GREEN ROUTES FOR THE SYNTHESIS OF INNOVATIVE BIODEGRADABLE OLIGOESTERS AND OLIGOESTERAMIDES

PhD Thesis – Summary

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by

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Abstract

The increasing global focus on environmental sustainability has led to an increased interest in synthesizing bio-based polymers and oligomers, using renewable resources and biocatalytic pathways for the synthetic process. Two of the most important classes of compounds in this regard are the class of polyesters and polyesteramides. Polyesteramides are especially of great interest nowadays since they combine the biodegradability and biocompatibility of polyesters with the robust thermal and mechanical properties of polyesteramides, the oligoesteramides being highly valuable compounds for various industrial and biomedical applications.

The two main objectives of this thesis were the enzymatic in vitro synthesis and comprehensive characterization of novel co-oligoesters of *ε*-caprolactone with bio-based hydroxy acids and amino acids, and the development and optimization of biocatalytic synthetic routes for innovative aliphatic and aromatic oligoesters and oligoesteramides derived from either *ε*-caprolactone or *ε*-caprolactam. A number of 14 new compounds were obtained *via* a green synthetic route and the characterization of the compounds was performed utilizing modern techniques such as MALDI TOF-MS mass spectrometry, size-exclusion chromatography (SEC), ^{1}H, ^{13}C, and 2D NMR spectroscopy, FT-IR spectroscopy or TG and DSC thermal analysis. All the synthetized compounds exhibited biodegradability properties in the presence of a Bega River inoculum from Timişoara, Romania.

The PhD thesis is developing the theme of bio-based oligoesters and oligoesteramides over four distinct sections, namely:

1. Literature Review: This section outlines the current research landscape in the synthesis of bio-based oligomers using enzymes as catalysts. The European Standard EN 16575 classifies bio-based materials as those derived from biomass, whereas biodegradable polymers are defined by their ability to decompose through microbial action in natural environments; furthermore, the term "biopolymer" broadly includes both biodegradable and non-biodegradable polymers sourced from biological origins, highlighting their significance in promoting environmental sustainability (Figure 1) [1]. Transitioning to renewable bio-based materials for polymer and oligomer synthesis is not only a sustainable approach to minimizing our reliance on fossil fuels but also fosters eco-friendly production practices that benefit the environment [2], [3], [4].

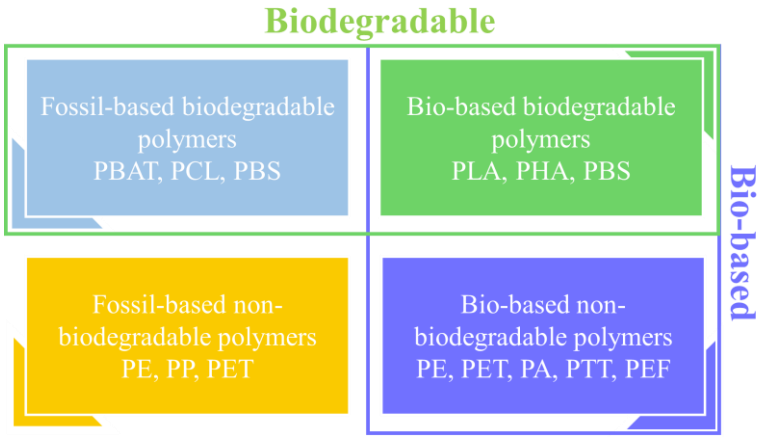

Figure 1. Classification of polymeric plastic materials based on the origin of the raw materials and their biodegradability property. Figure adapted from the *Synopsis report on the consultation on the policy framework on biobased, bio-degradable and compostable plastic* issued by the European Commission [1].

Furthermore, the use of enzymatic biocatalysis as a green alternative facilitates the precise synthesis of bio-based polymers or oligomers, unlocking a plethora of applications, especially in the biomedical field, where innovative solutions such as drug delivery systems are in high demand [2], [5], [6], [7]. The synthesis of bio-based polymers through biocatalytic pathways presents a sustainable and eco-friendly method, leveraging versatile enzymes such as lipases to achieve precise and well-defined polymer structures [8]. Bio-based aliphatic polyesters have been are important in the polymeric fields due to their biodegradability properties and the relative abundance of aliphatic monomers derived from renewable resources [9], [10]. In addition, polyesteramides possess both ester and amide bonds in their structure, providing a unique combination of properties [11]. The ester bonds contribute to the biodegradability and biocompatibility characteristic of polyesters, while the amide bonds impart the enhanced thermal and mechanical properties typical of polyamides [12]. Their synthesis *via* biocatalysis adds an additional layer of sustainability, aligning well with the growing emphasis on green chemistry and environmentally friendly manufacturing processes .

2. Original Contributions: This part presents the research findings, beginning with the thesis objectives. The focus is on the development and assessment of biocatalytic systems for producing oligoesters or oligoesteramides from renewable raw materials. Emphasizing green chemistry principles and the advantages of enzymatic catalysts, the experimental section of the thesis is exploring new synthetic pathways for obtaining novel materials from renewable feedstocks with various applications. This approach is aligning with the sustainability criteria driving the current progression of the bioeconomy.

2.1. Biocatalytic approach for novel functional oligoesters of ε-caprolactone and malic acid

The objective of this chapter was to enzymatically synthesize innovative functional oligoesters using ε-caprolactone (ε-CL) and D,L/L-malic acid (D,L-MA/L-MA) as primary building blocks. The end products of this reaction consisted of linear and cyclic co-oligomers that incorporated ε-CL and MA units. Notably, the integration of malic acid unit, characterized by pendant carboxyl groups, into the polycaprolactone oligomeric structure significantly expands the potential applications of these materials in various fields.

To achieve this, the catalytic process employed lipase B from *Candida antarctica*,

which was immobilized on acrylic resin (commonly known as Novozyme 435) or microporous ion-exchange resin (referred to as GF-CalB-IM). The reactions were executed at a temperature of 80 °C, a choice designed to maintain the biocatalyst's activity during extended reaction periods, allowing for effective multiple recycling cycles of the enzyme.

The structural analysis of the synthesized oligoesters was performed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and infrared (IR) spectroscopy. Additionally, the thermal characteristics of the products were investigated through thermogravimetric (TG) analysis and differential scanning calorimetry (DSC). Key parameters influencing product formation were also systematically studied, particularly focusing on the effect of the ε-caprolactone to malic acid (MA) molar ratio, alongside the impact of organic solvents on the synthesis process.

