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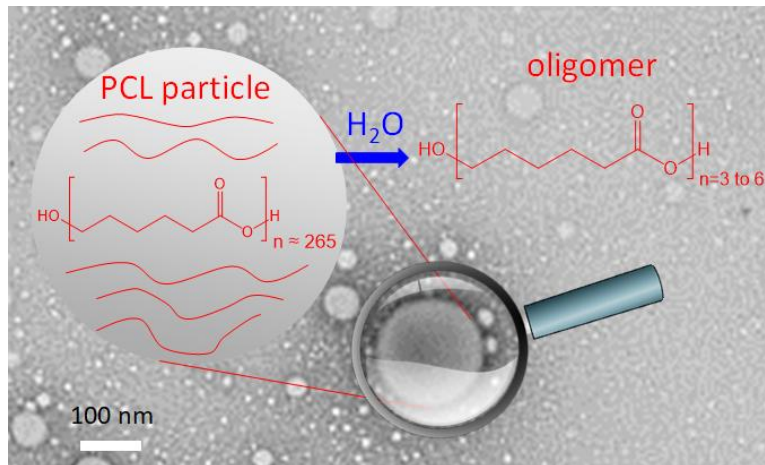
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Quantitative HPLC–mass spectrometry analysis shows the drastic impact of the composition of aqueous and biochemical media on the release of soluble hydrolysis products from submicron polycaprolactone

Maëva Boulée, Marie Carrière and Thierry Douki**

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ABSTRACT:

Accumulation of micro- and nanoplastics in the environment is a major concern, and biodegradable polymers are receiving growing interest as an alternative to more stable material. It remains yet to identify the degradation products of biodegradable plastics. In the present work, we studied polycaprolactone (PCL), a polymer used in a growing number of packaging and medical applications. We gathered quantitative data on the release of soluble oligomers from submicrometric PCL particles (100 to 1000 μm in diameter) throughout their degradation. This was made possible by the synthesis of well-characterized PCL oligomers, which were then used for the development of HPLC-mass spectrometry methods. The assay was first applied to degradation studies performed in aqueous media, in relationship to environmental degradation. We observed an influence of temperature and salinity, and obtained specific information on the mechanism of release of PCL hydrolysis products at the sub-micrometric scale. The same approach was then applied to study PCL degradation in cell culture media used in toxicity studies, Oligomers arising from the hydrolysis of PCL particles were released in both the presence and the absence of cells. The size distribution under these conditions was shifted to shorter oligomers than in water. This observation, supported by experiments on synthetic PCL oligomers, pointed to a significant contribution of fetal calf serum used as an additive in cell culture medium. The bulk of these results show that hydrolysis of submicrometric PCL particles and the subsequent production of oligomers is an efficient process that is strongly modulated by the degradation medium.

KEYWORDS

Polycaprolactone; hydrolysis; aqueous solutions; culture medium; biodegradation; oligomers

1. INTRODUCTION

The use of plastics constantly increases. The overall production was reported to be 400 and 413 million tons in 2022 and 2023, respectively [1, 2]. This value could reach 600 million tons in 2040, with 30 million tons released as waste in the environment either in water (69%) or on the land (31%) [3]. Because more than 80% of these plastics consists of polymers exhibiting stable C-C bonds, like in polyvinyl chloride, polyethylene, polypropylene and polystyrene, they do not degrade and accumulate in soil and water. The materials may undergo further transformation leading to the release of micro- and nanoplastics, which raises concerns in terms of toxicity to humans and to ecosystems [4, 5].

An alternative to these polluting materials is the use of plastics that can decompose in the environment as the results of chemical and biotic transformations, thereby avoiding accumulation [6-10]. However, this strategy will only be successful if the products released upon degradation are devoid of toxicity [11]. There is therefore a need for the identification of degradation products and for the assessment of associated hazards. Of particular interest are the soluble compounds that can easily migrate in various matrices, enter organisms and potentially penetrate cells where they may exhibit toxic properties.

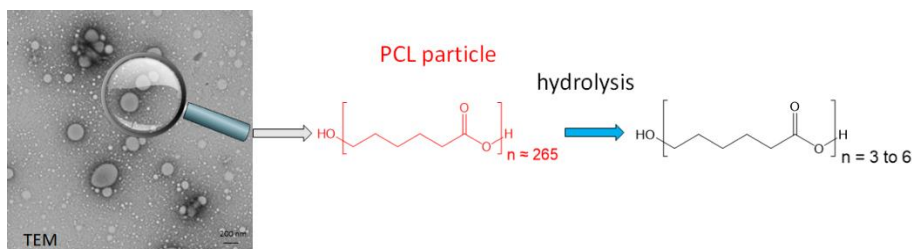
Several types of biodegradable plastics have been designed [7]. Those exhibiting a polyester backbone are among the most used, in particular polylactic acid (PLA) [12, 13]. A major interest of this material is that it is bio-sourced, originally produced from cornstarch. Polycaprolactone (PCL) is another biodegradable plastic that receives growing number of applications, for example in packaging, as drug carriers or in surgical processes [14-16]. Other medical applications used PCL for the preparation of functional hydrogel [17, 18] or nanocomposite scaffolds for bone regeneration [19].

From a structural point of view, PCL is a polymer of 6-hydroxyhexanoic acid (6-OH-HAc). However, it is produced on large scale by metal-catalyzed polymerization of ϵ -caprolactone. This precursor is not biosourced and is produced by oxidative ring expansion of cyclohexanone [20]. Hydrolysis of PCL and its copolymers have been already investigated [21-24]. In addition to its environmental significance, this process is also important to consider for medical applications [25, 26]. However, the bulk of the available data deal focus on the characterization of polymeric fraction, in particular in terms of loss of weight and loss of mass [27-31]. Some studies included the detection of the released oligomers but with limited characterization evidence and little quantitative information [32]. The accurate identification of the soluble fraction obtained upon PCL degradation is important since leachates from PCL particles were found to be toxic in zebra fish [33]. It can be added that the presence of oligomers following synthesis has been found to modulate the performances of PCL [34, 35]. This issue will not be addressed here but the developed analysis tools could be applied to it.

Better identification of the products released upon degradation of PCL is the major purpose of the present work. In addition, considering the issue of micro- and nanoplastics, we investigated PCL as submicrometric particles rather than in a bulkier form. In a previous work, we studied the soluble fraction released upon accelerated aging of aqueous suspensions of sub-micrometric particles of several polyester-based polymers, and in particular of PLA and PCL. The extensive characterization strategy applied was based on high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) [36]. We could show that, upon accelerated aging of PCL particles under environmental conditions (40°C and simulated equatorial sunlight), hydrolysis was the only degradation process leading to the release of small molecular weight

products (Scheme 1). The identified PCL degradation products were linear oligomers bearing between 2 and 8 monomeric units.

In the present work, we extended this qualitative approach to the collection of quantitative data on the formation of the different oligomers released upon hydrolysis of sub-micrometric pure PCL particles. This novelty was made possible by the synthesis of authentic standards. In order to gain information on the fate of PCL in aquatic environment, we first thoroughly investigated the release of hydrolysis products from PCL in water and seawater at different temperature. The results provided insights on the hydrolysis mechanisms. We then studied the behavior of PCL particles in cell culture medium, a key information for *in vitro* toxicological investigations. We could show that the components of the medium, especially fetal calf serum, accelerated the hydrolysis process.



Scheme 1: Structure of the PCL particles, polymer and hydrolysis products studied in this work. The particles exhibited a diameter of 100, 200, 500 or 1000 nm. They were made of 30 kDa polymers. The transmission electronic microscopic view of a suspension of 200 nm particles shows that particles are spherical. All particles consist of PCL polymeric chains of 30 kDa, namely 256 monomers in average. The work was based on the quantification of oligomers, made of 2 to 7 monomeric units, released upon hydrolysis of the particles.

2. EXPERIMENTAL PROCEDURES

2.1 Chemicals.

Ammonium formate (10 M in water), formic acid (HPLC-MS grade) and 6-OH-HAc were purchased from Aldrich (Saint Quentin-Fallavier, France). Acetonitrile (ACN) of HPLC MS grade was a Carlo Erba product (Val-de-Reuil, France). Water used for the preparation of HPLC mobile phases and suspensions of particles was purified on a Milli-Q system. PCL particles (100 to 1000

nm) were provided as an aqueous suspension by Phosphorex (Hopkinton, MA, USA). They were composed of 30 kDa polymer chains. The filtered seawater (Oligocean, Laboratoires Superdiet, France) contained 92 elements among them (in mg mL⁻¹) sodium 10.7, magnesium 1.3, sulfur 0.89, potassium 0.43, calcium 0.41, bromine 0.06 and (in µg mL⁻¹) boron 3.8, fluorine 1.28, phosphorus 0.727, lithium 0.151, iodine 0.064, zinc 0.016, cobalt 0.01, manganese 0.005, copper 0.005, iron 0.0006.

2.2 Synthesis of the PCL oligomers.

Pure 6-OH-HAc (30 mg) was weighted and placed in a sealed HPLC vial under air. The vial was then left for either 20, 63 or 186 h in a heating block set at 50°C. Samples were then removed from the block and 1 mL of water was added to quench the reaction. The vial was sonicated for 20 min and 500 µL of the resulting suspension was dissolved in water at a concentration of 1 mg mL⁻¹. The solution was analyzed by HPLC-MS/MS, both in the MS1 and the MS2 modes. MS1 peak areas were used to calculate the proportion of each oligomer and MS2 spectra provided unambiguous identification of the products. The 1 mg mL⁻¹ stock solution was diluted in water to obtain calibration solutions at 10 and 1 µg mL⁻¹ for HPLC-MS/SIM quantification. These solutions were diluted 10 times for MRM detection.

