

Poly(lactic acid) nanoplastics through laser ablation: Establishing a reference model for mimicking biobased nanoplastics in aquatic environments*.

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* supplementary material available.

Abstract

Due to the well-documented negative environmental impacts of conventional plastics, the use of bioplastics has been increasing. Poly(lactic acid) (PLA) is currently among the most common and industrially available bioplastics. Although PLA is compostable under industrial conditions and generally degrades more quickly than conventional plastics, its breakdown in typical environmental settings remains problematic. PLA's potential to contribute to plastic pollution through the release of microplastics and nanoplastics makes it crucial to understand how these particles behave, especially in marine environments. However, as for all nanoplastics, identifying, isolating, and quantifying PLA nanoplastics in water presents significant challenges. This study proposes a versatile approach to fabricate PLA nanoplastics through laser ablation in a water environment to mimic real-world samples. Commencing with bulk PLA films, this top-down method yields the formation of nanoplastics with an average diameter of 54.7 ± 26.7 nm. Surface and chemical analyses confirm the presence of carboxylic groups on their surface, potentially resembling the environmental degradation pathway of PLA under exposure to sunlight and humid environments. This indicates that the proposed process results in a PLA nanoplastics system that serves as an invaluable reference model, enabling realistic environmental scenario explorations and simulations for risk assessment evaluations on bio-based nanoplastics.

Keywords: biopolymers; water pollution; environmental fate; photoaging

Introduction

Since the advent of the first synthetic polymer, plastics have proliferated exponentially, driven by mass production and rapid expansion. Among the most prevalent are thermoplastic polymers, like polyethylene terephthalate (PET), polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polycarbonate (PC), and polystyrene (PS), all derived from non-renewable petrochemical sources ¹. These polymers, engineered to be low-cost and highly adaptable, exhibit resilience against various environmental factors such as microbial degradation, light exposure, and water permeation. This durability makes them indispensable for everyday applications, such as packaging and biomedical. However, their longevity poses a significant environmental threat when they are exposed to the environment in the form of plastic litter ²⁻⁴.

In fact, according to the Global Plastics Outlook database published in 2022 by the Organization for Economic Co-operation and Development (OECD), only 9% of the produced plastic waste undergoes recycling, while 19% is incinerated. ⁵ The remaining amount is either deposited in landfills (50%) or not managed with any of the processes mentioned above (22%), ultimately finding its way into aquatic ecosystems, where it accumulates over time ⁶. Specifically, long-term projections suggest an alarming escalation in mismanaged plastic waste, reaching over 270 million metric tons by 2060 ⁵. Such projections underscore the urgent need to address the pervasive issue of plastic waste accumulation and the resulting profound ecological ramifications.

These concerns surrounding the widespread use of petroleum-based plastics and their enduring presence in the environment have prompted efforts to explore alternative and more environmentally friendly materials, such as biobased and biodegradable plastics. One such alternative gaining traction is poly(lactic acid) (PLA), a thermoplastic polymer lauded for its bio-based origin and biodegradability under controlled conditions. PLA presents a promising solution to mitigate the environmental impact of petroleum-based plastics, offering similar versatility, processability, and functionality as conventional counterparts for specific applications ⁷. In fact, due to all these properties, PLA has earned the title of the "polymer of the 21st century" ^{8, 9}. However, studies reveal that the biodegradability of PLA is limited to specific composting conditions, requiring an oxygen-rich environment under controlled temperatures (58-80 °C) and humidity (> 60%) ¹⁰. This raises concerns about its eventual persistence in natural environments, resembling its petroleum-based counterparts, particularly in marine settings where temperatures are lower ². PLA waste in the environment may undergo various degradation pathways such as photo and thermal-oxidation, hydrolysis, mechanical fragmentation, etc. ^{2, 11, 12} to form microplastics (MPs) and nanoplastics (NPs). NPs are plastic fragments of size <1000 nm ¹³, with altered chemical and structural properties compared to their bulk, and are considered contaminants of emerging concern ^{13, 14}. As PLA utilization increases, it is important to expand our knowledge of the formation pathways of PLA NPs, their presence in the environment, and their environmental fate.

Multiple very recent studies have been conducted to explore the possible release of PLA MPs and NPs from commercial products such as PLA-lined paper cups ¹⁵ and tea bags ¹⁶, as well as the formation of secondary fragments under UV photoaging,

mechanical, biological, and enzymatic degradation of PLA macroplastic components ¹⁷⁻
¹⁹. Despite confirming the release of PLA NPs under environmental stressors in well-controlled experimental conditions, studies on the presence of PLA NPs in environmental samples present a multifaceted research challenge. This stems from the diversity of the properties of plastic sources, usage patterns, and different waste management strategies linked to the disposal of plastics. Consequently, there are numerous emission pathways for MPs and NPs. Therefore, the properties of the generated particles may also reflect these diversities ²⁰⁻²³, which, in combination with the various interactions that NPs may have with various environmental components, result in NPs with a wide range of physical and chemical properties in actual field samples. On top of that, studies have shown that the environmental concentrations of NPs are extremely low, ranging between 0.04-1.17 mg/L (depending on the NPs and water type) ²⁴. Considering also the fact that the environmental samples usually contain a great variety of organic matter makes the NPs identification and quantification akin to searching for a needle in a haystack ²⁵.

Recently, studies have begun to shed light on the effects of PLA MPs and NPs on organisms, using either commercially available particles ^{26, 27} or PLA NPs prepared through bottom-up chemical methods such as solvent evaporation combined with mini-emulsion techniques ^{28, 29}. Nevertheless, these studies may not fully capture the characteristics of environmentally relevant NPs, which often exhibit irregular shapes and complex surface chemistry. Despite the extensive endeavors to create representative reference NPs models using top-down techniques like milling processes ^{30, 31} and laser ablation ^{32, 33}, based on current knowledge, these are mainly focused on conventional plastics. In fact, there appears to be a gap in such efforts concerning commonly used

PLA, highlighting the necessity to broaden research endeavors toward constructing more representative reference models for studying the fate of PLA NPs. Understanding their distinctive properties and environmental behavior holds profound benefits for ecosystem health and human well-being in the foreseeable future.