The successful formation of co-oligoesters was confirmed by MALDI-TOF MS analysis (Figure 2), which allows the identification of cyclic and non-cyclic reaction products while also facilitating the determination of the chain lengths and their dispersity. The effect of varying the molar ratio of ε-CL to MA on the formation and composition of the oligomeric products was analyzed. Upon utilizing ε-CL in excess the conversions and relative content of co-oligomers increased. The operational stability of the biocatalysts, namely Novozyme 435 and GF-CalB-IM, was further assessed through multiple reaction cycles, consistently demonstrating remarkable stability throughout the process.

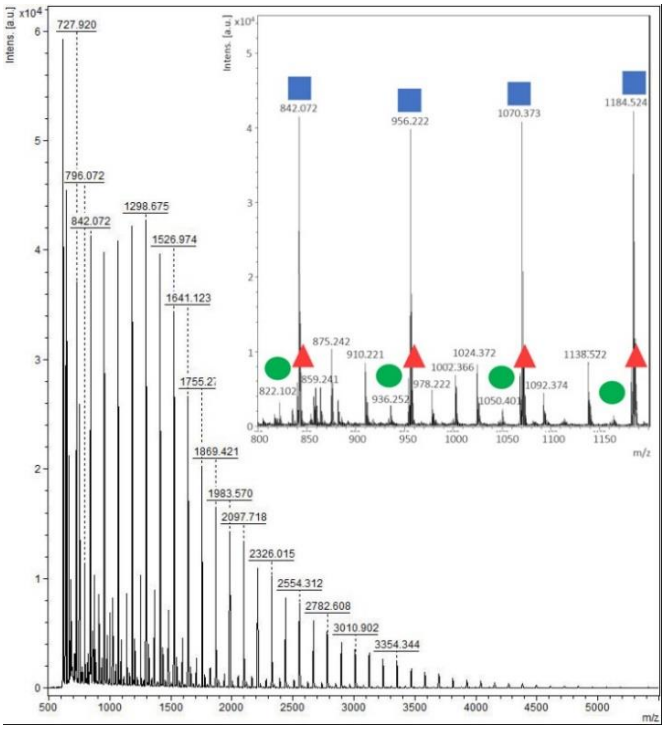

Figure 2. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF-MS) spectrum of reaction products synthesized from ε-caprolactone (ε-CL) and L-malic acid (L-MA) at a 2:1 molar ratio using GF-CalB-IM as the biocatalyst in solventless system at 80 °C. Inset: Detailed view of the *m/z* range 800–1200, highlighting the oligomers of interest (■ are linear oligoesters with 1 unit of MA, ● are cyclic oligoesters with 1 unit of MA, and ▲ are linear oligoesters with 2 units of MA).

For analyzing the influence of organic solvents on the enzymatic synthesis, the study incorporated solvents such as acetonitrile, toluene, and the eco-friendlier solvent 2-methyl-tetrahydrofuran (Me-THF) into the reaction scheme. The results indicated that the highest relative co-oligomer content was achieved within the solventless system, followed closely by the Me-THF environment. The temporal dynamics of the co-oligomerization of ε-caprolactone

with malic acid indicated that the peak conversion of malic acid occurred at around 24 hours of reaction time.

Further characterization of the resulting oligomer products using MALDI-TOF MS and FTIR spectroscopy provided compelling structural evidence supporting the formation of oligoesters. Thermal stability assessments of the oligoesters derived from malic acid and ε-CL were conducted through TG and DSC analyses, revealing that the co-oligomer exhibited diminished thermal stability compared to the polycaprolactone (PCL) homo-oligomer. The DSC results also highlighted that the co-oligomer was categorized as semicrystalline, which could influence its potential applications.

In summary, the synthesis of novel functional oligoesters using a green and sustainable process was achieved, demonstrating excellent operational stability of the biocatalysts throughout multiple reaction cycles. The thermal properties and structural characteristics of the reaction products were thoroughly investigated, elucidating the attributes that make these materials suitable for a variety of applications. Overall, the insights garnered from this study significantly contribute to the field of enzymatic synthesis of oligoesters, emphasizing the impact of selected reaction parameters on both the formation and properties of the resultant products. This work presents potential pathways for further innovations in the development of sustainable polymeric materials.

2.2. Biocatalytic synthesis of new oligoesteramides from ε-caprolactam and hydroxy acids

This chapter focuses on the environmentally friendly synthesis of novel oligomeric structures using *in vitro* biocatalysis and renewable starting materials. Specifically, the study investigates the production of innovative oligoesteramides (OEAs) synthesized from ε-caprolactam and four different hydroxy acids: L-malic acid, 3-hydroxybutyric acid, 16-hydroxyhexadecanoic acid, and 12-hydroxystearic acid. Utilizing a range of hydroxy acids introduces significant structural variety into the oligomeric products, which is expected to influence both the polymerization process and the properties of the resulting materials.

To synthesize these compounds, *Candida antarctica* lipase B (CalB), immobilized by adsorption, was selected as the biocatalyst. This choice stemmed from screening studies that identified it as a highly effective enzyme for such organic transformations, matching previous results regarding the synthesis of oligomers from ε-caprolactone. The reactions were conducted at a temperature of 80 °C, a condition intended to sustain the activity of the biocatalyst for prolonged periods, facilitating multiple recycle opportunities. Notably, this approach also allows for a reliable comparison between solventless systems and organic solvent-based systems.

The primary objective of this research was not to achieve specific molecular weights for the synthesized oligoesteramides, but rather to demonstrate the viability of using biocatalytic methods for their synthesis and to evaluate the properties of the resulting oligomers. Accordingly, the chapter also delves into exploring the influence of key reaction parameters such as the molar ratios of ε-caprolactam and hydroxy acids, the effect of organic solvents on the synthesis, and the reaction duration.

The presence of both ester and amide functionalities in the oligomeric structures was confirmed through multiple techniques including MALDI-TOF MS, FT-IR, and NMR spectroscopy. Size exclusion chromatography (SEC) played a crucial role in assessing average molecular weights and monomer conversion rates, while thermogravimetric (TG) and differential scanning calorimetry (DSC) analyses were conducted to evaluate the thermal properties of the synthesized oligoesteramides.

In the preliminary phases of the study, a screening process was carried out to determine the most suitable biocatalyst for the synthesis of oligoesteramides. As these reactions were

being conducted for the first time, it was imperative to identify an effective biocatalyst. The enzymatic reactions were examined under varying conditions to ascertain the formation of linear and cyclic co-oligomers. The characterization of the reaction products was achieved using MALDI-TOF MS analysis, which provided vital information regarding the molecular species formed during polymerization. For example, the MALDI-TOF MS spectrum of reaction products obtained from the polymerization of ε-caprolactam and 16-hydroxyhexadecanoic acid at a 1:1 molar ratio, conducted in a solventless environment at 80 °C for 24 hours, indicated the presence of cyclic products containing multiple ε-caprolactam units, along with linear co-oligomers.