2.3 HPLC-Mass spectrometry analyses.

Samples were injected into a ExionLC AD series chromatographic system (SCIEX, Framingham, MA) equipped with an octadecylsilyl silica gel column (Nucleodur C18 HiTec, 1.8 µm particle size, 2×100 mm ID, Macherey Nagel, Gutenberg, France). The flow rate was 0.35 ml min⁻¹ and the oven temperature 50°C. The mobile phases were a 2 mM ammonium formate aqueous solution containing 0.1 % formic acid (A) and ACN with 0.1 % formic acid (B). The initial composition of the gradient was pure A. The proportion of B was then increased to 10 % in

12 min, set to 60 % at 20 min and reached 100 % at 22 min. The latter composition was maintained for 3 min. After chromatographic separation, the flow was directed toward the TurboIonSpray source of a 6500+ QTrap mass spectrometer (SCIEX). The pressure of curtain gas, nebulization gas and heated sheath gas were set at 45, 60 and 60 psi respectively, while the source temperature was 550°C. The exhaust gas (air) was set at 25 L min⁻¹. The ion spray voltage was -4500 V. Single stage mass spectrometry experiments (MS1) were performed with the third quadrupole of the spectrometer used as a linear ion trap. Fragmentation data were recorded in a classical triple quadrupolar configuration. The pressure of collision gas was then set to “medium” while the collision energy was -30 V. Quantitative analyses were first performed in the single ion monitoring (SIM) mode with a quantification ion (QI-ion) and a confirmation ion (CI-ion) for each oligomer (Table 1). Detection in the multiple reaction monitoring mode (MRM) was also used. For this purpose, the signals corresponding to fragmentation of the targeted pseudo molecular ions into PCL monomer and dimer were monitored (Table 1). Quantitative data were inferred from external calibration curves. Limits of detection and quantification were calculated from the mean value and standard deviation of the noise determined in the chromatogram of a water blank over 1 min at the expected retention time of the analyte [37].

Table 1: *m/z* ratio of the ions used for the quantification of PCL oligomers by HPLC-mass spectrometry. Q-ion and C-ion are the ions used for quantification and confirmation, respectively in the single ion monitoring mode. Transition 1 and 2 are the fragmentation reactions monitored in the multiple reaction monitoring method. (n.a.: not applicable).

Method	SIM		MRM	
	Q-ion	C-ion	transition 1	transition 2
Monomer	113	85	131→85	n.a.
Dimer	245	131	245→131	245→85
Trimer	359	245	359→245	359→131
Tetramer	473	359	473→245	473→131
Pentamer	587	n.a.	587→245	587→131
Hexamer	701	n.a.	701→245	701→131

2.4 Aging of PCL particles under environmental conditions.

Particles were artificially aged in a test chamber (Q-SUN Xe-1, QLab) equipped with a xenon arc lamp filtered to emit light imitating the solar spectrum. The aging parameters were set based on the ISO 4892-2:2013 standard [38]. The irradiance was adjusted to $1.44 \text{ W m}^{-2} \text{ nm}^{-1}$ at 420 nm, and the temperature to 40°C, which corresponds to an equatorial zenithal exposure. For each aging experiment, 2 mL of particle suspensions were prepared at a concentration of 1 mg mL^{-1} in either Milli-Q ultra-pure water, PBS or seawater. The suspension was placed in quartz spectroscopy cuvettes with four transparent sides (Hellma Analytics). A magnetic bar was added and the cuvettes were then hermetically closed using a screw cap with a septum. The cuvettes were placed on a magnetic stirrer located in the test chamber. An aliquot fraction of the suspensions was sampled before aging (t_0) and the suspensions were irradiated for overall periods ranging between 88 to 96 hours. Aliquots of 100 μL were collected at regular intervals, typically every 24 hours. The aged suspensions were then stored at 4°C, not more than a week, before analysis.

2.5 Degradation of PCL particles in cell culture medium.

Caco-2 cells (ATCC HTB-37, passage 14 to 40) were seeded in 100 mm petri dishes (Falcon®) and maintained at 37°C in an atmosphere with 95% humidity and 5% CO₂ allowing them to proliferate and cover the surface of the dishes. The culture medium used was Dulbecco's Modified Eagle Medium (DMEM) + GlutaMAX™ (Gibco™) supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco™), 1% non-essential amino acids (MEM NEAA 100X, Gibco™), 50 units mL⁻¹ penicillin and 50 µg mL⁻¹ streptomycin (Sigma-Aldrich™). Cells were then collected and plated in 12 wells plates at a density of 80 000 cells cm⁻². When 70% confluency was reached after 24h, the culture medium was replaced by fresh medium containing 200 nm PCL particles (concentration 200 µg mL⁻¹). In other experiments, culture medium containing PCL particles, with or without FCS, were placed into wells that did not contain cells. In all cases, the medium was collected after 0, 24 and 48h. The medium was filtered at 0.2 µm and the filtrate was transferred into HPLC vials before analysis by HPLC-MS/MS (MRM mode) for their content in PCL oligomers.

2.6 Degradation of synthetic PCL oligomers

Solutions (1 µg mL⁻¹) of synthetic oligomers (oPCL) obtained after 186 h of incubation of 6-OH-HAc at 50°C were prepared by dilution in pure water and in seawater. A sample of these mixtures was collected and frozen immediately after preparation. Aliquot fractions (150 µL in 1.5 mL Eppendorf tubes) were placed either at 4°C in a fridge, at 20° in a temperature-controlled PCR block, or in incubators (without CO₂) set at either 37°C or 60°C. They were collected after increasing periods (24, 48, 72 and 96h) and frozen (-20°C) until HPLC-MS analysis performed after 0.2 µm filtration. In another experiment, 4 mL solutions of oPCL at 1 µg mL⁻¹ were prepared in complete culture medium containing FCS or not. Immediately after preparation, 900 µL of the

suspensions were collected (t0 h), filtered at 4°C using 10 kDa filters (5 min, 10,000 × g) and frozen. Aliquot fractions (500 µL) were placed in 6 wells (for each solution) of a 24-well plate and placed in an incubator at 37°C with 95% humidity and 5% CO₂. The samples were collected after either 24 or 48h. They were filtered (cut-off 10 kDa) and frozen. In all cases, solutions were then thawed, transferred into HPLC vials and injected as fast as possible to avoid uncontrolled degradation.

2.7 Statistical analyses.

Differences between the degradation yields and proportions of oligomers in aged PCL suspensions were based on the coefficients of a linear regression. Calculations were made in an Excel file. The standard error of the regression was used to calculate the standard error of the slope and of the intercept. These values were used to calculate the *t* parameter of a Student test that provided the probability *p* of the significance. Analysis of the cellular viability data involved a non-parametric Kruskal-Wallis multiple comparison analysis followed by comparison between selected conditions by use of a post-hoc Dunn's test (Prism-GraphPad software, San Diego, CA). Differences were considered statistically significant for $p < 0.05$ (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

3. RESULTS

3.1 Synthesis of PCL oligomers standards.

The synthesis took advantage of the property of 6-OH-HAc at undergoing fast dimerization, as shown by DFT calculations [39]. The one-pot protocol was very simple. 6-OH-HAc, which exhibits a melting point of 40°C, was used as a pure product and maintained at 50°C for increasing periods. The reaction was stopped by adding water. The resulting mixtures were analyzed by

HPLC-MS/MS under conditions used previously for the identification of PCL hydrolysis products [36]. When the reaction time increased, the content in monomeric 6-OH-HAc was found to rapidly decrease, while oligomers of increasing lengths were produced (Figure 1a,b). A complete mass spectrometry characterization was performed on the mixture obtained after 186h. The products of incubation of 6-OH-HAc were identified as PCL oligomers of length ranging between the dimer and the heptamer by comparison of their mass spectrometry features with those of compounds released by hydrolysis of PCL [36]. As illustrated for the tetrameric oligomer, the MS1 spectrum (Figure 1c) exhibited the expected deprotonated ion as the base peak (m/z 473.2). Similarly, the base peaks were observed at m/z 245.2, 359.1, 587.2, 701.3 and 815.3 for the dimer, trimer, pentamer, hexamer and heptamer of PCL. These observations show that the reaction products exhibit a molecular weight corresponding to that expected for the PCL oligomers. In addition, this result shows that the terminal hydroxyl and carboxylic groups are free. Thus, reactions with other molecules such as solvents can be ruled out since they would lead to a larger molecular weight than the native oligomers. Conversely, formation of cyclic oligomers by intramolecular esterification would result in the loss of a water molecule and a lower molecular weight. The MS1 spectra of the oligomers also exhibit ions corresponding to a source-mediated fragmentation leading to the loss of a 6-OH-HAc unit, as observed at m/z 359.1 for the tetramer (Figure 1c). The fragmentation spectra (MS2) of the pseudo-molecular ions of the reaction products provided additional structural information. They were characterized by a series of ions of decreasing m/z ratio separated from each other by 114 mass units (Figure 1d for the tetramer), which is the molecular weight of 6-OH-HAc. This successive loss of monomers confirms that the studied compounds are PCL oligomers. In addition, these results show that the oligomer is linear.

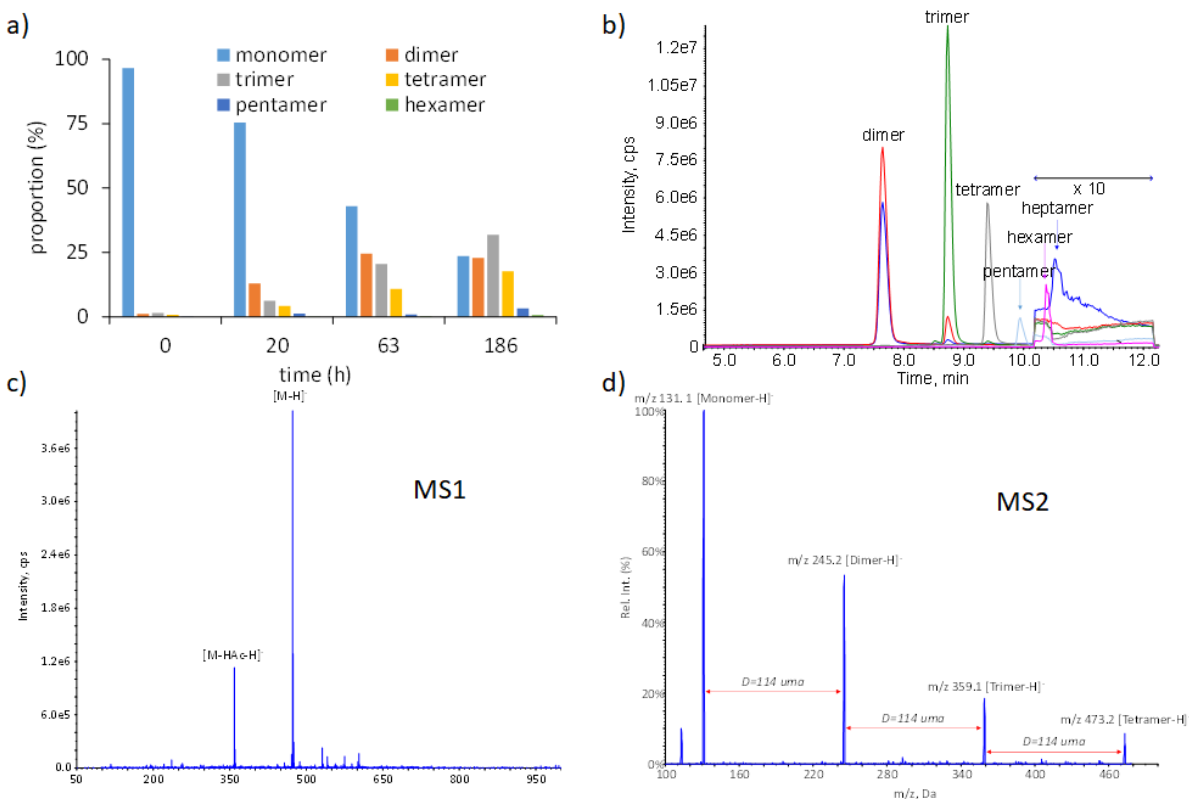


Figure 1: Formation of the PCL oligomers upon incubation of 6-OH-HAc at 50°C. a) Proportion of the different oligomers after increasing periods of reaction; b) HPLC analysis with mass spectrometry detection (MS1, m/z range 100-1000) of the sample incubated 186 h. The scale of intensity between 10.2 and 12 min was enhanced 10 times. The chromatograms correspond to the monitoring of the intensity of the pseudo-molecular ions of the different oligomers. They were extracted from the total ion current; c) single stage mass spectrum (MS1) of the PCL tetramer; and d) fragmentation mass spectrum (MS2) of the deprotonated pseudo-molecular ion of the PCL tetramer.