This study presents a versatile chemical-free approach to fabricating PLA NPs that closely mimic their expected properties in natural environmental conditions. Through laser ablation of a bulk PLA film immersed in water using an excimer pulsed laser with an excitation wavelength of 248 nm, PLA NPs with an average diameter of 54.7 ± 26.7 nm were successfully synthesized directly in the water medium. Chemical characterization of the resulting NPs reveals potential surface modifications consistent with the environmental degradation pathways of PLA. The as-fabricated PLA NPs serve as invaluable reference samples for studying the environmental fate of such particles and enable more realistic environmental assessments. Therefore, the presented outcomes contribute to advancing our understanding of PLA NPs and underscore the importance of considering bio-based polymeric fragments in environmental research.

Materials and Methods

Materials

Extruder grade PLA pellets (Grade: 2003D, Cas-No: 9051-89-2) were supplied from NatureWorks. UranylLess EM (electron microscopy grade) contrast stain solution used for transmission electron microscopy (TEM) studies was purchased from Delta Microscopies. Tris (hydroxymethyl) aminomethane hydrochloride salt (Trizma hydrochloride, Cas-No: 1185-53-1, pH 7.0-9.0, 0.002 M, purity $\geq 99\%$), phosphate buffered saline (Cas-No:

P3813, pH 7.4, 0.002 M), acetic acid (Cas-No: 64-19-7, purity $\geq 99.8\%$), sodium hydroxide (Cas-No: 1310-73-2, purity $\geq 98\%$), isopropanol (Cas-no: 67-63-0, purity $\geq 99.5\%$), as well as chloroform (CDCl_3 , Cas-no: 865-49-6, 99.9 atom % D) and deuterium oxide (D_2O , Cas-no: 7789-20-0, 99.9 atom % D) used for NMR analyses, were all purchased from Sigma-Aldrich.

Fabrication of PLA Films

To prevent interaction with air and moisture, the PLA pellets were initially dried in a vacuum oven at 60°C for 24 hours. Subsequently, the pellets were processed using an IKA Pilotina MC dry miller equipped with a sieve with a pore size of 3 mm. The resultant PLA powder, with a particle size of ≤ 3 mm, underwent an additional vacuum drying step under identical conditions, as previously referred, to ensure optimal dryness before film preparation. PLA films were then fabricated following a compression molding process using a Carver 4122 hot press. Precisely, 12 mg of the ground PLA powder were placed between two metal frames lined with Teflon sheets to prevent adhesion during the pressing process. This assembly was subsequently introduced between hot plates preheated to 180°C . Initially, the polymer was allowed to melt for 5 minutes without any applied pressure. Following this, a pressure of 4 metric tons was applied for 10 minutes to ensure proper molding and cohesion of the PLA. Once the pressing cycle was completed, the plates were left to naturally cool down to room temperature, forming the desired flat PLA films with a thickness of 1 mm and an area of 100 cm^2 .

Fabrication of PLA NPs by laser ablation

Before laser treatment, the produced PLA films were meticulously cleaned using isopropanol, followed by Milli-Q water washing steps. The laser ablation was executed by exposing a 6 cm² segment of the PLA film immersed in 8 mL of Milli-Q water to pulsed UV laser irradiation (Supplementary data Figure S1) using a KrF excimer laser (irradiation wavelength 248 nm, pulse duration of 20 ns, repetition rate of 10 Hz, Coherent-CompexPro 110) coupled with a micromachining apparatus (Optec-MicroMaster). The laser ablation was performed by 50 pulses, at a fluence of 3.5 J/cm², to produce an ablation spot of 450 × 450 μm. One complete ablation cycle consists of 144 ablation spots, arranged in a 12 × 12 grid format (Supplementary data Figure S2). To obtain an ablation product with a high number of NPs, 32 ablation cycles were executed, and the resultant dispersion was concentrated using a rotavapor set at 45 °C. For the chemical characterizations of the NPs, the concentrated samples underwent centrifugation (utilizing a Sartorius, Centristat G-16C centrifuge) at 21,000 x g for a duration of 10 minutes at 23 °C. After removal of the supernatant, the precipitated material was resuspended in approximately 20 μL of Milli-Q water and subsequently used for further characterization.

NPs Characterization

The morphology of the NPs was examined using transmission electron microscopy (TEM) (Jeol JEM 1400 Plus) imaging. Before the analysis, the concentrated samples were sonicated for 1 minute to ensure the uniform dispersion of particles. After sonication,

samples were stained with UranylLess EM contrast stain solution for 30 seconds and were deposited onto a copper (Cu) grid pre-coated with an ultra-thin layer of amorphous carbon film, ensuring optimal contrast and visualization during TEM analysis. Multiple images were captured and analyzed using the ImageJ and OriginPro 2020 software to obtain the size distribution of the observed NPs.

The hydrodynamic size distribution and the concentration of the formed NPs were quantified using nanoparticle tracking analysis (NTA) with the Malvern NanoSight Pro 300 (Malvern Panalytical), equipped with the NTA software version 3.4. Samples from different ablation cycles were analyzed to compare the repeatability across the ablation cycles. For this analysis, a volume of 1 mL from the vortexed sample was introduced into the NTA system via a syringe pump, operating at a rate of 50 AU. The particles were detected by blue laser ($\lambda = 488$ nm) camera-type sCMOS, providing the requisite sensitivity and resolution for accurate particle tracking and quantification.

Zetasizer Nano ZS (Malvern Instruments) was used to assess the surface charge characteristics of the produced NPs across varying pH values. To obtain the necessary pH values, aliquots of 300 μ L of the ablation product were mixed with 700 μ L of the different buffers prepared from tris(hydroxymethyl)aminomethane hydrochloride salt (Trizma hydrochloride) in Milli-Q water (pH 9–8, 0.002 M), phosphate buffer saline in Milli-Q water (pH 7–6, 0.002 M), and diluted acetic acid (0.002 M) in Milli-Q water with sodium hydroxide (pH 5–3, 0.002 M).

The NPs, concentrated through rotavapor and centrifugation, were resuspended in Milli-Q water and carefully drop-casted onto pre-cleaned and dried silicon wafer for Raman spectroscopy analysis (Supplementary data Figure S3). For a comparative

analysis, a pristine PLA film, cleaned with isopropanol and Milli-Q, was also examined. The Raman spectra were acquired utilizing the Renishaw inVia confocal Raman microscope, equipped with a CW DPSS laser with a wavelength of 532 nm. The spectral data were captured over the 600-3100 cm^{-1} range under the confocal settings using the 150x objective, with an accumulation time of 120 seconds at a laser power density of 50 kW/cm^2 . After data acquisition, data analysis and processing were executed using the built-in Wire 5.5 software platform.