As the oligomer synthesis progressed, the evaluation of molecular weight and polymerization degrees became increasingly pertinent. Size exclusion chromatography (SEC) was employed to quantify these parameters. Several key reaction parameters were systematically evaluated to ascertain their influence on the synthesis of oligoesteramides. Various molar ratios of *ε*-caprolactam and hydroxy acids were tested, specifically examining ratios of 2:1, 1:1, and 1:2 in both solventless and organic solvent environments. The results examining a solventless system are present in Figure 3, revealing that, when the enzymatic reaction was tested without a solvent the average molecular weights were relatively stable across the molar ratios.

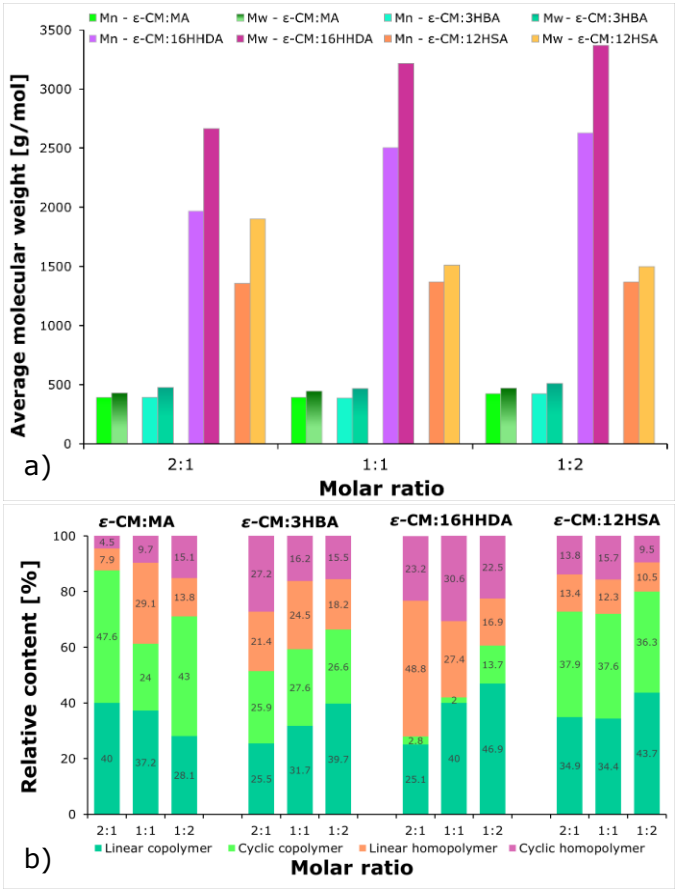

Figure 3. Influence of the molar ratio on the average molecular weights of the co-oligomers (**a**) and on the relative content of the reaction products obtained in the polymerization reaction of *ε*-caprolactam (*ε*-CM) with L-malic acid (MA), 3-hydroxybutyric acid (3HBA), 16-hydroxyhexadecanoic acid (16HHDA), 12-hydroxystearic acid (12HSA), respectively, at 80 °C, 24 h, under solventless conditions, in the presence of Novozyme 435 as the catalyst (**b**).

However, for systems incorporating the hydroxy acids (MA and 3HBA), a slight increase in average molecular weights was discerned when the hydroxy acid was used in excess,

influencing the co-oligomeric structure. This phenomenon can be attributed to the greater molecular weight of hydroxy acid monomers compared to ε-caprolactam. Conversely, when employing 12-hydroxystearic acid as the co-monomer, the restrained reactivity from ε-caprolactam caused the average molecular weights to be higher when ε-caprolactam was present in excess. This underscores a trend whereby specific structural characteristics of the hydroxy acids dictate their polymerization dynamics. The relative composition of reaction products demonstrated significant variations depending on the specific hydroxy acid used. Notably, L-malic acid yielded the highest co-oligomer content due to its lower reactivity and short carbon chain, while other hydroxy acids resulted in co-oligomer contents ranging from 60% to 80% when used in excess.

The operational stability of Novozyme 435 was a focal point of the study. The results indicated that the biocatalyst retained its effectiveness over multiple reaction cycles. The findings indicate that the biocatalyst maintains comparable performance even after undergoing 8 consecutive reaction cycles (Figure 4). This analysis provided insights into the biocatalyst's operational efficiency, suggesting that Novozyme 435 can sustain activity over numerous cycles without significantly degrading performance, thus holding promise for scaling the enzymatic synthesis of oligoesteramides to a larger scale.

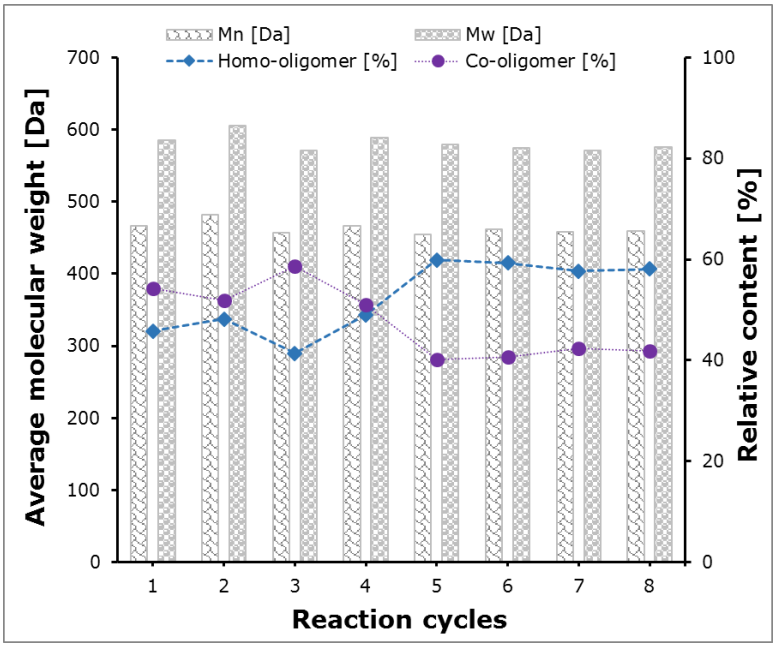

Figure 4. The operational stability of the Novozyme 435 and biocatalyst after 8 successive reaction cycles for the synthesis of oligoesteramides based on ε-caprolactam (ε-CM) and 3-hydroxybutyric acid (3HBA) obtained at 80°C, 24h, in a solvent-free system, at a molar ratio ε-CM:3HBA of 1:1.