3.2 Optimization of quantitative HPLC-mass spectrometry assays for PCL oligomers.

The crude reaction mixture obtained after 186 h was used to prepare calibrated solutions of standards. The concentration of monomer was determined by injecting of solutions of pure 6-OH-HAc, while that of the longer oligomers was calculated based on the initial amount of 6-hydrohexanoic acid used in the synthesis and the ratio between the area of the corresponding peak of the HPLC-MS1 chromatogram. The solution of PCL oligomers (oPCL) was used for the optimization of the source and detection parameters of the mass spectrometer. As previously reported [36], a much better sensitivity was achieved in the negative than in the positive ionization

mode. Two methods were developed. First, analyses of aged aqueous suspensions were performed using detection in the single ion monitoring mode (SIM). Chromatographic separations were carried out on a reverse phase UHPLC column with a gradient of ACN in acidified aqueous ammonium formate. The limit of quantification was similar for all oligomers, in the range of 1 ng injected (Table 2), which was well suited for the analysis of the aged suspensions of PCL particles. A good stability of the signal was observed, even in long runs of injections. External calibration curves were found to be linear over a wide range of concentrations (Figure 2a). Second, for experiments involving cells and oPCL at low concentrations, a multiple reaction monitoring method (MRM) was developed because small amounts of oligomers were anticipated. The chromatographic conditions were identical to the SIM method. Settings of the mass spectrometer were optimized for each oligomer to achieve the most sensitive as possible monitoring of the fragmentation of the pseudo-molecular ions into fragments at $m/z=131$ and $m/z=245$. A drawback of the assay is the dependence of the sensitivity on the length of the oligomers (Table 2). The limit of detection was in the range of 100 fg injected for the dimer, trimer and tetramer, but was 50 times larger for the pentamer. Excellent linearity and repeatability were obtained (Figure 2b).

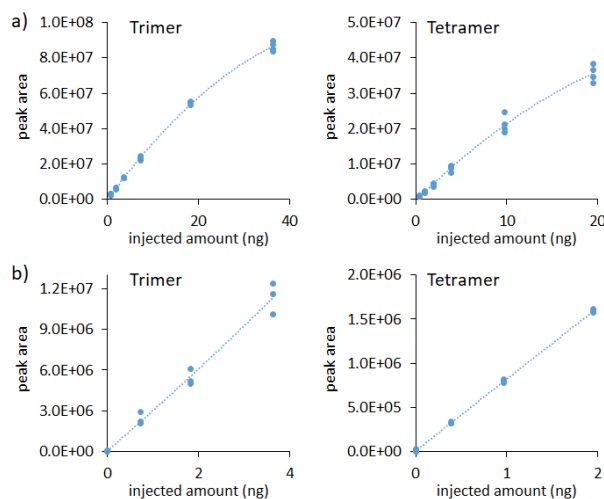


Figure 2: Calibration curve of the HPLC mass spectrometry assays for PCL oligomers. Data are shown for the trimer and tetramer in a) the Single Ion monitoring mode (SIM) and b) the multiple reaction monitoring mode (MRM).

Table 2: Limits of detection and quantification of the PCL oligomers by HPLC-mass spectrometry. Two methods were applied: single ion monitoring (SIM) and multiple reaction monitoring (MRM). LOD is the limit of detection and LOQ the limit of quantification.

Method	SIM		MRM	
	LOD (ng)	LOQ (ng)	LOD (fg)	LOQ (fg)
Dimer	0.78	0.96	33	64
Trimer	0.55	0.68	45	105
Tetramer	0.58	0.64	121	251
Pentamer	1.12	1.26	5446	15457

3.3 Degradation of PCL particles in pure water.

The designed HPLC-MS assay was first applied to the aging of PCL particles in pure water under environmentally relevant conditions. Experiments were performed on commercially available pure PCL particles of 100, 200, 500 and 1000 nm in diameter. The particles were spherical (Scheme 1) and no evidence of the presence of 6-OH-HAc dimer reported to be present in large amounts in numerous PCL batches was obtained [34]. Their size was verified by TEM and found to be 78, 204, 503 and 939 nm, respectively (data not shown). Two main types of information were gathered. The overall yield of release of hydrolysis products could be determined by summing the concentration of the different oligomers. Emphasis was also placed on the relative proportions of the different hydrolysis products. A first series of experiments was performed at 40°C in pure deionized water with particles of increasing size and at a concentration of 1 mg mL⁻¹. A first information was that the time-course of hydrolysis products release was linear (Figure 3a). In addition, the particles size did not affect the overall yield of formations of the oligomers within experimental error (Figure 3b). In all cases, the overall yield of hydrolysis products was in the range of 0.25% after 96h aging. The HPLC-MS detection also allowed us to gather quantitative data on the proportion of the different oligomers. For the four investigated particle sizes, the

decreasing order of concentration in the soluble fractions of suspensions aged at 40°C was pentamer > tetramer > hexamer > trimer. The monomer, dimer and heptamer were produced only in minute amounts. The proportions of the oligomers in the liquid phase were similar for the four particle sizes at each time point. The values were thus averaged to improve the precision of the determination of distribution in length of the hydrolysis products. The proportion of the different oligomers was not constant during the time-course study. We observed a trend at the increase of the amount of trimer and tetramer at the expense of pentamer and hexamer when aging time increased (Figure 4a).

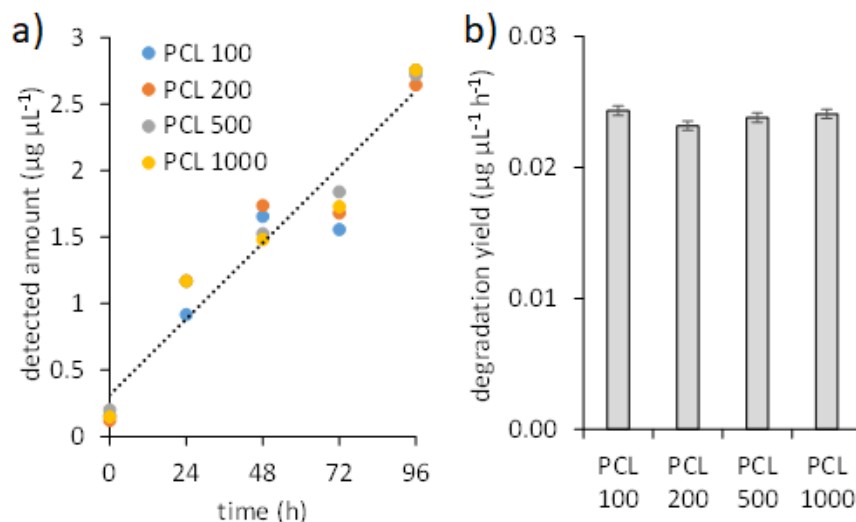


Figure 2: Yield (w/w) of formation of oligomers (dimer to hexamer) upon aging of PCL particles of increasing size (100 to 1000 nm). Total concentration of oligomers (in $\text{ng } \mu\text{L}^{-1}$) was determined upon duplicate analyses of two replicates. Yields were calculated by linear regression of the amounts of oligomers released at either 0, 24, 48, 72 or 96 h. Reported values are the slopes \pm standard error of the slopes. Degradation yields were compared by a *t* test and no statistical significance was observed ($p > 0.05$).

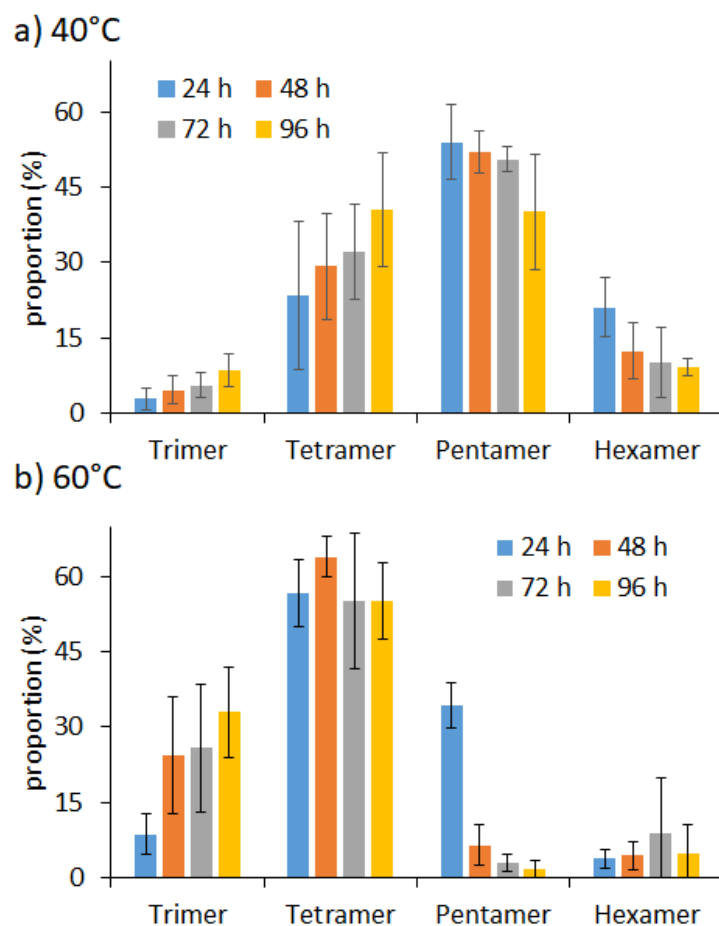


Figure 3: Release of the main oligomers upon aging of sub-micrometric PCL particles exposed to simulated sunlight. Reported values were average of experiments performed with 200, 500 and 1000 nm particles. The temperature was set at either a) 40°C, or b) 60°C. Suspensions were made in pure water at a concentration of 1 mg mL⁻¹. Proportions are expressed in % of the amounts of oligomers determined upon duplicate analyses of two replicates. Error bars are the standard deviations. The concentrations determined in untreated samples were subtracted.

