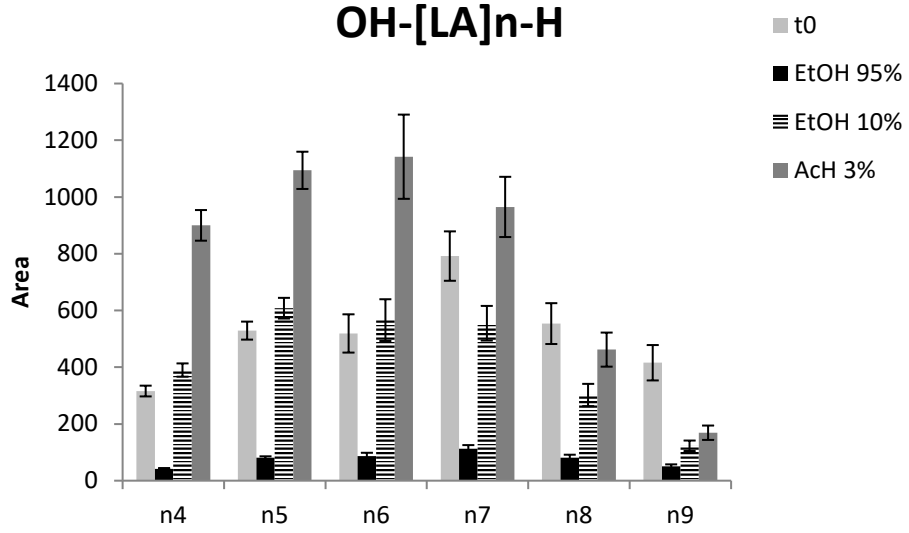

700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
ng/g simulant
Mig EtOH 95%
Mig EtOH 10%
Mig AcH 3%
n2
n3
n4
n5
n6
n7
n8
n9
n10
n11
n12
n13
n14
n15


400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
ng/g simulant
Mig EtOH 95%
Mig EtOH 10%
Mig AcH 3%
n2
n3
n4
n5
n6
n7
n8
n9
n10
n11
n12
n13
n14
n15