Characterization of synthesized oligoesteramides employed various techniques, including MALDI-TOF MS, FT-IR, and NMR analysis, revealing a wealth of structural information. The MALDI-TOF MS spectra allowed for the identification of different oligomeric structures, confirming the presence of linear and cyclic co-oligomers, as well as oligomers stemming solely from the hydroxy acid raw materials. FT-IR spectroscopy further confirmed the chemical architecture of oligoesteramides, with observed shifts in fundamental absorption bands confirming the formation of ester and amide bonds during the synthesis. For instance, in the spectrum of the oligomer derived from ε-caprolactam and 16-hydroxyhexadecanoic acid, the characteristic bands representing C=O groups were found to shift significantly, indicating successful interactions between the co-monomers. NMR analysis

demonstrated the successful formation of both amide and ester linkages. The distinct chemical shifts observed for the oligomers aligned perfectly with those anticipated for the expected ester and amide functionalities.

To evaluate the thermal properties of the synthesized oligoesteramides, both thermogravimetric (TG) and differential scanning calorimetry (DSC) analyses were carried out in a controlled nitrogen atmosphere. This investigation revealed varying degradation profiles across different co-oligomers, with ε-CM and 16-hydroxyhexadecanoic acid exhibiting superior thermal stability compared to those with shorter-chain hydroxy acids. The thermal degradation characteristics were closely linked to the molecular weight of the synthesized products, with higher weight materials generally displaying enhanced thermal stability. An interesting observation was the increased degradation steps observed in materials comprising hydroxy fatty acids, indicating a complexity in thermal responses attributed to the presence of both ester and amide groups influencing stability.

This chapter delineates the successful enzymatic synthesis of novel oligoesteramides derived from ε-caprolactam and various hydroxy acids using an eco-friendly biocatalytic approach. Findings from the study highlight the importance of reaction parameters, such as molar ratios and solvent environments, in influencing the properties and behavior of the synthesized materials. Novozyme 435 emerged as the catalyst of choice, demonstrating significant potential for industrial scale-up due to its stability and efficiency. The oligoesteramides synthesized through this sustainable method exhibited promising thermal stability and structural characteristics, opening avenues for further exploration in applications across fields like nanomaterials and cosmetics. Overall, this synthesis and process optimization not only expands the scope of enzymatic polymerization but also emphasizes the growing potential of biocatalytic synthesis as a viable route toward developing innovative and sustainable materials.

2.3. Enzymatic synthesis and optimization of aromatic oligoesteramides starting from ε-caprolactam and 5-hydroxy-furancarboxylic acid

This chapter presents the synthesis of aromatic oligoesteramides synthetized through an enzymatic pathway that utilizes ε-caprolactam (ε-CM) and 5-hydroxymethyl-2-furancarboxylic acid (5HMFCA) as monomers. The polymerization reactions were conducted using a range of biocatalysts, including commercially available immobilized forms of *Candida antarctica* lipase B such as Novozyme 435 and GF-CalB-IM, as well as native enzymes immobilized in the laboratory through sol-gel entrapment methods. The experimental approach was designed to take place within a solvent-free system under conditions that included an equimolar ratio of ε-CM to 5HMFCA, a fixed reaction time of 24 hours, and a temperature maintained at 80°C, reflecting previously established optimal conditions for similar oligoesteramide syntheses.

MALDI-TOF MS analysis confirmed the successful incorporation of both ester and amide linkages into the oligomeric chains resulting from the polymerization process. The investigation into biocatalysts highlighted the efficiency of using sol-gel entrapment methods to stabilize lipases, contributing to higher yields of aromatic oligoesteramides with an observed relative co-oligomer content between 45% and 58% and average molecular masses ranging from 380 Da to 530 Da (Table 1). Both the commercially available and laboratory-entrapped lipases demonstrated capability in producing oligomer products (Table 2).

Table 1. The molecular weights and monomer conversion of the reaction products of ε-caprolactam with 5HMFCA, synthesized at 80 °C, 24 h reaction time in a solventless system, in the presence of various lipases as catalysts, determined by SEC.

Immobilized biocatalyst	Molar ratio ε-CM:HA	M_n [Da]	M_w [Da]	Đ[a]	ε-CM Conv. [%]	5HMFCA Conv. [%]
Candida antartica lipase B (Novozyme 435)	1:1	420	430	1.02	14.6	18.1
Candida antartica lipase B (GF-CalB-IM)	1:1	490	530	1.08	13.4	19.6
Candida antartica lipase B (sol-gel entrapment)	1:1	380	390	1.03	15.5	17.8
Pseudomonas stutzeri lipase (sol-gel entrapment)	1:1	460	470	1.02	14.4	19.0

[a]Đ-Dispersity.

Table 2. The relative composition of the reaction products of ε-caprolactam with 5HMFCA, synthesized at 80 °C, 24 h reaction time in a solventless system, in the presence of various lipases as catalysts, determined by MALDI-TOF MS.

Immobilized biocatalyst	Molar ratio ε-CM:HA	Relative content of the reaction products [%]				DP Max[f]
		LC[b]	CC[c]	LH[d]	CH[e]	
Candida antartica lipase B (Novozyme 435)	1:1	36	22	16	26	6
Candida antartica lipase B (GF-CalB-IM)	1:1	32	24	21	23	7
Candida antartica lipase B (sol-gel entrapment)	1:1	48	-	52	-	4
Pseudomonas stutzeri lipase (sol-gel entrapment)	1:1	22	23	23	32	5

[b]LC-Linear co-oligomer; [c]CC-Cyclic co-oligomer; [d]LH-linear homo-oligomer; [e]CH-Cyclic homo-oligomer; [f]DP Max_n- Maximal degree of polymerization of the co-oligomer.

Further research focused on dissecting the impact of the molar ratio of the monomers and the reaction medium on the resulting structure and composition of the oligoesteramides. Distinct settings for the enzymatic ring-opening polymerization were evaluated; these included both organic (toluene) and solventless systems. Comparable average molecular weights in toluene to were also recorded in solvent-free systems, with molecular weights ranging from 360 Da to 530 Da. The emphasis was placed on the total co-oligomer content, which differed significantly between the two environments. The highest relative oligoesteramide content reached 61% in solventless conditions, suggesting a favored reaction environment for enzymatic polymerization when not using solvents.

The detailed analysis of different molar ratios indicated that utilizing an excess of 5HMFCA favored co-oligomer formation, while a molar excess of ε-CM promoted co-oligomerization in the solventless environment. When examining the equimolar ratio, the resultant co-oligomer content was positioned within the intermediary range observed across both solvent conditions.