The surface composition of NPs was elucidated through X-ray photoelectron spectroscopy (XPS) using a Kratos Axis UltraDLD instrument. The concentrated NPs were drop-casted on a precleaned and dried indium (In) substrate. A comparative analysis was also performed on the PLA film, which was cleaned with isopropanol and followed with Milli-Q water. A monochromatic Al $K\alpha$ source acquired both survey and high-resolution spectra, operating at 20 mA and 15 kV. The survey spectra were obtained with a pass energy set at 160 eV, an energy step of 1 eV, and an analyzed area spanning $300 \times 700 \mu\text{m}^2$. Concurrently, high-resolution spectra were recorded within the same designated area, utilizing a pass energy of 20 eV and an incremental energy step of 0.1 eV. The Kratos charge neutralizer system was consistently applied across all samples to ensure accurate data interpretation. Furthermore, for precise binding energy scale calibration, the position of the dominant C 1s peak was standardized at 285 eV, representative of C-C bond configurations. Subsequent data analysis and interpretation were conducted utilizing the Casa XPS software.

The NMR analysis was also performed on the ablation product to identify the molecular components and to confirm the chemical nature of the formed NPs. To allow the

characterization of the solid fraction and considering that polymer chains form this, the concentrated NPs (prepared following the same protocol explained previously) were resuspended in CDCl_3 and D_2O in a 1:1 ratio. The obtained dispersion was subsequently centrifuged for 30 minutes at 3200 rpm to separate the D_2O part from the CDCl_3 and separately collect the two phases. The CDCl_3 can dissolve PLA and is expected to contain all the molecular polymeric components in its phase. In contrast, the D_2O phase may contain the polar molecular components of the ablation product distributed in the water medium during the ablation process. For the comparative studies, 5 mg of pristine PLA was dissolved in 500 μL of CDCl_3 and was vortexed for 30 minutes. The ^1H NMR spectra of the PLA film and the ablation product in CDCl_3 were recorded using Bruker FT NMR Neo 600 MHz spectrometer, equipped with a 5 mm CryoprobeTM QCI $^1\text{H}/^{19}\text{F}$ $^{13}\text{C}/^{15}\text{N}$ -D and an automatic sample changer, SampleJetTM, with temperature control. The spectra were collected with the Bruker zg30 sequence, with a 30-degree flip angle, TD 64 K, a spectral width of 30 ppm, an acquisition time of 1.835 seconds, a number of 2048 scans, and a relaxation delay of 4 seconds.

Results and Discussion

As shown in Figure 1 (a), the TEM analysis of the concentrated ablation product reveals the presence of spherical-like particles and some particles of irregular shape. The particle size distribution presented in Figure 1 (b) shows that particles have a nanoscale size ranging between 10-125 nm, with an average diameter of 54.7 ± 26.7 nm. TEM

images were captured from different spots across the sample (Supplementary data Figure S4) to ensure an objective size analysis.

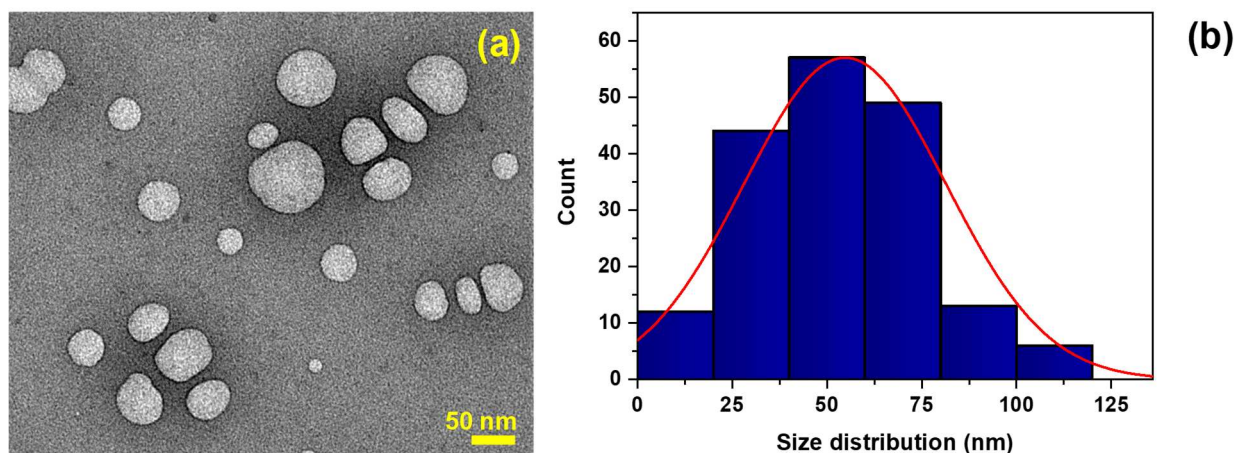


Figure 1. (a) TEM micrograph of the formed nanoparticles. (b) The size distribution of nanoparticles was calculated from TEM micrographs using ImageJ software on a total of 180 particles.

To further explore the size distribution and quantify the number of particles in the ablation product, the NTA analysis was employed. As revealed by the study, each ablation cycle yielded around $1.29 \times 10^9 \pm 6.46 \times 10^7$ particles/mL. Furthermore, as depicted in Figure 2, in agreement with the TEM study, the produced nanoparticles have heterogeneous size distribution, with most of them presenting hydrodynamic sizes ranging between 50-200 nm, with a mean size of around 127.4 ± 42.9 nm in the aqueous medium. Furthermore, a substantial proportion, encompassing about 90% of the particles, has diameters not exceeding 185 nm. In contrast, a minor fraction, constituting 10% of the particles detected, exhibited a hydrodynamic diameter of less than 75 nm. To explore if the adopted method results in a reproducible fabrication process, the NTA analysis was

performed on samples from different ablation cycles. As shown in Table S1 of the Supplementary data file, the concentration and size distributions were comparable across NPs produced from different ablation cycles, confirming the repeatability of the fabrication process.