We also investigated the effects of temperature. For this purpose, aging experiments were repeated at 60°C, the temperature applied in industrial composters, and compared with results obtained at 40°C. The illumination conditions were the same in both experiments. The degradation yield was twice as large at 60°C as at 40°C for the three studied sizes (200, 500 and 1000 nm). For example, the rates of formation of oligomers in a 1 mg mL⁻¹ suspension of 200 nm PCL were 0.11 and 0.05 ng μL⁻¹ h⁻¹ at 60°C and 40°C, respectively. Differences were also observed in the distribution of the length of oligomers produced upon hydrolysis (Figure 4b). A first observation

was that the trimer and the tetramer were the major oligomers at 60°C, while the tetramer and the pentamer predominated at 40°C. In addition, the proportion of the trimer was doubled at 60°C. In contrast, the amount of hexamer was roughly three times lower at the highest temperature. Another result was that the content in pentamer rapidly dropped for aging times longer than 24h while that of trimer strongly increased. In order to accurately quantify the time-dependent evolution of the proportions of the different oligomers, we performed a linear regression of the data (Table 3). The slope provided the rate of increase or decrease in the proportion of the products. The intercept of the linear regression also provided interesting data since it represents the distribution of oligomers immediately after hydrolysis before they were further transformed in solution during the aging experiment. We could thus assess that the shortest oligomers were initially released in larger amounts from PCL particles at 60°C than at 40°C.

Table 3: Linear regression analysis of the evolution of the proportion of oligomers upon aging of PCL particles at either 40 or 60°C. Slope are expressed in difference % per hour and the intercept in %. Results are values \pm standard errors calculated from the standard error of the linear regression. Statistical significance of the difference between samples obtained at either 40°C or 60°C was determined by a t test (*: $p < 0.05$; ***: $p < 0.001$).

	Trimer	Tetramer	Pentamer	Hexamer
40°C				
slope	0.075 \pm 0.027 *	0.226 \pm 0.113	-0.179 \pm 0.075 *	-0.159 \pm 0.058 *
intercept	0.84 \pm 0.75	17.7 \pm 3.2	59.8 \pm 2.18	22.6 \pm 1.6
60°C				
slope	0.309 \pm 0.102	-0.057 \pm 0.092	-0.422 \pm 0.090	0.033 \pm 0.066
intercept	4.4 \pm 2.9	61.2 \pm 2.6 ***	36.7 \pm 2.5 ***	3.5 \pm 1.8 ***

3.4 Degradation in different aqueous environments.

In a subsequent step, we repeated the aging of PCL suspensions under different conditions. We used 200 nm PCL particles and first compared the overall yield of degradation in pure water, PBS

and seawater. As shown in **Figure 4**, the yields were similar in water and PBS. In contrast, the amount of released oligomers was 2.3 times larger in seawater than in pure water (Figure 5). A pH effect can be ruled out since the yield was the same in PBS and seawater, which have the same pH. Another difference between the results of aging in pure water and seawater was the time-dependent proportions of the oligomers (Figure 6a). In pure water, the proportion of pentamer in pure water decreased between 24h and 88h, while those of trimer and tetramer slightly increased. Scattering of data prevented the observation of a clear trend for the hexamer. A much stronger effect was observed in seawater. The proportion of pentamer was divided by a factor of 2 during the experiment and the amount of hexamer was close to the limit of detection at 88h. On the contrary, the proportion of tetramer and trimer increased by respective ratios of 1.9 and 1.3 between 24h and 88h of aging. Like for the experiments in water, extrapolation at t0h was used to determine the proportion of oligomers that were present immediately after hydrolysis of the particles. A similar distribution was obtained in pure water and in seawater (Figure 6b).

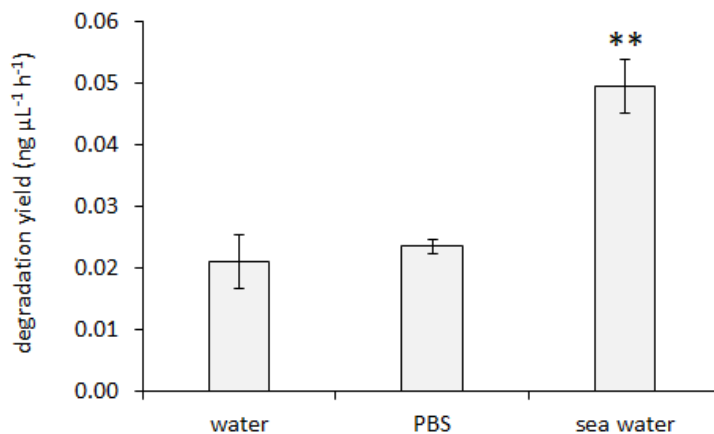


Figure 4. Rate of degradation of 200 nm PCL particles aged at 40°C in suspension in either water, PBS or seawater. The concentration was 1 mg mL⁻¹. Total concentration of oligomers (in ng μL^{-1}) was determined in two replicates. Reported results were obtained by linear regression of time-course study and are the slopes \pm standard error of the slopes. Statistical significance of the results was determined by a t test (**: $p < 0.01$).

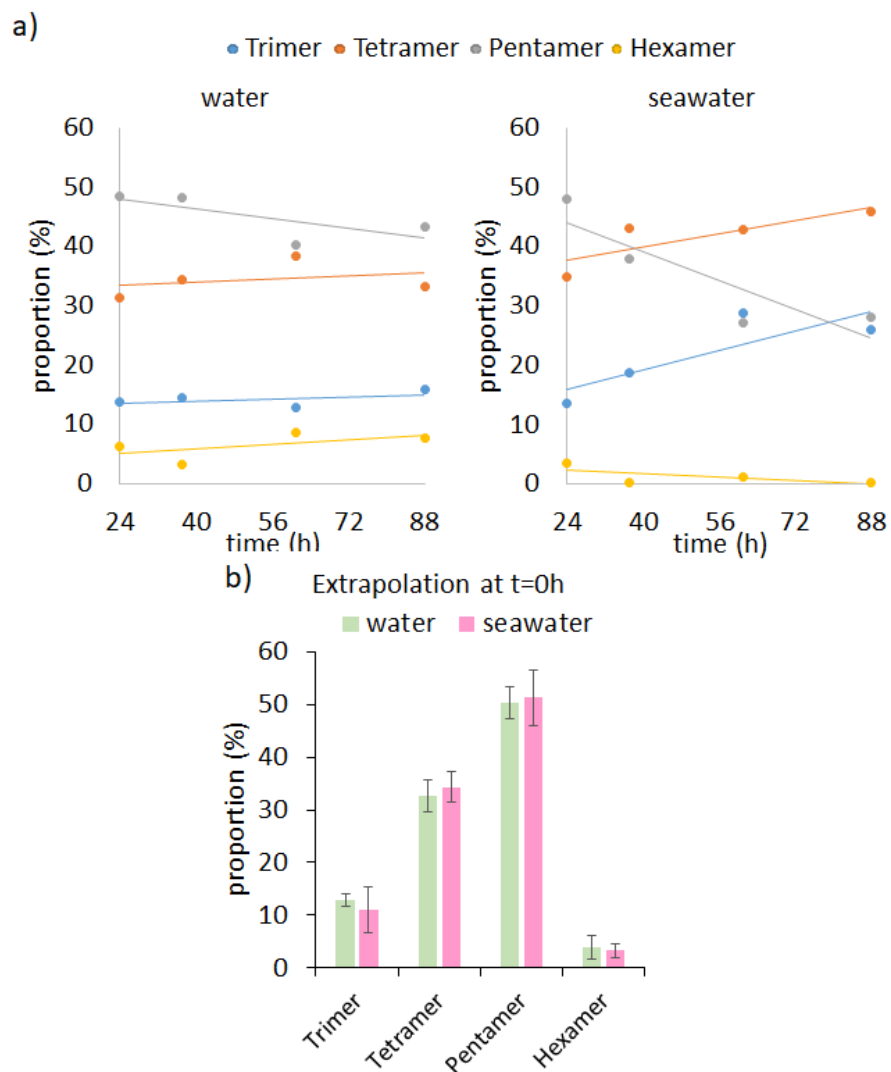


Figure 5: Proportion of the different PCL oligomers during aging of 200 nm particles at 40°C in either pure water or seawater; a) time-course study and b) extrapolation at t0h of the proportion of the different oligomers. Suspensions were prepared at a concentration of 1 mg mL⁻¹. Proportions are expressed in % of the amount of oligomers determined in two replicates. The concentrations determined in untreated samples were subtracted. Reported values extrapolated at t0h are the intercepts ± standard error. Results were compared by a t test and no statistical significance was observed ($p > 0.05$).

Further characterization of the temperature and electrolyte effects was performed on purified synthetic oligomers. Solutions of oPCL (1 $\mu\text{g ml}^{-1}$) prepared either in pure water or in sea water were incubated for 96h at 4, 20, 37 or 60°C. The proportion of the shortest oligomers was found to be the largest in sea water, especially at 60°C where the monomer represented 98.9 % of the oligomers while the corresponding value was 62.1% in pure water (Figure 7a). Another interesting observation deals with the yield of the various oligomers (Figure 7b). At 20°C in pure water, a

small formation of monomer and a small decrease in the concentration of tetramer were observed. No significant trend was observed for the other oligomers. The absolute values for the variation in concentrations of monomer and the pentamer were slightly increased at 60°C, and the formation of the dimer was detectable. In seawater at 20°C, degradation was mostly observed for the trimer while the monomer and the dimer were produced. At 60°C, both rates of reaction were larger in absolute values. Both the dimer and the trimer were degraded while the monomer was produced in large yield.