In an effort to optimize the synthesis process further, the Design of Experiments (DoE) methodology was applied, using Novozyme 435 as the primary enzyme. Employing the Box-

Behnken design via the Unscrambler software, three key parameters were systematically varied: temperature, biocatalyst proportion relative to the total weight of the monomers, and the molar ratio of the monomers. The range of experiments required comprised 15 trials, each carried out for a duration of 24 hours at 1200 rpm, with the aim of elucidating how these factors influenced the average molecular weights and the relative compositions of the synthesized products.

The optimization results are represented graphically in Figure 5, showcasing the combined impact of the various factors on the linear co-oligomer content and average molecular weight of the final product.

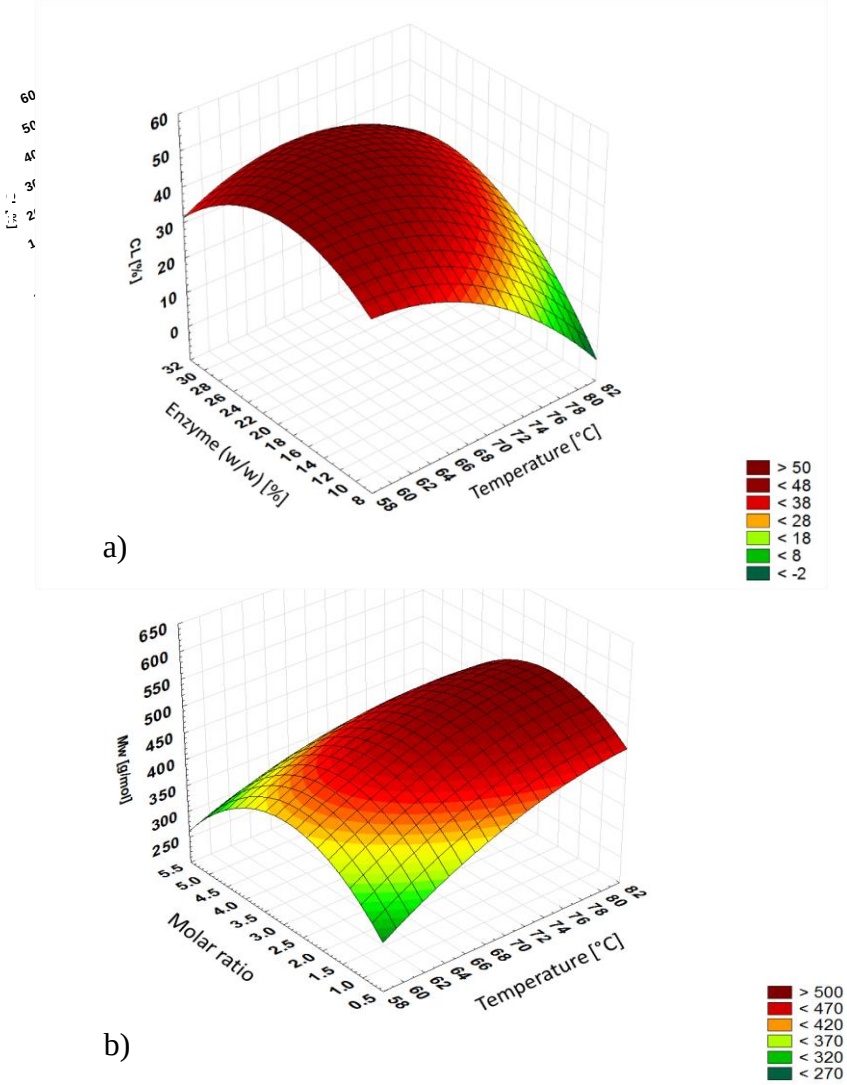

Figure 5. Combined effects of the reaction parameters on the composition and molecular weight of the enzymatically synthesized oligoesteramide product; (**a**) combined effect of the enzyme amount and temperature on the linear co-oligomer content (CL, %; (**b**) combined effect of the molar ratio of ε-CM:5HMFCA and temperature on the weight-average molecular weight (M_w).

Notably, while the overall impact of biocatalyst quantity was determined to be negligible. The optimal conditions were established, indicating that the highest linear co-oligomer content was achieved at approximately 72°C with a biocatalyst amount of 22% relative to the monomers. Furthermore, the data suggested that elevated average molecular weights were attained with a molar ratio of around 3.5:1 (ε-CM:5HMFCA) at temperatures exceeding 70°C.

In conclusion, the chapter offers comprehensive insights into the enzymatic synthesis

of aromatic oligoesteramides utilizing modern analytical techniques such as MALDI-TOF MS and SEC for characterization of the copolymerization products. The screening of biocatalysts, along with a focus on influential parameters, including the molar ratio of monomers and reaction media viscosity, demonstrated a minimal effect on the overall polymerization reaction. The effectiveness of applying the DoE statistical method in optimizing the synthesis process of ε-CM-based aromatic oligomers is facilitating superior outcomes in the generation of well-defined oligoesteramide structures.

2.4. Enzymatic synthesis of oligoesters and oligoesteramides starting from ε-caprolactone and amino acids

In this chapter, the synthesis of oligomers through the reaction of hydroxy acids or lactones with amino acids containing amino groups in their side chains was investigated. Specifically, the focus is on the enzymatic and chemical synthesis of oligoesteramides using ε-caprolactone (ε-CL) in conjunction with the amino acids lysine (Lys) and arginine (Arg). Additionally, serine (Ser) is employed as a co-oligoester to incorporate amino acid units into the oligomeric structures. The resulting possible reaction products include various linear and cyclic co-oligomers, alongside linear and cyclic homo-oligomers derived from both amino acids and ε-CL, produced as secondary outcomes.

The methodology employed two types of catalysts: the hydrolytic enzyme alcalase and the chemical catalyst stannous octoate ($Sn(Oct)_2$). MALDI-TOF MS analysis confirmed the incorporation of amino acid units within the ε-caprolactone oligomeric chains, with the presence of both ester and amide functionalities. To analyze the co-oligomer samples, solubility tests were performed in standard solvents for oligomers such as tetrahydrofuran (THF), methanol, and dimethylsulfoxide (DMSO). The results revealed that certain co-oligomers, specifically ε-CL-Ser, ε-CL-Lys, and ε-CL-Arg, exhibited solubility in these solvents depending on the catalyst used. The only soluble products included:

- ε-CL-Ser synthesized in the presence of alcalase.
- ε-CL-Lys synthesized in the presence of $Sn(Oct)_2$.
- ε-CL-Arg synthesized in the presence of either alcalase or $Sn(Oct)_2$.