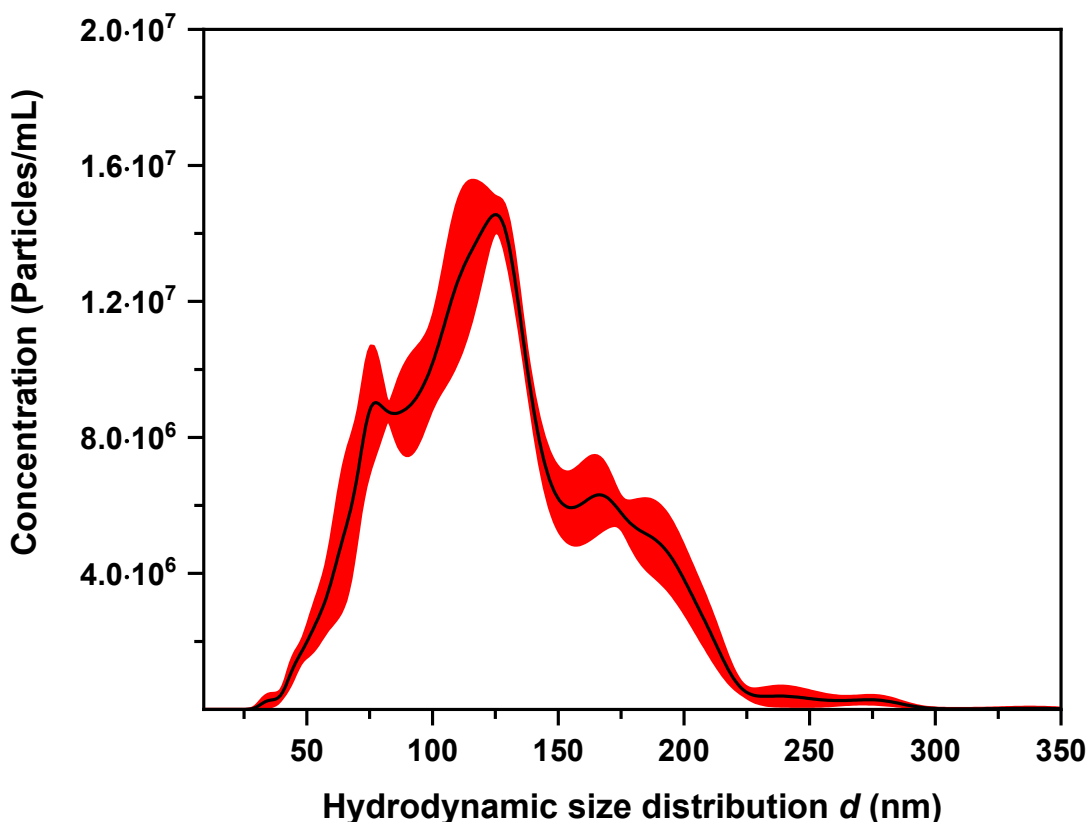


Figure 2. The total concentration of the NPs produced in the aqueous dispersion and their hydrodynamic size distribution derived from NTA analysis as observed from five consecutive measurements on the same sample, with the black line representing the mean hydrodynamic diameter and the red showing the standard deviation.

The TEM and NTA analyses confirm the formation of nanoparticles as the result of interaction between the high-energy UV laser beam and the polymeric surface. The

formation of these particles, starting from the polymer film immersed in water, may be attributed to one of the two main ablation pathways: condensation or fragmentation. Condensation is a purely thermal pathway that follows the atomization of polymeric matter upon photon absorption. The carbon vapor formed during the heating phase condenses upon saturation in the plume, primarily forming carbon-based nanoparticles.³⁴ On the other hand, fragmentation is a mechanical effect attributed to the hydrodynamical expansion of the heated volume of the polymer target. It breaks the polymeric material into small particles due to the rapid thermoelastic expansion and tensile. Therefore, it may be a low-temperature pathway preserving the chemical structure of the polymer, mainly resulting in the formation of polymer-like nanoparticles.³⁴ It is essential to note that the properties of the resulting particles are intricately linked to the inherent characteristics of the polymer and the specific operational parameters of the laser system employed.^{35, 36} It should also be emphasized that there is no ionization and decomposition of water, as it is less likely to happen in a nanosecond (ns) laser processing underwater³⁷.

To evaluate the chemical nature of the formed particles, Raman analysis was performed on the ablation product, and the obtained spectrum was compared with that of the pristine PLA film. As shown in Figure 3, the Raman spectrum of pristine PLA depicts all the characteristic peaks of the PLA³⁸, with the prominent peaks at 873 and 1768 cm^{-1} to indicate the out-of-phase C-O-C and carbonyl stretching of the polymer chain, respectively. The two peaks at 1044 and 1129 cm^{-1} denote the C-CH₃ bond stretch³⁸, while the vibrational signatures associated with the symmetric and asymmetric deformation modes of CH₃ groups are visible at 1392 cm^{-1} and 1455 cm^{-1} , respectively. Furthermore, the symmetric and asymmetric stretching modes of CH₃ groups were

observed at 2944 cm^{-1} and 3002 cm^{-1} , respectively ³⁹. Finally, the symmetric stretching mode of the C-H bonds is evident at 2866 cm^{-1} . A comprehensive analysis of the ablation product spectrum underscores that the generated particles retain all the distinctive peaks representative of the PLA polymer, proving that the formed particles have the PLA polymeric nature, suggesting the formation of NPs through the fragmentation ablation pathway. Interestingly, additional peaks also appear, postulated to arise from the degradation effects induced by the laser ablation of the film in water, targeting the ester bonds within the PLA matrix, confirming the formation of different chemical groups not initially part of the PLA polymer ⁴⁰. Specifically, a closer look at the Raman spectrum shows additional peaks between the 1620-1680 cm^{-1} range, which can be attributed to C=C and the out-of-the-phase stretching of carboxylic groups ⁴¹. The appearance of the double-bonded carbon peak indicates the presence of alkenes or cyclic compounds ⁴².