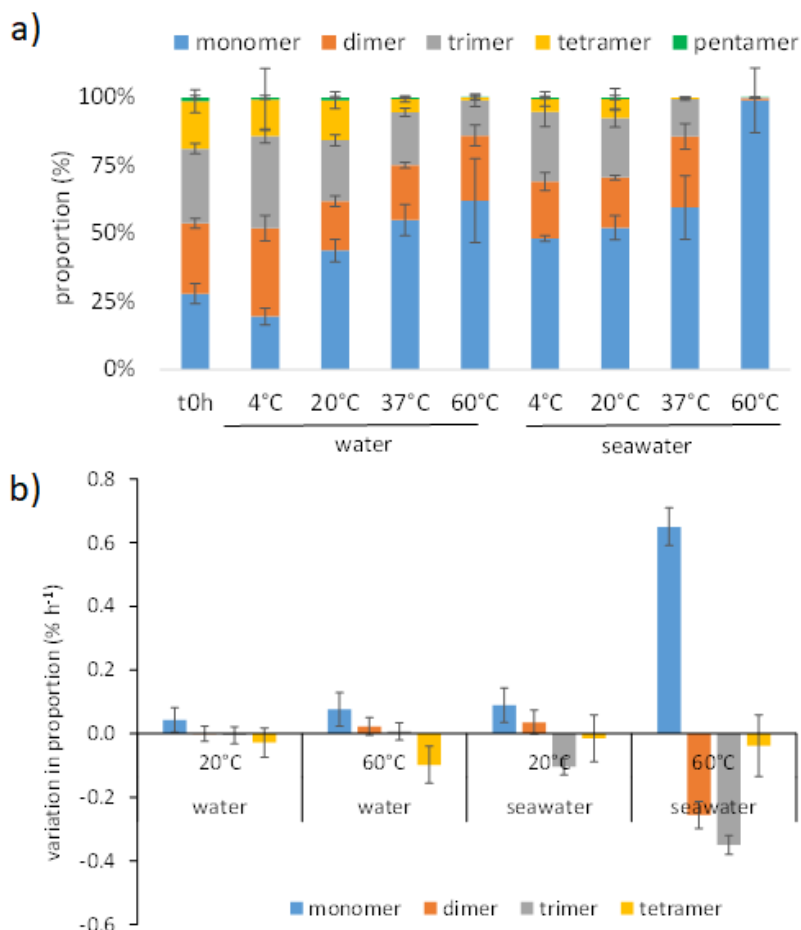


Figure 6: Degradation of synthetic PCL oligomers in either pure water or seawater. a) Proportion (expressed in %) of the different species after 96h of incubation (mean \pm standard deviation, $n=3$), except the first values determined immediately after preparation of the solution and b) rate of reaction (expressed in h^{-1}) for the formation or degradation of oligomers calculated between 0 and 96h (slope \pm standard error of the slope, $n=3$). Results are mean \pm standard deviation ($n=3$).

3.5 Degradation of PCL particles in cell culture medium.

Experiments were then designed to determine the fate of PCL particles and oligomers under conditions typically used in *in vitro* experiments. In order to mimic a contamination by ingestion, we used the intestinal cell line Caco-2. For this study, either PCL particles or synthetic oligomers (oPCL) were added to culture medium in the presence or absence of cells. The concentration of hydrolysis products was then quantified after increasing incubation times (Figure 8). A first result was that, in either the presence or the absence of cells, incubation of 200 nm PCL particles in complete culture medium led to the immediate release of oligomers (Figure 8, Table 4). Interestingly, the monomer was by far the major hydrolysis product, which was not the case upon hydrolysis of PCL200 in water. It may be suggested that components of the medium are involved in this strong degradation. Another striking observation is the rapid increase of the content in monomer (Figure 8) and the concomitant degradation of the longer oligomers in complete medium, both in the presence or absence of cells. This is indicative that a component of the medium has the ability to degrade PCL particles. When the experiment was performed in medium devoid of fetal calf serum (FCS), this increase in monomer level, as well as the decreases in the concentration in longer oligomers (Table 4), was no longer observed.

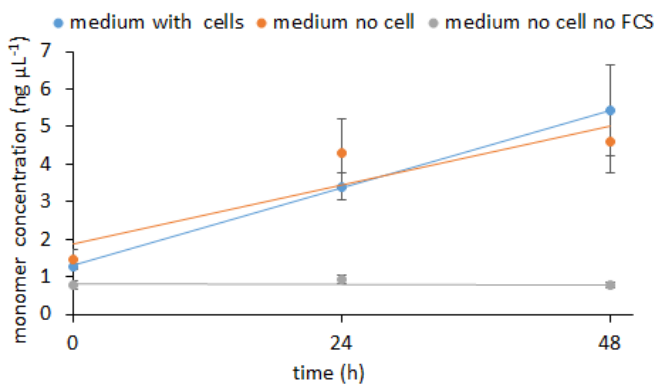


Figure 7: Amounts of PCL monomer detected in cell culture medium treated with PCL particles (200 nm) for increasing periods, in the presence or absence of Caco-2 cells, by 200 ng/ μL of 200 nm PCL. Results are mean \pm standard deviation ($n=3$).

Table 4: concentration (in ng/ μ L) of PCL oligomers measured either in DMEM culture medium without FCS or in PBS after addition of 200 μ g/mL of 200 nM PCL particles. Results are mean \pm standard deviation ($n=3$).

Condition	Time	monomer	dimer	trimer	tetramer
medium with cells	0h	1.286 \pm 0.061	0.030 \pm 0.008	0.062 \pm 0.005	0.006 \pm 0.003
	24h	3.408 \pm 0.373	0.002 \pm 0.000	0.003 \pm 0.001	0.001 \pm 0.000
medium no cell	0h	1.465 \pm 0.251	0.041 \pm 0.011	0.087 \pm 0.026	0.007 \pm 0.003
	24h	4.286 \pm 0.919	0.007 \pm 0.004	0.008 \pm 0.002	0.001 \pm 0.000
medium no cell no FCS	0h	0.779 \pm 0.101	0.045 \pm 0.002	0.016 \pm 0.004	0.001 \pm 0.001
	24h	0.936 \pm 0.114	0.064 \pm 0.006	0.022 \pm 0.003	0.001 \pm 0.001

In order to confirm the role of FCS in the degradation of oligomers, we incubated the synthetic oPCL in complete medium with or without serum (Figure 9). The concentration in monomer was high in the presence of FCS already immediately after the beginning of the treatment, while those of the dimer and trimer decreased. In contrast, the concentrations in monomer, dimer and trimer remained constant in the absence of FCS.

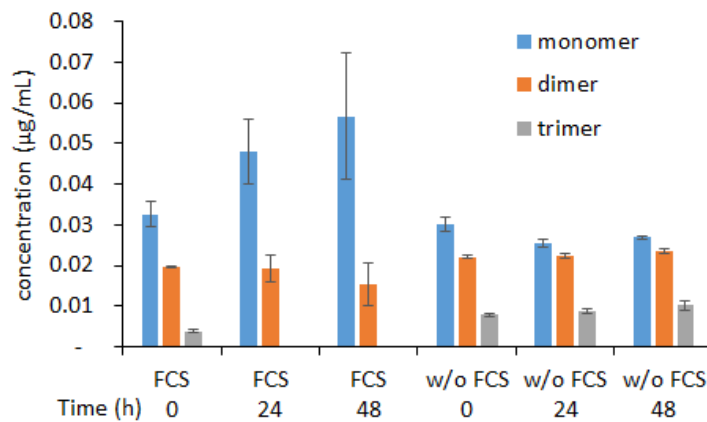


Figure 8: Amounts of PCL oligomers in solution of synthetic oPCL in DMEM culture medium in the presence (FCS) or the absence (w/o FCS) of fetal calf serum. Results are concentration (mean \pm standard deviation, $n=3$)

4. DISCUSSION

The purpose of this work was to quantitatively study the release of oligomers upon hydrolysis of PCL, in aqueous suspensions and in a cellular context. For this purpose, we used HPLC coupled to mass spectrometry with electrospray ionization. This technique has been applied to the degradation of several types of plastics, including polylactic acid [36, 40], which exhibits a polyester backbone, like PCL. Degradation of PCL has also been studied by mass spectrometry but mostly by MALDI-based approaches [41]. We have recently shown that HPLC-MS/MS was a highly relevant technique for the identification of soluble products generated upon aging of PCL and other polyesters [36]. We presently extended this approach to the quantification of the different oligomers produced upon hydrolysis in various media, both environmentally-relevant and relevant in toxicity studies. Development of the method was made possible by the synthesis of pure standards from 6-OH-HAc. With these products in hand, we could develop specific and accurate HPLC-mass spectrometry assays providing quantitative results by external calibration. This allowed us to provide novel data on the yield of degradation and on the distribution of the different oligomers in the liquid phase of PCL suspensions aged under different conditions.

The first part of the work was devoted to the study of the impact of the aging conditions on the yield and length of the released oligomers. A specificity of our investigation is that PCL particles were in the sub-micrometric range while available data mostly concern films and bulky materials [21, 42]. Results have been also reported on short polymers in mechanistic studies [43]. Yet, degradation of sub-micrometric particles type of material has never been extensively studied. Our experiments were first performed in pure water at 40°C and 60°C. A first observation was that the size of the particles did not change the yield of oligomeric hydrolysis products in mass ratio. Yet, particle specific surface area increases when the diameter decreases. The density of the spherical

PCL particles used in this work is 1.145 g cm^{-3} , as specified by the supplier. Considering that the particles are not porous, their specific surface area would therefore be 52.3, 26.3, 10.6 and $5.2 \text{ m}^2 \text{ g}^{-1}$ for diameters of 100, 200, 500, 1000 nm, respectively. A direct link between specific surface area and degradation rate has been established for bulk materials [42, 44]. Consequently, the degradation should be faster with small compared to large particles, which was not the case with presently studied sub-micrometric particles. This observation means that the degradation of the latter species is not only a surface process. This could be explained by an efficient diffusion of water molecules within the particles, as previously reported [14, 21, 45], and a degradation not only at the surface but also in the core.