The composition and structure of the reaction products were established *via* MALDI-TOF MS analysis, which allowed for the identification of potassium adducts (K^+) corresponding to synthesized oligomers. For instance, the peaks identified in the mass spectra corresponded to co-oligomers containing varying units of ε-CL units linked to amino acids, demonstrating the successful formation of oligomeric structures. The reaction products were characterization by FT-IR spectroscopy in comparison with the original monomeric components used during polymerization. The presence of vibrational bands associated with the N-H group and carbonyl functionalities indicated successful synthesis of oligoesteramides containing arginine. Additionally, FT-IR spectra corresponding to products derived from serine and lysine showcased similar patterns, revealing the characteristic shifts in the functional groups that correspond the formation of ester and amide linkages.

The thermal stability of the synthesized products was evaluated using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The result indicated that the newly formed oligoesteramides displayed reduced stability compared to poly(ε-caprolactone) (PCL) homo-oligomers. The DSC curves illustrated the thermal properties of the synthesized products, with the oligoesteramides showing a melting temperature falling between the respective melting points of the corresponding monomers.

The average molecular weights and relative content of the soluble reaction products are presented in Table 3. Notably, the incorporation of serine into oligoesteramides resulted in relatively high molecular weight products, but with approximately 90% of the product consisting of homo-oligomers. Conversely, the products synthesized from lysine displayed lower average molecular weights, paired with a higher content of homo-oligomers, while

arginine yielded much higher contents of oligoesteramides despite similar average molecular weights. Conversely, when arginine was used as a comonomer, the co-oligomer content was increased, up to 77%. The comparison of enzymatic methods with traditional chemical catalysts like $Sn(Oct)_2$ demonstrated that while the co-oligomer content was comparable, the chemical catalyst resulted in higher average molecular weights and increased cyclic oligomer contents compared to those obtained through alcalase catalysis.

Table 3. Average molecular weights and relative content of the polymerization products based on ε-CL and amino acids calculated from MALDI TOF-MS spectra.

Co-substrate	Catalyst	M_n [Da]	M_w [Da]	$Đ_M$[a]	Relative content of the reaction products [%]			
					LC[b]	CC[c]	LH[d]	CH[e]
ε-CL – Ser	Alcalase	1590	1780	1.12	10	-	88	2
ε-CL – Lys	$Sn(Oct)_2$	780	960	1.23	10	7	80	3
ε-CL – Arg	Alcalase	800	820	1.03	59	18	20	3
ε-CL - Arg	$Sn(Oct)_2$	1360	1410	1.04	46	31	19	4

[a]Đ-Dispersity, [b]LC-Linear co-oligomer; [c]CC-Cyclic co-oligomer; [d]LH-linear homo-oligomer; [e]CH-Cyclic homo-oligomer.

In summary, this investigation effectively demonstrates the possibility of synthesizing oligoesters and oligoesteramides derived from ε-caprolactone and various amino acids (arginine, serine, and lysine) through both enzymatic and chemical approaches. The utilization of MALDI-TOF MS analysis confirmed the formation of the oligomeric products with one or more amino acid units integrated into the structure. Characterization of the products through FT-IR spectroscopy and thermal behavior analyses highlighted the synthesis outcomes.

2.5. Efficient biotransformation of biobased raw materials into novel polyesters/polyesteramides; comparative study of enzymatic synthesis of block and random copolymers and terpolymers

The present chapter studied the enzymatic synthesis of innovative polymerization products mostly derived from renewable monomers, such as ε-caprolactone (ε-CL), dimethyl itaconate (DMI), dimethyl adipate (DMA), 1,8-octanediol (ODO), and 1,8-octanediamine (ODA). The objective was to evaluate and develop synthetic pathways within the framework of enzymatic polymerization, including techniques like direct copolymerization, which yields random copolymers, and a more complex two-step block copolymerization process. The research's overarching goal was to generate co-polyesters and co-polyesteramides from various combinations of these monomers, thus diversifying the function of available bio-based materials. The synthesis methodologies not only featured a comparative analysis between random and block copolymerization but also innovatively combined ring-opening polymerization with polycondensation techniques. This dual approach represents a significant advancement in the field, suggesting pathways to produce novel polymer architectures through sustainable practices.

A major focus of the investigation was the impact of various molecular ratios among the monomers on the synthesis of the random ε-CL:DMI:ODO terpolymer. The study evaluated different molar ratios of ε-CL, DMI, and ODO during the polymerization process. The results demonstrated compelling evidence that the molar ratio significantly influenced the conversion

rates of the constituent monomers. While the conversion rates for DMI were consistently high at 99% and ε-CL surpassed 95%, the reactivity of ODO varied noticeably with its molar ratios. This variability underlines the specificity of enzymatic action in regulating polymer structure through aspects of molecular ratio and highlights the importance of careful experimental design in optimizing polymerization processes.

The synthesis of the required homopolymerization and copolymerization products for the block structures was examined. The initial steps involved the enzymatic ring-opening polymerization of ε-caprolactone. The study evaluated various initiators, including water and several alcohols, for their effectiveness in catalyzing the polymerization. The results revealed substantial variations in molecular weights based on the type of initiator employed, with the distilled water as the most efficient initiator, leading to significant increases in both the number-average molecular weight (M_n) and weight-average molecular weight (M_w). Following the optimization of the homopolymerization reaction, the copolymerization of DMI and DMA with ODO and ODA was investigated, underscoring how the selection of co-monomers directly influenced the properties of the co-polymerization products formed.

The synthesis of random and block copolymers was not only an experimental procedure but an engineered process utilizing specific monomer combinations in equimolar ratios. This allowed for to the production of diverse polymer structures, characterized under optimized conditions of enzymatic polymerization at 85°C for 48 hours. Differences in solubility properties emerged among the synthesized products, which permitted further characterization through methods such as Size Exclusion Chromatography (SEC), Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS), and Nuclear Magnetic Resonance (NMR) spectroscopy. The comprehensive evaluation of molecular weights and the respective structural compositions underlined the intricate distinctions between random and block copolymers. Structural characterization was a performed by MALDI-TOF MS and NMR analysis to substantiate the formation of ester and amide bonds within the polymers. For example, the MALDI TOF-MS characterization results presented in Figure 6, indicate that the molecular weights values fall within the range of 3860 to 5170 Da for the ε-CL:DMI:ODO system.