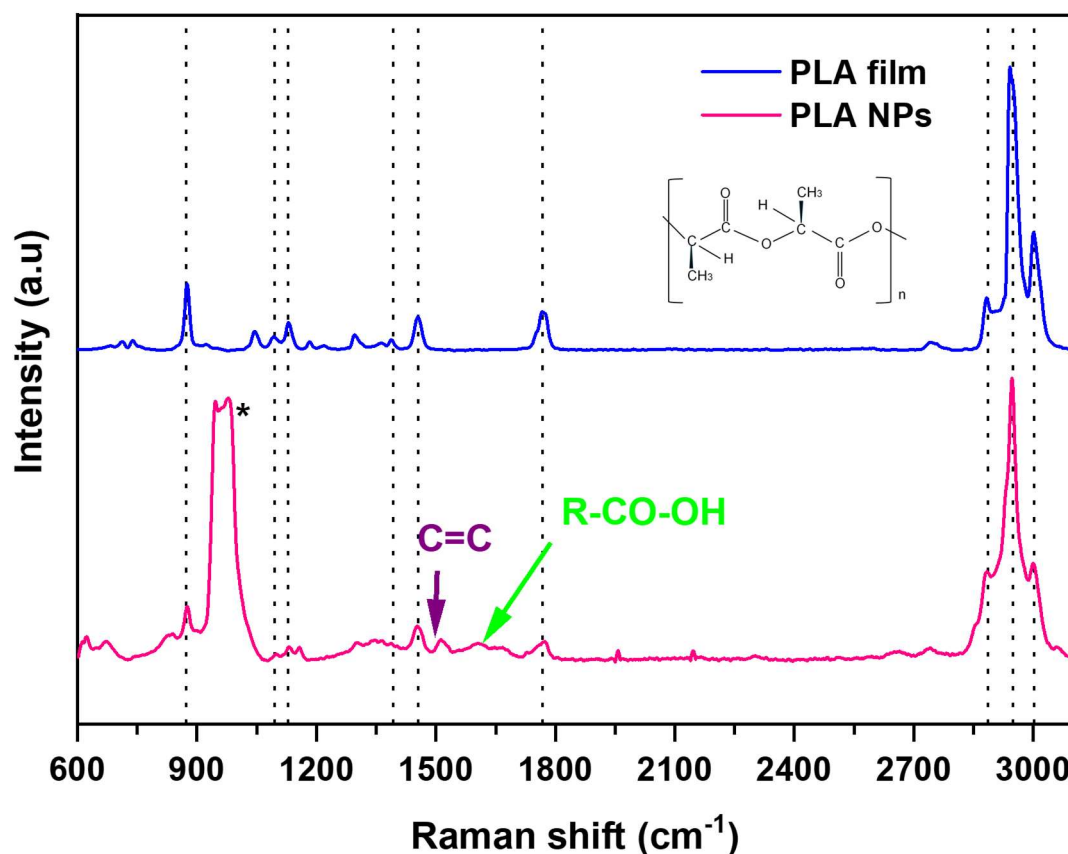


Figure 3. The comparative Raman spectra of pristine film and the PLA NPs. The * corresponds to the Si peak, the substrate on which samples were deposited for the analysis.

The presented results so far effectively confirm the formation of PLA NPs upon laser ablation of PLA films. Nonetheless, NPs originating from different environmental degradation pathways are proven to comprise diverse surface chemistry^{14, 43}. To explore the surface properties of the formed PLA NPs and any resemblance with those anticipated during the aging of environmentally exposed samples, the surface charge and the XPS analyses of the NPs were performed. The zeta potential of the as-generated PLA NPs was -37.6 ± 4.6 mV in the water dispersion after the laser ablation process, with the pH ranging between 6.5-7, in agreement with what was observed by Banaei et al.¹⁶ in PLA

NPs (~ 280 nm) released from commercial tea bags, and Zhang et al.⁴⁴ on photo-aged PLA NPs. Through the pH modification of the NPs medium, it can be observed that the zeta potential increases in absolute values as the pH increases. These changes are more evident in the 5-8 pH range. This behavior is characteristic of the presence of weak acidic groups on the NPs surface, possibly arising from the surface oxidation of the PLA and cleavage of polymeric chains induced upon exposure to UV-pulsed laser irradiation during ablation⁴⁵. Such processes can form weak acidic groups on the NPs surface, imparting a negative surface charge at the NP-water interface⁴⁶. As shown in Figure 5, by increasing the pH of the solution, the deprotonation of the weak acidic groups leads to the formation of a more negative surface charge. On the contrary, these groups undergo protonation under acidic conditions, shifting the surface charge towards more positive values.

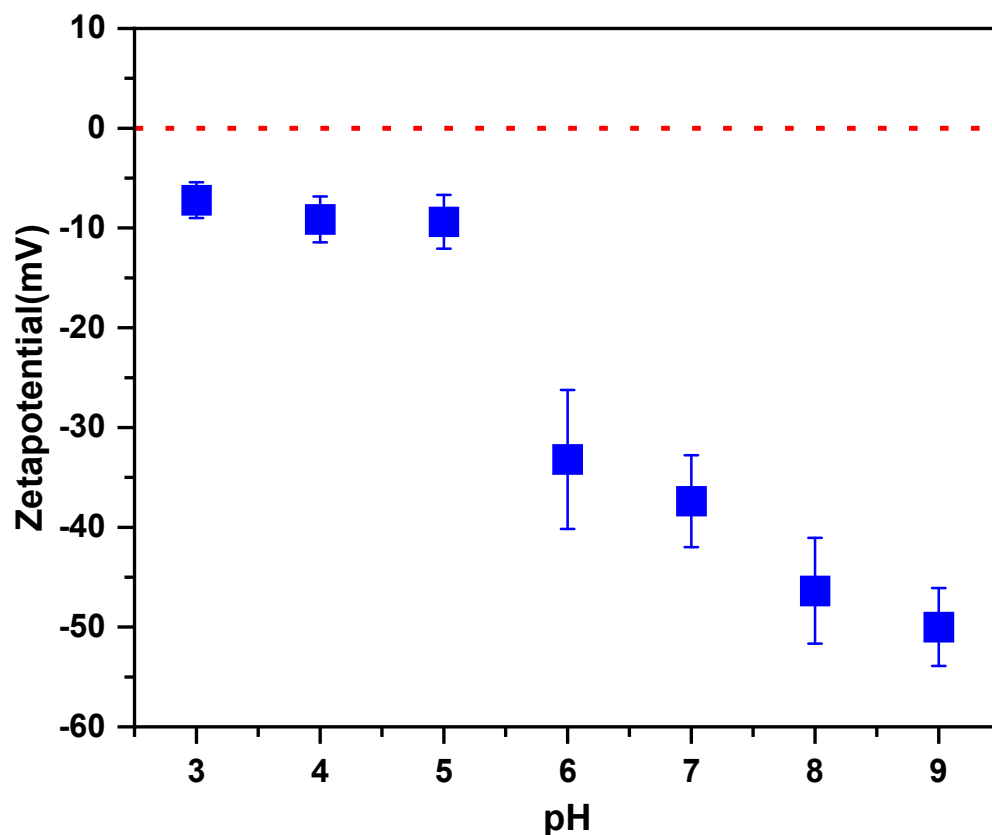


Figure 4. Zeta potential of the NPs in aqueous environments of different pH values.