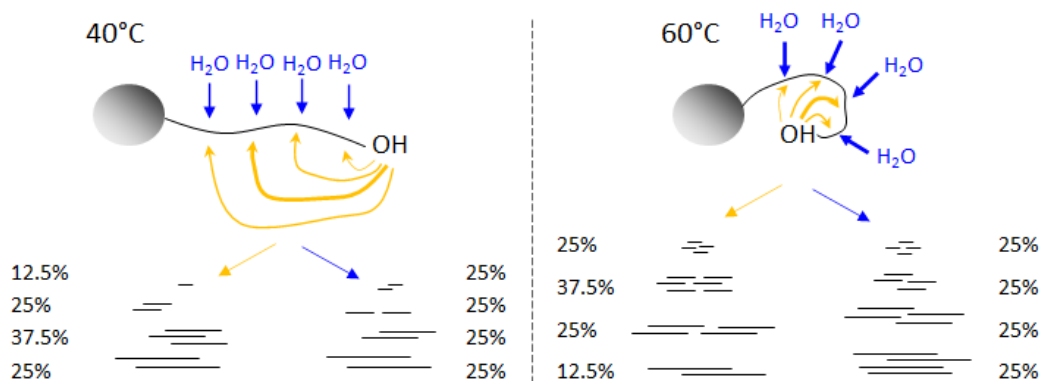
In contrast to the size, the temperature had a major impact on the production of oligomers from PCL particles as previously reported for bulk objects [46]. Increasing it from 40 to 60°C doubled the rate of formation of oligomers. This information may not be sufficient for the complete description of degradation processes. Indeed, degradation of PCL [21], like that of other plastics, is often described in terms of a first phase that corresponds to the breakage of the polymer chains and a decrease of their molecular weight, followed by a second phase involving the release of small fragments and/or soluble products from the material. In our case, quantification of the hydrolysis products in the liquid fraction provided information only on the latter step. However, the fact that we did not observe a lag phase in our time-course study, while it is expected in bulk PCL [42], may suggest that these two phases (loss of molecular weight & loss of mass) occur almost simultaneous in the sub-micrometric particles.

In addition to the determination of the degradation yield, precise quantification of the different oligomers released upon aging of PCL provided information on their respective proportions. Oligomers ranging between the trimer and the hexamer represented the overwhelming majority of

the hydrolysis product released in aqueous suspensions. The proportions between the different species were similar for the different particle sizes, but the respective proportion of the oligomers of different length differed at 40 and 60°C. For all time points, the most frequent oligomer at 40°C was the pentamer. In contrast, it was the tetramer at 60°C. In addition, the proportion was not constant during the aging experiments and the proportion of the shortest oligomers increased at the expense of the longest. At 40°C, the average length (in monomeric units) was 4.9 after 24h aging and 4.4 after 96h. The corresponding values were 4.3 and 3.8 at 60°C. It has been proposed that cleavage of ester bonds in the bulk of PCL could lead to a local increase in density of free carboxylic groups. This would locally decrease the pH and consequently increase the hydrolysis rate, leading to an exponential degradation of the polymer [14, 46]. We rather observed a linear time-course release of oligomers, suggesting on the opposite that the hydrolysis products rapidly diffuse out from the sub-micrometric particles. This is in line with the ease of diffusion of water molecules within the particles proposed above. Both phenomena are likely explained by the small size of the PCL particles investigated.

The distribution of oligomers resulting from the sole hydrolysis of the particles was inferred from the extrapolation of the time-dependent data at t0h. With these calculated values, the average length of oligomers (in monomeric units) is 5.0 and 4.3 at 40°C and 60°C, respectively. The fact that the distribution of the oligomers released in the first step of the degradation process depends on the temperature provides interesting mechanistic information (Scheme 2). Two mechanisms have been proposed for the hydrolytic decomposition of polyester chains like PCL [21]. The first one is a random reaction of water molecules with the ester bonds within the polymer. In this process, the hydrolysis rate of diester bonds may be affected by the temperature in absolute values but their relative reactivity is not likely to be affected. The second pathway, observed for PLA [47]

and proposed for PCL [48], involves a major role of the terminal hydroxyl groups of the polymer that may react with the adjacent diesters bonds with a probability that depends on the local flexibility of the chains. The size distribution of the released oligomers is thus likely to be affected by the temperature, which induces structural changes in the polymer chains. This is particularly true for PCL, which exhibits a melting temperature of approximately 60°C. Our results are in line with the later hypothesis since they can be explained by a structural modification of the PCL polymer making the terminal hydroxyl more accessible to the vicinal ester groups and thereby leading to a favored formation of shorter oligomers at 60°C compared to 40°C.

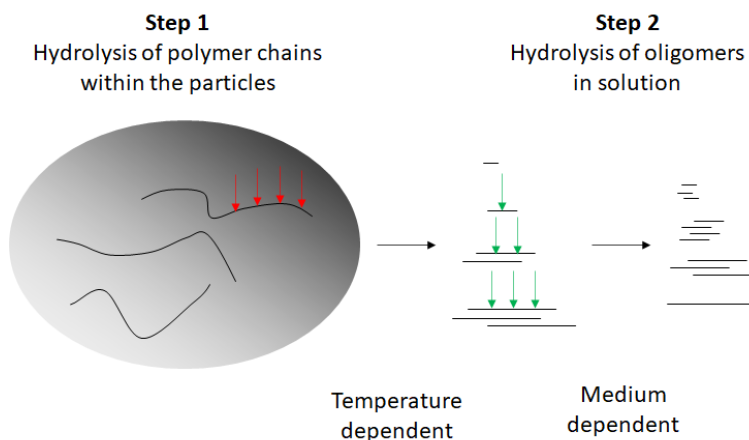


Scheme 2: Proposed explanation for the observed difference in the proportion of oligomers at 40 and 60°C. Two mechanisms can be involved. The first (bleu arrows) is mediated by water molecules that randomly react along the polymer chain. When the temperature increases, the rate of reaction increases (bold arrows) but relative proportion of the oligomers (symbolized by straight lines of different size) is the same as at 40°C. The second mechanism (orange arrows) involves the terminal hydroxyl group of the polymer chain. The distribution of oligomers is defined by the accessibility of the OH group to the ester bonds (arrows of different thickness). When the temperature increases, the polymer is more flexible at the accessibility of the terminal OH of within the polymer changes. Closer ester groups are thus more prone to reaction. Consequently, the distribution of oligomers is enriched in shorter species compared to 40°C. The reported proportions are only illustrative.

In a subsequent step, we investigated the release of hydrolysis products from PCL particles in other aqueous solutions containing various electrolytes at a constant temperature of 40°C. We first compared the degradation of PCL in pure water and in PBS. The purpose was to determine whether the well-known acidification of deionized water [49], which may result from the saturation in CO₂, could affect our results. In addition, PBS exhibits an ionic strength and a pH close to physiological

conditions and may be an appropriate medium to foresee the fate of PCL particles *in vivo*. A similar yield of hydrolysis products was determined in the aqueous phase of both types of suspension after aging. Another study was performed in seawater, a medium highly relevant to the environmental pollution by micro- and nanoplastics [50-52]. The yield of oligomer release was 2.5 times larger in seawater than that in pure water. This result is in agreement with other studies that characterized the degradation of the bulky fraction of PCL [51-55]. These results can be explained by the high salt concentration in seawater (465 mM in Na⁺ in seawater compared to 137 mM in PBS). It must also be pointed out that seawater is characterized by the presence of divalent cations that are absent from buffered salt solutions like the PBS that was used in this study. A major contribution of these species can thus be proposed. Interesting observations can be made regarding the size distribution of the oligomers. First, the proportions of oligomers extrapolated at t₀h are similar in water and in seawater. As explained above, these values reflect the initial distribution of oligomers after hydrolysis of the particles. The fact that they are similar in both media is likely because both experiments were performed at the same temperature. In contrast, the conversion of the longest oligomers into the shortest ones is much faster in seawater than in pure water. It may be concluded that the content in PCL hydrolysis products is accounted for by two processes: the degradation of the particles followed by the hydrolysis of the released oligomers into shorter species. The first step is mostly temperature-dependent while the second is strongly modulated by the nature of the medium. Accordingly, the degradation yields of the synthetic oligomers of different sizes were found to be similar in water at 20°C and 60°C. When comparisons were made at the same temperature in water and seawater, much larger values of reaction rates were obtained in the latter medium. Interestingly, the data obtained with oPCL in seawater, and to a lesser extent in water, showed that the longest oligomers exhibited a strong degradation rate while the shortest one, and

in particular the monomer, were readily produced. These observations provided further support for a contribution of the hydrolysis of the products initially released from the PCL particles to the final size distribution (Scheme 3).



Scheme 3: Proposed mechanism of release of hydrolysis product in the soluble fraction of aged suspensions of PCL particles. The first step (red arrows) is the cleavage of ester bonds within the polymer. The second step (green arrows) corresponds to the hydrolysis of the oligomers (symbolized by straight lines of different size) produced in the first step. Step 1 is dependent on the temperature while step 2 strongly depends on the composition of the aqueous solution as shown by experiments in seawater.

After these experiments in aqueous suspension, we investigated the release of hydrolysis products from PCL under cell culture conditions. PCL is known to be hydrolyzed not only by chemical reactions but also by biotic processes involving enzymes such as esterases or lipases [56]. This property is one of its advantages over non-biodegradable plastics. This topic of the biodegradability of plastics is often discussed in terms of microbial activity [57]. However, the toxic impact of PCL degradation products is also of importance in human, in particular with respect to ingestion. Consequently, numerous *in vitro* studies are performed with intestinal models such as the Caco-2 cell line. We thus extended our investigation of the modulation of the degradation of PCL in different aqueous solution to cell culture media, in the presence or absence of cells. Similar works have been reported [58, 59] but focused on the degradation of the polymer rather

than the release of hydrolysis products. Additional data were also provided by the study of the degradation of pure synthetic oligomers.

A general comment is that the size distribution in all the experiments under cell culture conditions was shifted toward shorter species compared to water, PBS and seawater. Under the latter conditions, detected products were larger than the trimer, while in culture medium, the major oligomers were the monomer or the dimer. Accordingly, a first observation is that large amounts of PCL monomer were detected in medium soon after the preparation of the suspensions. This likely results from pre-existing hydrolysis products of particles present in the PCL stock solution. The concentration in oligomers, and mostly monomer, then rapidly increased, reaching a factor of 5 at 48h compared to the initial amount. Surprisingly, the same observation was made in both the presence and the absence of cells. These results suggest a role for some components of the medium on the degradation of PCL and of the oligomers. Interestingly, release of hydrolysis products was no longer observed when particles were incubated in medium without FCS. This provides evidence for active participation of FCS in particle degradation. Accordingly, serum has been shown to contain lipase activities [60] that can degrade PCL particles and hydrolyze PCL oligomers in short fragments [22, 61-63]. Lipase and esterase activities were also identified in Caco-2 cells [64, 65] but our observation of similar amounts of oligomers in the medium independently of the presence of cells shows that these cellular activities do not contribute to a large extent to our results. It remains to be shown whether PCL hydrolysis products released within the cells can be extracted. A major input of our study is the necessity to consider the artefactual culture medium-mediated degradation processes in the study of the cellular impact of plastics micro- and nanoparticles.