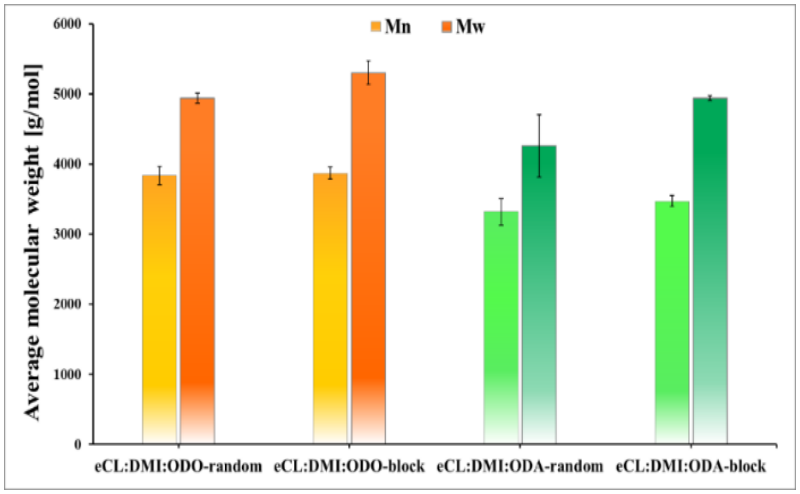

Figure 6. Average molecular weights for the ε-caprolactone, dimethyl itaconate, and 1,8-octanediol random and block copolymers, and the ε-caprolactone, dimethyl itaconate and 1,8-octanediamine random and block copolymers, respectively, catalyzed by Novozyme 435 in a solventless system, at 85 °C for 48 h.

Thermal stability assessments conducted via Thermogravimetric Analysis (TGA) provided additional insights into the thermal performance of the synthesized polymers, revealing variations in degradation profiles and thermal stability associated with the monomers

and structures obtained, most of the compounds being thermally stable up to 300 °C.

In conclusion, this extensive investigation is highlighting the potential of enzymatic synthesis to generate novel and functional polyesters and polyesteramides from renewable resources. The results entail that the targeted selection of monomers, especially when combined in a systematic approach, can alter the characteristics of the final polymerization products, particularly in terms of thermal stability and biodegradability. Random configurations of terpolymers generally displayed superior properties compared to their block counterparts, emphasizing how structural arrangements can substantially enhance material performance.

2.6. Assessment of microbial biodegradability of bio-based oligomers

The synthetized materials were investigated for their potential to undergo microbial depolymerization, a process mostly facilitated by the cleavage of ester linkages that are inherently present in their structures. These ester bonds exhibit susceptibility to enzymatic hydrolysis, the process by which microorganisms break down complex substances into simpler metabolites, rendering these bio-based materials suitable candidates for biodegradation [13]. A critical parameter in assessing the biodegradability of these polymers is the biochemical oxygen demand (BOD), which quantifies the oxygen consumed by microorganisms during degradation [14]. To evaluate the biodegradability of the enzymatically synthesized oligoesters and oligoesteramides, BOD measurements were performed using OxiTop® control S6 systems, specifically designed for monitoring aerobic biodegradation. These systems allow for continuous tracking of the oxygen consumption at 24-hour intervals, providing insights into the efficiency with which the oligomers derived from renewable sources can be broken down by microbial action.

For the co-oligomers created from ε-caprolactone and L-malic acid and the homo-oligomers of ε-caprolactone. The results demonstrated that both product types exhibited high biodegradability, after correlation with the BOD values. Notably, the incorporation of malic acid into the ε-caprolactone structure led to an increase in the oxygen demand for the complete oxidation. In addition, further investigations into the biodegradation behaviors of the oligoesteramides synthesized from ε-caprolactam and various hydroxy acids revealed a similar trend. The metabolic interactions with microorganisms present in river water reflected a high degree of biodegradability, with different hydroxy acids influencing the BOD values recorded. Specifically, the oligoesteramides produced from ε-caprolactam and 5-hydroxymethyl-2-furancarboxylic acid showcased the highest BOD values, further confirming their biodegradation potential.

In the case of the terpolymers synthesized from ε-caprolactone, both block and random copolymers were evaluated for their biodegradability. The results presented in Figure 7 indicated that block copolymers obtained from ε-caprolactone and dimethyl adipate with 1,8-octanediol or 1,8-octanediamine achieved notably high BOD measurements. This finding is significant as it suggests that copolymers integrating a substantial fraction of polycaprolactone segments are ultimately more susceptible to microbial degradation, mirroring the patterns observed with the homo-oligomer. In terms of reaction conditions affecting biodegradability, random copolymers based on ε-caprolactone and dimethyl itaconate demonstrated the most favorable performance, achieving high percentages of biodegradation. This observation emphasizes the role of specific monomers, in this case, dimethyl itaconate, in facilitating biodegradation.

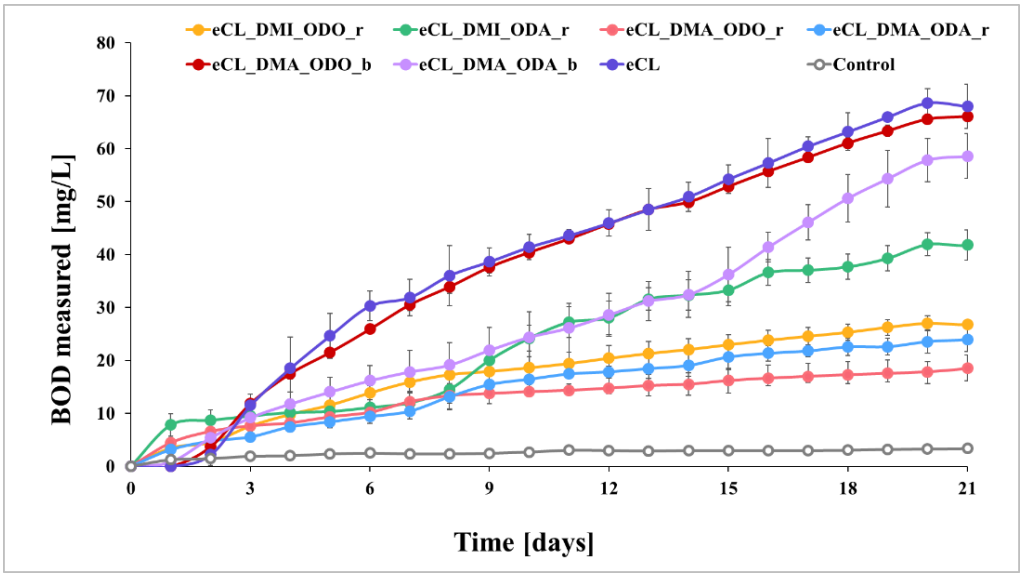

Figure 7. Biochemical oxygen consumption (BOD) monitored using the OxiTop systems over a period of 30 days to monitor the microbiological degradation of the random products of ε-caprolactone copolymerized with dimethyl itaconate and either 1,8-octanediol (orange) or 1,8-octanediamne (green), the random products of ε-caprolactone copolymerized with dimethyl adipate and either 1,8-octanediol (pink) or 1,8-octanediamne (light blue), the block products of ε-caprolactone copolymerized with dimethyl adipate and either 1,8-octanediol (red) or 1,8-octanediamne (dark blue), and the ε-caprolactone homo-oligomer in comparison to the control sample (grey).