The surface chemistry of the produced PLA NPs was also explored by XPS. As shown in Figure 5 (a), the deconvolution of the C1s of the pristine PLA film indicates the contribution of three characteristic peaks at 285.0, 287.0, and 289.0 eV attributed to the $\underline{\text{C}}\text{-C}$ or to the $\underline{\text{C}}\text{-H}$ bonds, to the $\underline{\text{C}}\text{-O-C=O}$, and to the $\text{O}=\underline{\text{C}}\text{-O}$ bonds respectively ⁴⁷ (also refer supplementary data S2). In the case of the PLA NPs, as in Figure 5 (b), in addition to the characteristic peaks of PLA, two additional peaks at 285.7 and 287.2 eV appear, corresponding to the hydroxyl (-COH) and carboxyl (-COOH) polar functional groups, confirming the observations of the zeta potential analysis. Analogous changes were observed in PLA films treated with ns (248 nm) and fs (515 nm) laser pulses, ^{48, 49}, with UV lamps ⁵⁰, and environmental factors (light, humidity, and temperature) ⁵¹, indicating

that these changes can be attributed to the aging of PLA under the influence of laser irradiation in the presence of water.

On the other hand, the O1s spectra of pristine PLA film and PLA NPs presented in Figure 5(c),(d) possess two characteristic peaks at 532.2 and 533.2 eV corresponding to the ester ($\text{O}=\text{C}-\text{O}-\text{C}$) and ether ($\text{C}-\text{O}-\text{C}$) in the main polymer chain (please refer also to Table S2, Supplementary data).⁴⁷ In the deconvolution of the O1s, the atomic percentages (Supplementary data Table S3) of C-O decrease while C=O increases in the PLA NPs compared to the pristine PLA. These changes and the presence of additional surface groups indicate the modifications in PLA due to the adopted laser ablation process.

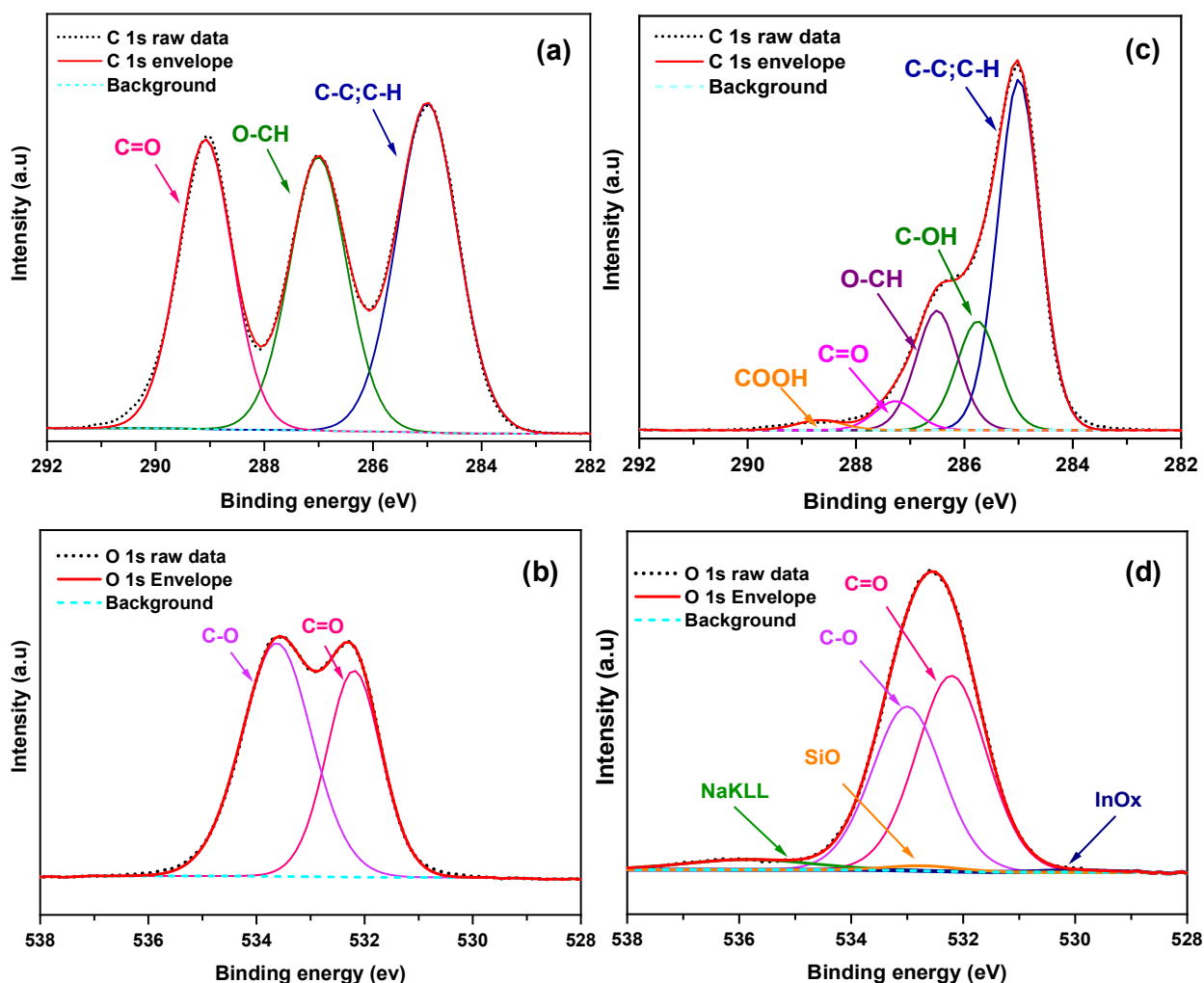


Figure 5. XPS spectra; (a-b) C 1s and O 1s spectra of PLA film. (c-d) C 1s and O 1s spectra of PLA NPs. Additional oxygen bonds are attributed to the substrate (InOx) and possible impurities during the sample preparation process (see also Supplementary data Figure S5).