5. CONCLUSION

In summary, the developed analytical tools first provided a series of data on the hydrolytic degradation of PCL that point to specificities at the sub-micrometric scale. We observed a lack of influence of the particle specific area, in contrast to reports on bulk materials. We also established that the distribution of the oligomers present in aged aqueous suspensions depends both on the hydrolysis of the particles and on the degradation of the resulting oligomers in solution. The former process is strongly influenced by the temperature while the latter depends mostly on the presence of electrolytes. We also gathered information on the degradation of PCL particles and oligomers in cultures medium of human intestinal cells and obtained evidence for efficient acellular enzyme-based degradation processes.

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Note

The authors declare no competing financial interest.

▪ ABBREVIATIONS

6-OH-HAc, 6-hydroxy hexanoic acid; ACN, acetonitrile; CI-ion, confirmation ion; HPLC-MS/MS, high performance liquid chromatography coupled to tandem mass spectrometry; MS1, single stage mass spectrometry analysis; MS2, fragmentation mass spectrometry analysis; PCL, polycaprolactone; PLA, polylactic acid; QI-ion, quantification ion.

▪ REFERENCES

- [1] OECD (2022) Global plastics outlook: economic drivers, environmental impacts and policy options, OCDE Publishing, Paris. <https://doi.org/10.1787/de747aef-en>
- [2] Plastics Europe (2024) The circular economy for plastics - A European analysis.
- [3] OECD (2024) Policy scenarios for eliminating plastic pollution by 2040, OCDE Publishing, Paris. <https://doi.org/10.1787/76400890-en>.
- [4] S.S. Ali, T. Elsamahy, E. Koutra, M. Kornaros, M. El-Sheekh, E.A. Abdelkarim, D.C. Zhu, J.Z. Sun, Degradation of conventional plastic wastes in the environment: A review on current status of knowledge and future perspectives of disposal, *Sci. Total Environ.* 771 (2021) 144719. <https://doi.org/10.1016/j.scitotenv.2020.144719>.
- [5] J.P. da Costa, A. Avellan, C. Mouneyrac, A. Duarte, T. Rocha-Santos, Plastic additives and microplastics as emerging contaminants: Mechanisms and analytical assessment, *TrAC Trend Anal Chem* 158 (2023) 116898. <https://doi.org/10.1016/j.trac.2022.116898>.
- [6] J. Augusta, R.J. Muller, H. Widdecke, Biodegradable polymers - Test methods and assessment standards, *Chem. Ing. Tech.* 64 (1992) 410-415. <https://doi.org/10.1002/cite.330640504>.
- [7] R. Kumar, K. Sadeghi, J. Jang, J. Seo, Mechanical, chemical, and bio-recycling of biodegradable plastics: A review, *Sci Total Environ* 882 (2023) 163446. <https://doi.org/10.1016/j.scitotenv.2023.163446>.
- [8] T.D. Moshood, G. Nawanir, F. Mahmud, Sustainability of biodegradable plastics: a review on social, economic, and environmental factors, *Crit Rev Biotechnol* 42 (2022) 892–912. <https://doi.org/10.1080/07388551.2021.1973954>.
- [9] T.D. Moshood, G. Nawanir, F. Mahmud, F. Mohamad, M.H. Ahmad, A. AbdulGhani, Sustainability of biodegradable plastics: New problem or solution to solve the global plastic pollution?, *Current Res Green Sustainable Chem* 5 (2022) 100273. <https://doi.org/10.1016/j.crgsc.2022.100273>.
- [10] M.C. Shen, B. Song, G.M. Zeng, Y.X. Zhang, W. Huang, X.F. Wen, W.W. Tang, Are biodegradable plastics a promising solution to solve the global plastic pollution?, *Environment Pollution* 263 (2020) 114469. <https://doi.org/10.1016/j.envpol.2020.114469>.

- [11] B. Gewert, M.M. Plassmann, M. MacLeod, Pathways for degradation of plastic polymers floating in the marine environment, *Environ Sci-Proc Imp* 17 (2015) 1513–1521. <https://doi.org/10.1039/c5em00207a>.
- [12] W. Ali, H. Ali, S. Gillani, P. Zinck, S. Souissi, Polylactic acid synthesis, biodegradability, conversion to microplastics and toxicity: a review, *Environ. Chem. Lett.* 21 (2023) 1761–1786. <https://doi.org/10.1007/s10311-023-01564-8>.
- [13] R.A. Ilyas, S.M. Sapuan, M.M. Harussani, M. Hakimi, M.Z.M. Haziq, M.S.N. Atikah, M.R.M. Asyraf, M.R. Ishak, M.R. Razman, N.M. Nurazzi, M.N.F. Norrrahim, H. Abral, M. Asrofi, Polylactic Acid (PLA) Biocomposite: processing, additive manufacturing and advanced applications, *Polymers-Basel* 13 (2021) 1326. <https://doi.org/10.3390/polym13081326>.
- [14] M.A. Woodruff, D.W. Hutmacher, The return of a forgotten polymer-Polycaprolactone in the 21st century, *Prog Polym Sci* 35 (2010) 1217–1256. <https://doi.org/10.1016/j.progpolymsci.2010.04.002>.
- [15] C.I. Idumah, Poly (α -caprolactone) (PCL) biopolymeric bionanoarchitectures for tissue engineering applications, *Int. J. Polym. Mater. Polym. Biomat.* (2024) 2372795. <https://doi.org/10.1080/00914037.2024.2372795>.
- [16] M. Thakur, I. Majid, S. Hussain, V. Nanda, Poly(ϵ -caprolactone): A potential polymer for biodegradable food packaging applications, *Packag. Technol. Sci.* 34 (2021) 449–461. <https://doi.org/10.1002/pts.2572>.
- [17] F. Afkhami, H. Eslami, A. Sabzevari, M. Ansari, Fabrication and characterization of injectable nanocomposite hydrogel containing PEG-PCL-PEG copolymer/Montmorillonite nanoparticles for bone tissue regeneration, *Arab J Sci Eng* (2025). <https://doi.org/10.1007/s13369-024-09912-8>.
- [18] A.D. Firoozabadi, H. Eslami, A. Sabzevari, M. Ansari, Removal of tetracycline antibiotic as a hospital waste by ph-sensitive degradable composite hydrogel using Fenton-like system, *J Polym Sci* 63 (2025) 1236–1248. <https://doi.org/10.1002/pol.20240945>.
- [19] Y. Kaviani, H. Eslami, M. Ansari, S.A. Poursamar, 3D printed magnetoactive nanocomposite scaffolds for bone regeneration, *Biomed. Mater.* 20 (2025). <https://doi.org/10.1088/1748-605X/ad9f04>.
- [20] M. Labet, W. Thielemans, Synthesis of polycaprolactone: a review, *Chem. Soc. Rev.* 38 (2009) 3484–3504. <https://doi.org/10.1039/b820162p>.
- [21] M. Bartnikowski, T.R. Dargaville, S. Ivanovski, D.W. Hutmacher, Degradation mechanisms of polycaprolactone in the context of chemistry, geometry and environment, *Prog Polym Sci* 96 (2019) 1–20. <https://doi.org/10.1016/j.progpolymsci.2019.05.004>.
- [22] S. Mirtabaei, M. Mollajavadi, M. Ketabi, The pore size effect on the degradation, tensile properties and cell viability of polycaprolactone/starch scaffold: experimental study, *Iran. Polym. J.* 34 (2025) 571–581. <https://doi.org/10.1007/s13726-024-01397-4>.
- [23] J. Anaya-Mancipe, A. de Figueiredo, L. Rabello, M. Dias, R. Thiré, Evaluation of the polycaprolactone hydrolytic degradation in acid solvent and its influence on the electrospinning process, *J Appl Polym Sci* 141 (2024) 16. <https://doi.org/10.1002/app.55662>.
- [24] M. Kupczak, A. Mielanczyk, D. Neugebauer, The influence of polymer composition on the hydrolytic and enzymatic degradation of polyesters and their block copolymers with PDMAEMA, *Materials* 14 (2021) 10. <https://doi.org/10.3390/ma14133636>.

- [25] L. del-Mazo-Barbara, J. Gómez-Cuyàs, L. Martínez-Orozco, O. Pérez, E. Bou-Petit, M. Ginebra, In vitro degradation of 3D-printed polycaprolactone\biomimetic hydroxyapatite scaffolds: Impact of the sterilization method, *Polym Test* 139 (2024) 15. <https://doi.org/10.1016/j.polymertesting.2024.108566>.
- [26] H. Ramaraju, A. Verga, B. Steedley, A. Kowblansky, G. Green, S. Hollister, Investigation of the biodegradation kinetics and associated mechanical properties of 3D-printed polycaprolactone during long-term preclinical testing, *Biomaterials* 321 (2025) 14. <https://doi.org/10.1016/j.biomaterials.2025.123257>.
- [27] N. Erdal, G. Lando, A. Yadav, R. Srivastava, M. Hakkarainen, Hydrolytic Degradation of Porous Crosslinked Poly(ϵ -Caprolactone) Synthesized by high internal phase emulsion templating, *Polymers-Basel* 12 (2020) 14. <https://doi.org/10.3390/polym12081849>.
- [28] A. Høglund, M. Hakkarainen, A. Albertsson, Degradation profile of poly(ϵ -caprolactone) -: the influence of macroscopic and macromolecular biomaterial design, *J Macromol Sci Part A-Pure Appl Chem* 44 (2007) 1041–1046. <https://doi.org/10.1080/10601320701424487>.
- [29] I. Castilla-Cortázar, J. Más-Estellés, J. Meseguer-Dueñas, J. Ivirico, B. Marí, A. Vidaurre, Hydrolytic and enzymatic degradation of a poly(ϵ -caprolactone) network, *Polym. Degrad. Stabil.* 97 (2012) 1241–1248. <https://doi.org/10.1016/j.polymdegradstab.2012.05.038>.
- [30] C. Lam, M. Savalani, S. Teoh, D. Hutmacher, Dynamics of in vitro polymer degradation of polycaprolactone-based scaffolds:: accelerated versus simulated physiological conditions, *Biomed. Mater.* 3 (2008) 15. <https://doi.org/10.1088/1748-6041/3/3/034108>.
- [31] M. Jenkins, K. Harrison, The effect of crystalline morphology on the degradation of polycaprolactone in a solution of phosphate buffer and lipase, *Polym. Adv. Technol.* 19 (2008) 1901–1906. <https://doi.org/10.1002/pat.1227>.
- [32] L. Bosworth, S. Downes, Physicochemical characterisation of degrading polycaprolactone scaffolds, *Polym. Degrad. Stabil.* 95 (2010) 2269–2276. <https://doi.org/10.1016/j.polymdegradstab.2010.09.007>.
- [33] B. James, A. Medvedev, S. Makarov, R. Nelson, C. Reddy, M. Hahn, Moldable plastics (polycaprolactone) can be acutely toxic to developing zebrafish and activate nuclear receptors in mammalian cells, *ACS Biomater. Sci. Eng.* 10 (2024) 5237–5251. <https://doi.org/10.1021/acsbiomaterials.4c00693>.
- [34] C. Gong, Y. Gu, X. Wang, C. Yi, Oligomer Content Determines the Properties and Application of Polycaprolactone, *Macromolecules* 55 (2022) 5342–5352. <https://doi.org/10.1021/acs.macromol.2c00275>.
- [35] C.H. Gong, J.X. Li, C.W. Yi, S.L. Qu, Catalytic Regulation of oligomers in polycaprolactone, *Mol Catal* 508 (2021). <https://doi.org/10.1016/j.mcat.2021.111594>.
- [36] T. Douki, V. Bard, M. Boulée, M. Carrière, Extensive HPLC tandem mass spectrometry characterization of soluble degradation products of biodegradable nanoplastics under environmentally relevant temperature and irradiation conditions, *Environment Sci.Nano* 11 (2024) 3956-3965. <https://doi.org/10.1039/d3en00960b>.
- [37] A. Shrivastava, V. Gupta, Methods for the determination of limit of detection and limit of quantitation of the analytical methods, *Chronicles of Young Scientists* 2 (2011) 21. <https://doi.org/10.4103/2229-5186.79345>.