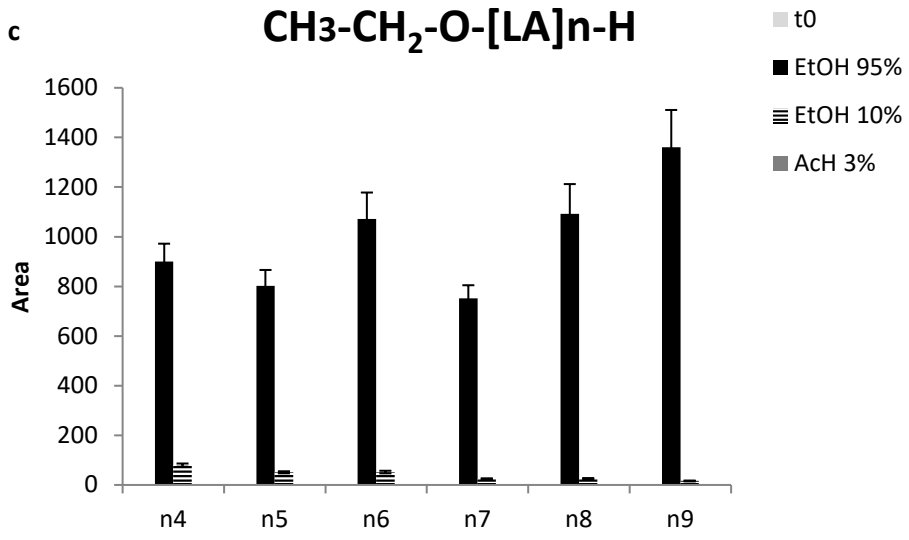
a

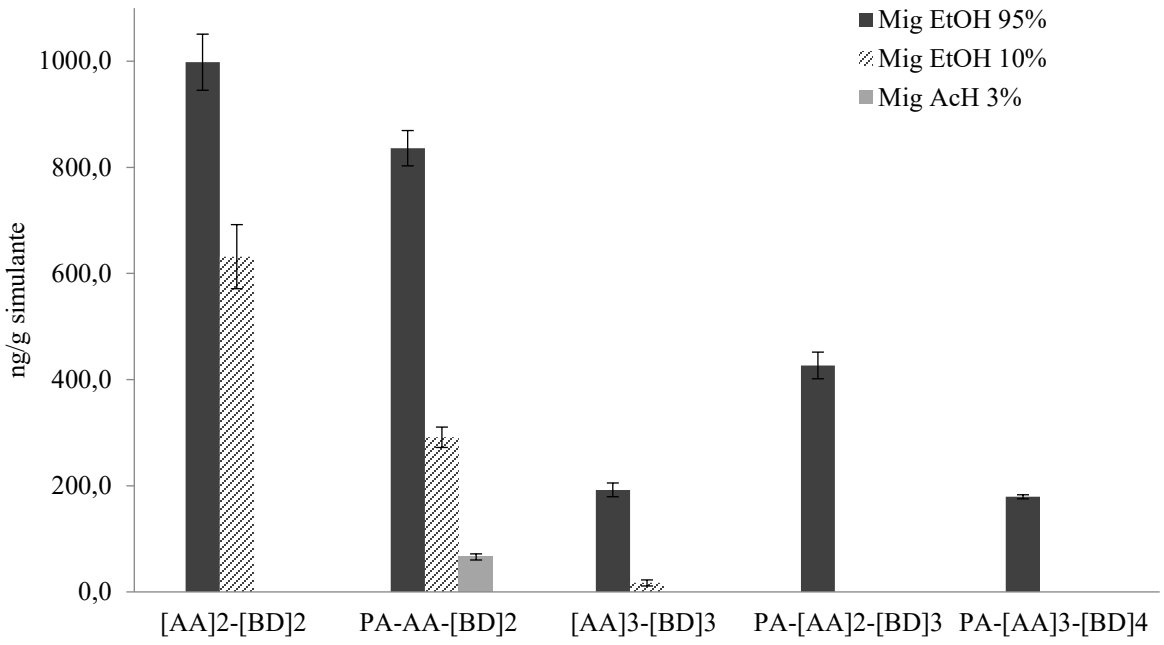

[LA]n
t0
EtOH 95%
EtOH 10%
AcH 3%
Area
14000
12000
10000
8000
6000
4000
2000
0
n4
n5
n6
n7
n8
n9

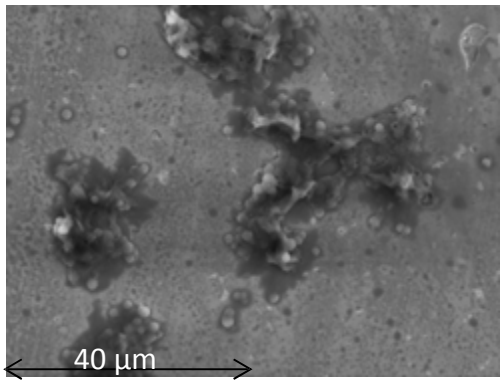
b


OH-[LA]n-H
t0
EtOH 95%
EtOH 10%
AcH 3%
Area
1400
1200
1000
800
600
400
200
0
n4
n5
n6
n7
n8
n9

c


CH3-CH2-O-[LA]n-H
t0
EtOH 95%
EtOH 10%
AcH 3%
Area
1600
1400
1200
1000
800
600
400
200
0
n4
n5
n6
n7
n8
n9


Mig EtOH 95%
Mig EtOH 10%
Mig AcH 3%
ng/g simulante
1000,0
800,0
600,0
400,0
200,0
0,0
[AA]2-[BD]2
PA-AA-[BD]2
[AA]3-[BD]3
PA-[AA]2-[BD]3
PA-[AA]3-[BD]4



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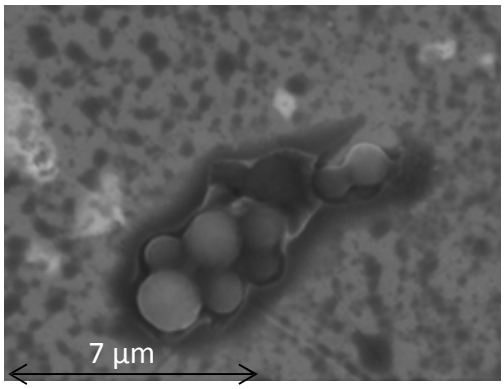
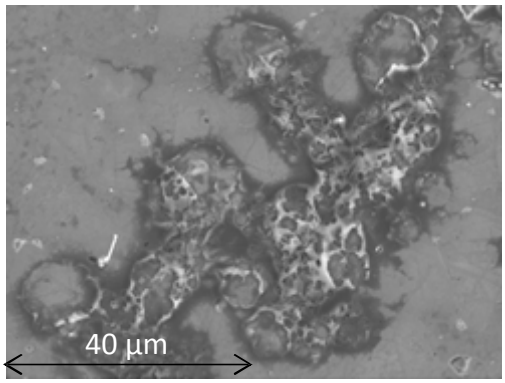
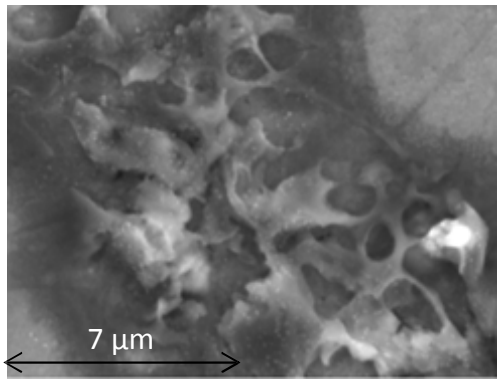


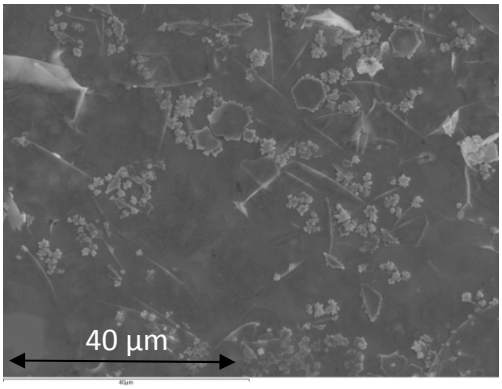
2-a



1-b



2-b



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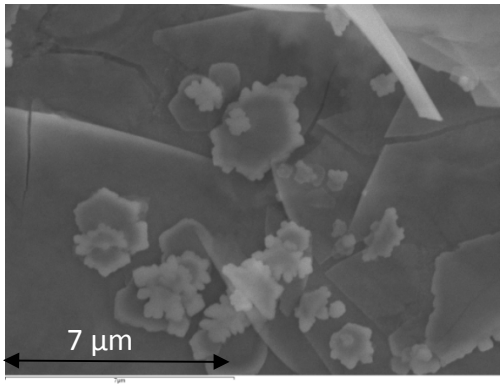

4