To further investigate the chemical nature of the formed particles in the water, NMR analysis was also performed. According to Figure S6 in the supplementary data, the NMR spectrum of the pristine PLA film dissolved in CDCl_3 is in accordance with what was reported in the literature^{52, 53}. Some additional signals observed can be attributed to the different forms of the PLA, such as poly(L-PLA), poly(rac-PLA), isotactic PLA, or

syndiotactic PLA ^{46, 52}. Similarly, as shown in Figure 6(a), the NMR spectrum of the ablation product in CDCl₃ resembles the spectrum of the pristine PLA film, confirming, as in the case of the Raman study, that the formed NPs are of PLA nature ⁵³. Additional peaks attributed to the D₂O residue and the CDCl₃ solvent are also present in the spectrum of PLA NPs. Furthermore, a peak attributed to formic acid is also evident, in agreement with Theriasis et al., who also detected formic acid in minor proportions after the photo-oxidation of PLA along with other low molecular weight photo products ⁵⁴, as well as with what was observed by Magri et al. ³² in PET NPs fabricated by laser ablation. In the aliphatic region of the spectrum (Figure 6(b)), other than the expected signals corresponding to PLA, many additional signals appear, which could be attributed to the possible presence of different chain lengths and forms of PLA. Interestingly, as depicted in Figure 6(c), some unexpected signals relating to the aromatic molecules were observed (Supplementary Figure S7), which could be attributed to the production of aromatic molecules due to the possible thermal degradation of PLA [44], as reported previously. In particular, Lomakin et al., evaluating the photodegradation of PLA under UV radiation, observed weak signals corresponding to the double bond of the C=C-C(O)O- group ⁵⁵, which were absent in the pristine PLA. However, in the present case, the concentration of these substances is too low to allow their identification through 2D NMR experiments and/or ¹³C NMR analysis. To sum up, the NMR analysis of the ablation product confirms that the NPs formed by laser ablation are indeed PLA-based NPs, preserving the main structural fragments of PLA along with different polymeric units of varying chain lengths and molecular structures due to the possible degradation process during their formation.

The NMR analysis of the D₂O fraction (supplementary Figure S8) showed the presence of lactic acid; the different NMR signals could be due to the presence of various forms of lactic acid, like monomer, dimer, and trimer. Also, we observed different solvent residual impurities in this spectrum, like ethanol, methanol, acetone, and acetic acid, at different concentrations, probably due to the sample handling while using rotavapor. Only the NMR signals of formic acid and CDCl₃ are visible in the aromatic region.

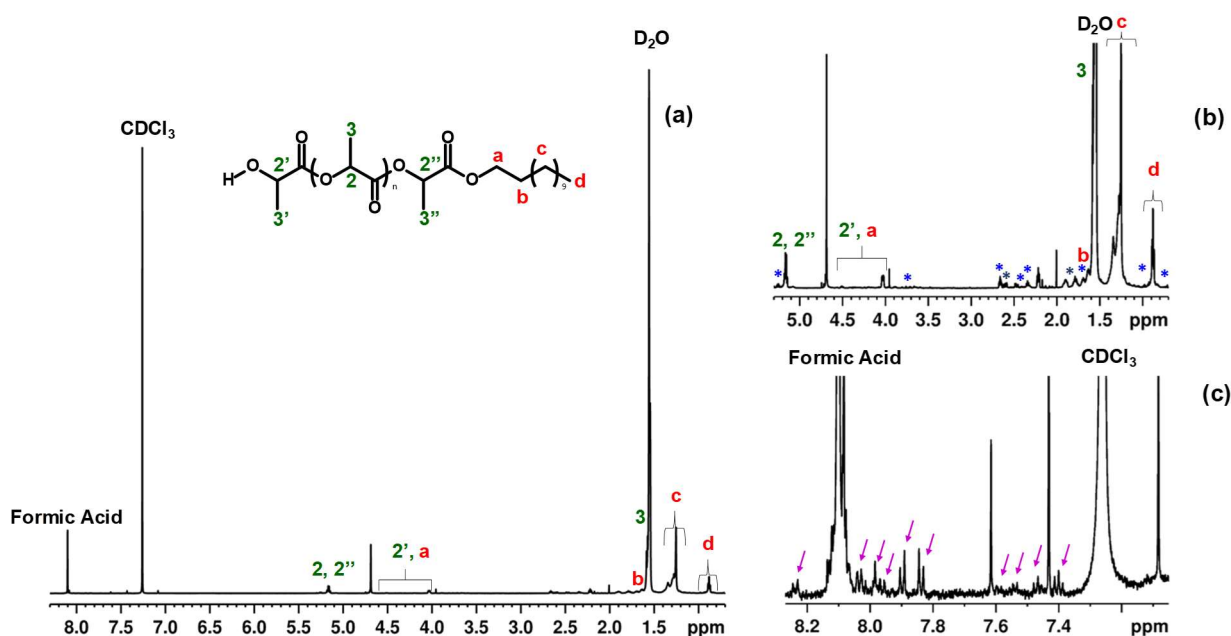


Figure 6. (a). The ¹H NMR spectra of the PLA NPs in CDCl₃. Inset: the molecular structure of PLA. The numbers indicate the methyl (-CH₃) and methenyl (=CH-) protons backbone, and the letters correspond to the protons of the lactic acid unit bearing the hydroxyl end group. (b). Zoom-in of the aliphatic region; blue (*) indicates possible signals of PLA of different chain lengths. (c). Zoom-in of the aromatic region; purple arrows indicate some signals of aromatic compounds.

The so-far performed analysis of the formed NPs reveals their PLA chemical nature and a significant surface chemistry modification. The low concentration of the potential by-products hindered the ability to conduct supporting analyses necessary for defining a specific degradation mechanism during the adopted fabrication process. It should be noted that further studies are necessary to fully elucidate the byproducts to gain a comprehensive understanding of the extent and precise mechanisms underlying these degradation pathways of PLA NPs formed through laser ablation.

However, it is essential to note that the typical environmental degradation pathways of PLA have been well-established in the literature ^{2, 10, 42}. Excluding biodegradation, according to Figure 7, the degradation of PLA involves hydrolysis (pathway A), photo-oxidation (pathway B), thermo-oxidation (pathway C), or a combination of these processes. ².

According to pathway (A), the hydrolytic degradation of PLA under the influence of water and heat occurs via chain cleavage of ester groups, preferentially in the amorphous regions, ⁵⁶ leading to an increase in the concentration of carboxylic acid end chains. These carboxylic end groups and their oligomers can catalyze the breakage of ester linkage during hydrolysis, forming acid groups. In the present study, the detection of additional polar groups (-COOH and -COH) on the surface of the NPs, along with their negative charge, could be indicative of the formation of carboxylic end-group compounds due to the hydrolytic degradation of the PLA during ablation.