The degree of biodegradability (Dt), calculated post-21 days of incubation, illustrated that the enzymatic polymerization products produced using bio-based monomers demonstrate substantial biodegradability. For instance, the block copolymers containing dimethyl adipate and 1,8-octanediol achieved full degradation, confirming their efficacy as biodegradable materials. This indicates significant potential for these polymers to be employed in applications with low environmental impact, such as packaging materials or matrices intended for the encapsulation of bioactive compounds.

2.8. Evaluation of the novel oligoesteramides for drug-carrying nanoparticles

The potential use of the bio-based polymers for creating drug-carrying nanoparticles was assessed at the Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences in Budapest, Hungary. The experiments concerning the preparation of nanoparticles, encapsulation of a drug compound and its controlled release were performed for the ε-CM:16HHDA oligoesteramide product, which is characterized by favorable structural properties and lack of solubility in water. They were included in a PhD Thesis defended at the University Pannonia from Veszprém (Hungary) and consequently are not part of the present thesis.

3. Experimental section: This section lists all the methodologies and materials employed for the PhD thesis in a comprehensive manner.

4. Final conclusions: This section is summarizing the most important outcomes of this study, namely:

1. The literature overview, stated at the beginning of the PhD thesis, emphasized the relevance of biocatalysis in the industrial production of polyesters/oligoesters and

polyesteramides/oligoesteramides when developing a sustainable production process in the context of an emerging bioeconomy.

2. Novel linear and cyclic oligoesters were obtained over enzymatic catalysis utilizing ε-caprolactone (ε-CL) and D,L/L-malic acid (D,L-MA/L-MA).

3. Novel oligoesteramides (OEAs) derived from ε-caprolactam and four distinct hydroxy acids (L-malic acid - MA, 3-hydroxybutyric acid - 3HBA, 16-hydroxyhexadecanoic acid - 16HHDA, and 12-hydroxystearic acid - 12HSA) were synthesized exploring various reaction conditions.

4. The enzymatic synthesis potential of aromatic oligoesteramides using ε-caprolactam (ε-CM) and 5-hydroxymethyl-2-furancarboxylic acid (5HMFCA) as monomers was achieved.

4.1. Identification of co-polymerization products for ε-CM and 5HMFCA was enabled through MALDI-TOF MS spectrometry and SEC chromatography.

5. The enzymatic and chemical synthesis of polymers incorporating ε-caprolactone (ε-CL) and amino acids (serine, lysine, arginine) with outcomes including linear or cyclic oligomers was realized.

6. The enzymatic synthesis of random and block terpolymers from bio-derived or potentially bio-derived units, namely ε-caprolactone (ε-CL) with dimethyl itaconate (DMI) or dimethyl adipate (DMA) and 1,8-octanediol (ODO) or 1,8-octanediamine (ODA) was successfully achieved.

7. The microbial biodegradation of 14 oligomers and terpolymers was evaluated over a period of 21 days utilizing a water inoculum from the Bega River, Timisoara, Romania, with all the reaction products exhibiting biodegradability, up to complete biodegradation.

8. The ε-CM:16HHDA oligoesteramide product is showcasing a potential pharmaceutical application of the synthesized oligoesteramides as effective and novel drug encapsulation carrier, upon being tested by a partner research team.

Original contributions

1. Using raw materials sourced from renewable resources such as ε-caprolactone, ε-caprolactam, D,L/L-malic acid, 3-hydroxybutyric acid, 16-hydroxyhexadecanoic acid, and 12-hydroxystearic acid, 5-hydroxymethyl-2-furancarboxylic acid, dimethyl adipate, and dimethyl itaconate, the enzymatic synthesis of 14 novel co-oligomers and random/block terpolymers was accomplished, the products not being previously reported in literature, as well as the enzymatic synthesis of 6 homo-oligoesters.

2. The enzymatic synthesis of oligoesteramides derived from ε-caprolactam was achieved for the first time.

3. The evaluation of 15 enzymatic formulations, commercially available or stabilized in laboratory, for the synthesis of ε-caprolactam derived oligoesteramides with hydroxy acids.

4. Demonstrating the selectivity of lipases (*Candida antartica* lipase B and *Pseudomonas stutzeri* lipase) for the synthesis of oligoesteramides species, the immobilized *Pseudomonas stutzeri* lipase being used for the first time in the synthesis of oligoesteramides.

5. The first comparative study of the enzymatic polymer synthesis by random and block copolymerization and also an innovative approach of combining ring-opening polymerization with polycondensation.

6. The enzymatic ring-opening polymerization of ε-caprolactone was optimized after identifying the addition of 10% water initiator at 85°C for 24 h as the most efficient reaction conditions for obtaining enzymatically synthetized polycaprolactone.

7. The structural confirmation of the synthesized oligomeric/polymeric products was conducted through advanced methodologies such as MALDI TOF-MS mass spectrometry and size-exclusion chromatography.

8. The presence of both ester and amide bonds in the chemical architecture of the polyesters/oligoesters and polyesteramides/oligoesteramides was assessed utilizing ^{1}H, ^{13}C and 2D NMR spectroscopy.

9. The chemical reactivity in the enzymatic polymerization reaction of the vinyl group embedded in the dimethyl itaconate structure was clarified, observing that this functional group is preserved when co-polymerized with diols and reactive when co-polymerized with diamines, forming pyrrolidone structures.

10. The operational stability of *Candida antartica* lipase B immobilized on acrylic resin in the synthesis of oligoesters and oligoesteramides was demonstrated for 8 to 13 reaction cycles.

11. The parameters influencing the synthesis of oligoesters/polyesters and oligoesteramides/polyesteramides were evaluated and optimized in order to increase the efficiency of the process, by means of modern analytical techniques, including also statistical methods such as the Design of Experiments (DoE).

12. The thermal properties of the newly synthetized co-oligomers and random/block terpolymers were analyzed demonstrating a thermal stability of up to 360 °C for the block structures.

13. A number of 14 of the enzymatically synthetized oligomers and terpolymers products are displaying promising biodegradability properties, after 21 days of evaluation for the microbial decomposition.

14. The most efficient oligoesteramide system derived from *ε*-caprolactone, namely *ε*-CM:16HHDA, also demonstrates a good potential for being utilized in pharmaceutical applications as innovative carrier for drug encapsulation, following testing by a collaborating research team.

The results obtained in this thesis have been published in three scientific articles (two as first author) in high-ranked international journals (Processes, Reactive and Functional Polymers, International Journal of Biological Macromolecules), and have been presented at 6 international conferences in the field of biocatalysis and industrial biotechnology, as shown in the Publication List.

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