- [38] International Organization for Standardization (2023) *Plastics — Methods of exposure to laboratory light sources Part 2: Xenon-arc lamps*, ISO 4892-2:2013, International Organization for Standardization.
- [39] B. Wu, X.D. Feng, W. Du, Z.X. Li, G. Qian, X.Z. Duan, X.G. Zhou, Z. Liu, J. Zhang, Continuous synthesis of ϵ -caprolactone in a microreactor and kinetics insights into its side reactions, *J. Flow Chem.* 14 (2024) 337–347. <https://doi.org/10.1007/s41981-023-00301-0>.
- [40] M. Aznar, S. Ubeda, N. Dreolin, C. Nerín, 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* 1583 (2019) 1–8. <https://doi.org/10.1016/j.chroma.2018.10.055>.
- [41] P. Rizzarelli, S. Carroccio, Modern mass spectrometry in the characterization and degradation of biodegradable polymers, *Anal. Chim. Acta* 808 (2014) 18–43. <https://doi.org/10.1016/j.aca.2013.11.001>.
- [42] A. Chamas, H. Moon, J.J. Zheng, Y. Qiu, T. Tabassum, J.H. Jang, M. Abu-Omar, S.L. Scott, S. Suh, Degradation rates of plastics in the environment, *Acs Sustain Chem Eng* 8 (2020) 3494–3511. <https://doi.org/10.1021/acssuschemeng.9b06635>.
- [43] M. Suzuki, Y. Tachibana, K. Kasuya, Biodegradability of poly(3-hydroxyalkanoate) and poly(ϵ -caprolactone) via biological carbon cycles in marine environments, *Polym. J.* 53 (2021) 47–66. <https://doi.org/10.1038/s41428-020-00396-5>.
- [44] J.A. Tamada, R. Langer, Erosion kinetics of hydrolytically degradable polymers, *P Natl Acad Sci USA* 90 (1993) 552–556. <https://doi.org/10.1073/pnas.90.2.552>.
- [45] C. Shih, Chain-end scission in acid catalyzed hydrolysis of poly (d,l-lactide) in solution, *J Controlled Release* 34 (1995) 9–15. [https://doi.org/10.1016/0168-3659\(94\)00100-9](https://doi.org/10.1016/0168-3659(94)00100-9).
- [46] A. Leroux, T.N. Nguyen, A. Rangel, I. Cacciapuoti, D. Duprez, D.G. Castner, V. Migonney, Long-term hydrolytic degradation study of polycaprolactone films and fibers grafted with poly(sodium styrene sulfonate): Mechanism study and cell response, *Biointerphases* 15 (2020) 061006 <https://doi.org/10.1116/6.0000429>.
- [47] S.J. de Jong, E.R. Arias, D.T.S. Rijkers, C.F. van Nostrum, J.J. Kettenes-van den Bosch, W.E. Hennink, New insights into the hydrolytic degradation of poly(lactic acid): participation of the alcohol terminus, *Polymer* 42 (2001) 2795–2802. [https://doi.org/10.1016/S0032-3861\(00\)00646-7](https://doi.org/10.1016/S0032-3861(00)00646-7).
- [48] T. Ouhadi, C. Stevens, P. Teyssie, Study of poly- ϵ -caprolactone bulk degradation, *J Appl Polym Sci* 20 (1976) 2963–2970. <https://doi.org/10.1002/app.1976.070201104>.
- [49] L.A. Melnik, D.A. Krysenko, Ultrapure water: properties, production, and use, *J Water Chem Techno+* 41 (2019) 143–150. <https://doi.org/10.3103/S1063455x19030020>.
- [50] J.P. Da Costa, A.R. Nunes, P.S.M. Santos, A.V. Girao, A.C. Duarte, T. Rocha-Santos, Degradation of polyethylene microplastics in seawater: Insights into the environmental degradation of polymers, *J Environ Sci Heal A* 53 (2018) 866–875. <https://doi.org/10.1080/10934529.2018.1455381>.
- [51] A. Nakayama, N. Yamano, N. Kawasaki, Biodegradation in seawater of aliphatic polyesters, *Polym. Degrad. Stabil.* 166 (2019) 290–299. <https://doi.org/10.1016/j.polymdegradstab.2019.06.006>.

- [52] G.X. Wang, D. Huang, J.H. Ji, C. Völker, F.R. Wurm, Seawater-degradable polymers—fighting the marine plastic pollution, *Adv Sci* 8 (2020) 2001121. <https://doi.org/10.1002/adv.202001121>.
- [53] H. Tsuji, Environmental degradation of biodegradable polyesters 1. Poly(ϵ -caprolactone), poly[(R)-3-hydroxybutyrate], and poly(L-lactide) films in controlled static seawater, *Polym. Degrad. Stabil.* 75 (2002) 347–355. [https://doi.org/10.1016/S0141-3910\(01\)00240-3](https://doi.org/10.1016/S0141-3910(01)00240-3).
- [54] B. Lu, G. Wang, D. Huang, Z. Ren, X. Wang, Z. Zhen, W. Zhang, J. Ji, Comparison of PCL degradation in different aquatic environments: Effects of bacteria and inorganic salts, *Polym. Degrad. Stabil.* 150 (2018) 133–139. <https://doi.org/10.1016/j.polymdegradstab.2018.02.002>.
- [55] M. Rutkowska, M. Jastrzebska, H. Janik, Biodegradation of polycaprolactone in sea water, *React. Funct. Polym.* 38 (1998) 27–30. [https://doi.org/10.1016/s1381-5148\(98\)00029-7](https://doi.org/10.1016/s1381-5148(98)00029-7).
- [56] J.H. Lai, H.Q. Huang, M.W. Lin, Y.Q. Xu, X.T. Li, B.G. Sun, Enzyme catalyzes ester bond synthesis and hydrolysis: The key step for sustainable usage of plastics, *Front Microbiol* 13 (2023) 1113705. <https://doi.org/10.3389/fmicb.2022.1113705>.
- [57] G. Lear, S.D.M. Maday, V. Gambarini, G. Northcott, R. Abbel, J.M. Kingsbury, L. Weaver, J.A. Wallbank, O. Pantos, Microbial abilities to degrade global environmental plastic polymer waste are overstated, *Environ. Res. Lett.* 17 (2022) 043002. <https://doi.org/10.1088/1748-9326/ac59a7>.
- [58] A. Yeo, B. Rai, E. Sju, J.J. Cheong, S.H. Teoh, The degradation profile of novel, bioresorbable PCL-TCP scaffolds: an in vitro and in vivo study, *J Biomed Mater Res A* 84a (2008) 208–218. <https://doi.org/10.1002/jbm.a.31454>.
- [59] C.X.F. Lam, D.W. Hutmacher, J.T. Schantz, M.A. Woodruff, S.H. Teoh, Evaluation of polycaprolactone scaffold degradation for 6 months in vitro and in vivo, *J Biomed Mater Res A* 90a (2009) 906–919. <https://doi.org/10.1002/jbm.a.32052>.
- [60] N.W. Tietz, D.F. Shuey, Lipase in serum - the elusive enzyme - an overview, *Clin Chem* 39 (1993) 746–756. <https://doi.org/10.1093/clinchem/39.5.746>.
- [61] Y. Tokiwa, T. Suzuki, K. Takeda, 2 types of lipases in hydrolysis of polyester, *Agricult Biol Chem* 52 (1988) 1937–1943. <https://doi.org/10.1080/00021369.1988.10868966>.
- [62] M. Mochizuki, M. Hirano, Y. Kanmuri, K. Kudo, Y. Tokiwa, Hydrolysis of polycaprolactone fibers by lipase - Effects of draw ratio on enzymatic degradation, *J Appl Polym Sci* 55 (1995) 289–296. <https://doi.org/10.1002/app.1995.070550212>.
- [63] B. Almeida, P. Figueiredo, A. Carvalho, Polycaprolactone enzymatic hydrolysis: a mechanistic study, *ACS Omega* 4 (2019) 6769–6774. <https://doi.org/10.1021/acsomega.9b00345>.
- [64] T. Imai, M. Imoto, H. Sakamoto, M. Hashimoto, Identification of esterases expressed in Caco-2 cells and effects of their hydrolyzing activity in predicting human intestinal absorption, *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 33 (2005) 1185–1190. <https://doi.org/10.1124/dmd.105.004226>.
- [65] J.H. Spalinger, E.G. Seidman, D. Ménard, E. Levy, Endogenous lipase activity in Caco-2 cells, *Biochim Biophys Acta* 1393 (1998) 119–127. [https://doi.org/10.1016/S0005-2760\(98\)00068-X](https://doi.org/10.1016/S0005-2760(98)00068-X).