In addition, considering pathway (B), the photo-oxidative degradation of PLA happens via the dissociation of C-O and C=O bonds in the presence of UV radiation below 280 nm. ⁵⁷ The photodegradation of PLA mainly follows the Norrish type II mechanism, in

which an excited state carbonyl abstracts an α -H to yield carboxylic acid and vinyl chain ends groups. The main chain scission in PLA can also lead to the formation of C=C bonds, where the electron transition at C=O triggers the reaction ⁵⁸. Lomakin et al. have also reported a similar formation of C=C bonds with the participation of CH₃ groups associated with the photodissociation of PLA following the Norrish II mechanism ⁵⁵. However, the weak signals corresponding to these peaks in the analyses could indicate the weakly expressed nature of this mechanism. In addition to Norrish type II products, hyperperoxide intermediates can also be formed from the activation of the methine group of the PLA backbone and the insertion of O₂, which can subsequently degrade to carboxylic acids, aldehydes, and diketones ⁵⁹. The presence of carboxylic acid groups on the NP surface observed in Raman and XPS analyses, along with the C=C bonds in the Raman spectrum and the NMR analysis, could indicate the photodegradation pathway of PLA upon interaction with a UV laser.

Finally, PLA can also undergo thermal degradation during laser ablation, predominantly by random chain scissions through non-radical reactions along the polymer backbone, as shown in the pathway (C). Depending on the point in the backbone at which the reaction occurs, the final product could be a lactide molecule, an oligomeric ring with more than two repeat units, or acetaldehyde plus carbon monoxide ^{42, 60-62}. In addition, the presence of oxygen can also induce thermo-oxidative degradation, inducing chain scission mechanisms, the formation of free radicals, and ester and carbonyl groups. ¹⁰. PLA is also reported to form a series of oxidation, fracture, and double bond products after photoaging under low-temperature thermal degradation conditions, including molecular chain fracture, depolymerization, and inter and intramolecular exchange reactions ⁶³. As

observed in the NMR analysis of the NPs, the presence of very weak peaks at the aromatic region may be, indeed, due to the formation of aromatic molecules (like O-phthalic acid)⁴² as a result of thermo-oxidative degradation, as mentioned above. Overall, from the presented analyses, it may be concluded that the degradation process is a combined mechanism of all three pathways. This leads to the aging of the polymer, causing deterioration of chemical properties⁶⁴ by the chain disruption at the ester group and the formation of small-sized compounds and other molecules⁸.

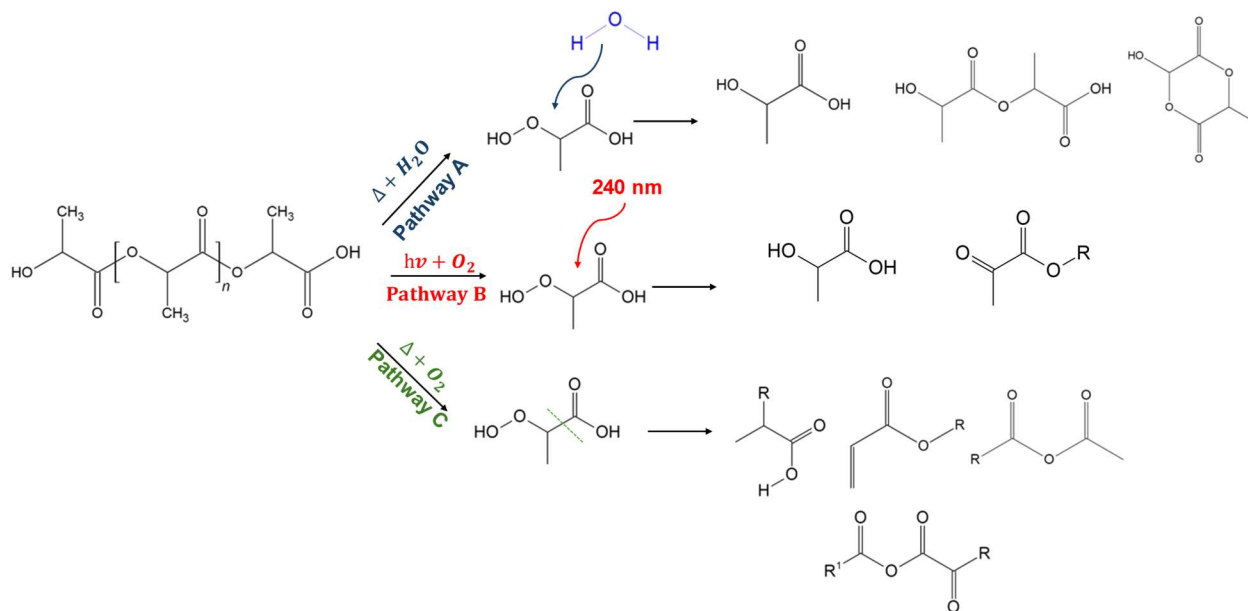


Figure 7. Common by-products in the hydrolytic (pathway A), photo-oxidative (pathway B), and thermo-oxidative degradation (Pathway C) pathways of PLA².

Conclusions

In conclusion, we present an attempt to mimic PLA NPs that would naturally form in aqueous environments due to the environmental degradation of macroplastics. This was succeeded through the fabrication of PLA NPs via laser ablation in a water environment, yielding particles characterized by a nearly spherical geometry and heterogeneous size distribution with an average diameter of 54.7 ± 26.7 nm. The NTA analysis also confirms this heterogeneity of the NPs, with 90% of the particles detected falling below 200 nm of hydrodynamic diameter. The analytical techniques adopted corroborated the chemical nature of the developed NPs with some modifications attributed to the primary chain polymer backbone cleavage, with physicochemical attributes corresponding to those of their expected naturally occurring counterparts. Moving forward, the herein fabricated NPs present a valuable model for investigating the environmental implications of a biobased polymer like PLA, especially their interactions and potential complex formations with other environmental pollutants.

Data Availability

Data will be made available from the corresponding author upon request.

Author Contributions

Malavika Manju Sudheer: Investigation, methodology, visualization, writing-original draft. Arezou Fazli: Methodology, writing-review and editing; Stefania Sganga: investigation, Writing-review and editing; Nicolo Tirelli: resources; Riccardo Carzino: Investigation, writing-review and editing; Marina Veronesi: Investigation, writing-review

and editing; Kirill Khabarov: Investigation, Athanassia Athanassiou: funding acquisition, Writing- review and editing; Despina Fragouli: Conceptualization, methodology, supervision, validation, visualization, writing-review and editing,

Conflict of interest

The authors declare no conflicts of interest.

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