SYNTHESIS, CHARACTERIZATION AND DEGRADATION OF NEW OLIGOESTERS FROM RENEWABLE RAW MATERIALS

PhD Thesis - Summary

To obtain the scientific title of PhD of
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Biopolymers synthesized from renewable resources are included in White Biotechnology field, and can be obtained by either isolation of natural biopolymers from plants or microorganisms, or by chemical or enzymatic synthesis *in vitro* using renewable monomers. Bio-based polymers have various applications in medical and pharmaceutical field, food industry, varnish and paints industries, textiles, as well as in agriculture and horticulture.

Enzyme-catalyzed synthesis of biopolymers is currently an alternative method to chemical polymerization due to the renewable origin of the biocatalysts, their biodegradability, efficient catalytic activity in mild reaction conditions and low impact on the environment.

The doctoral thesis is divided into four parts:

1. **A literature study** detailing the current state of the art of biopolymer synthesis using enzymes as catalysts

Nowadays almost every conventional plastic has an ecological alternative. The biopolymers industry is constantly developing, having the potential to solve the economic problems related to the depletion of resources and to reduce the harmful impact of non-biodegradable plastics on the environment. There are three classes in which biological polymeric materials are divided [1]:

- partially or totally non-biodegradable bioplastics;
- Biodegradable bioplastics;
- Biodegradable fossil-based plastics.

The use of enzymes as catalysts for polymerization reactions has numerous advantages as these biocatalysts come from renewable resources, are biodegradable, can operate under mild reaction conditions and have a low impact on the environment.

Insertion of monomeric units into the main chain or side chain of natural polymers is a promising way to improve their properties. Polymers with novel functions are thus obtained that can be used in various applications, especially in the medical and food field.

A bibliographic study was also carried out on the renewable raw materials used in the Original contributions part, which can be obtained from biomass using enzymatic or chemical catalysis. Over time, researchers have described synthesis under mild conditions of many renewable monomers that can be used in the polymer industry.

At the same time, oligomer and polyester polymerization methods have been described as well as enzyme-catalyzed types of polymerization: enzymatic ring opening polymerization and enzymatic polycondensation.

One of the most effective lipases for biocompatible and biodegradable polymer synthesis is lipase B from *Candida antarctica*, as confirmed by numerous literature studies [2] - [6]. The main characteristics of the lipases are enantioselectivity, regioselectivity and chemoselectivity, and they proved to be ideal biocatalysts for *in vitro* synthesis of polymers, resulting in polyesters, polycarbonates, polyamides or graft copolymers on different polymeric scaffolds [7].

Also, numerous studies have been presented regarding the synthesis of polyesters containing ε-caprolactone [8], [9], 5-hydroxymethylfurfural derivatives [10] - [12] and itaconic acid derivatives [13], [14].

One of the main concerns of polymers is their biodegradability. In this respect, a series of researches were mentioned concerning the enzymatic and microbial degradation of some polymers [15] - [17].

Although theoretical calculations on polymers is a narrower field of literature, more and more research groups use this method because of the valuable information that can be obtained through these methods. In this respect, several reports were specified on the molecular modeling of some polymers.

2. Original contributions, presenting the results obtained during the doctoral research, start with the presentation of the objectives of the PhD thesis. The doctoral thesis is focused on enzymatic synthesis (using lipases) or chemical synthesis of new biopolymers based on renewable raw materials, their characterization and theoretical studies regarding their energetic or chemical stability. Copoliesterification is a way to improve the properties of some known polymers.

Synthesis and characterization of copolymers with ε-caprolactone and 5-hydroxymethyl-2-furoic acid

In this chapter, novel oligomers containing ε-caprolactone (ECL) and 5-hydroxymethyl-2-furoic acid (5OH2FA) were enzymatically synthesized. The copolymers formation was demonstrated by MALDI TOF-MS spectrometry, and copolymers with several 5OH2FA units inserted into the poly(ε-caprolactone) (PCL) chain could be identified.

The reactions were performed in the temperature range 40-80°C and three commercially available immobilized enzymes were tested: Novozyme 435, Lipozyme CalB and GF-CalB-IM. The best results were obtained for lipase GF-CalB-IM.

The influence of water on the formation of reaction products was studied, demonstrating the negative effect of higher water content on the average molecular weights of copolymers.

The structure of the synthesized copolyesters was confirmed by FT-IR and NMR spectroscopy, and the thermal behavior was evaluated by TG and DSC.

The reuse of the biocatalyst in 4 batch cycles showed a fairly good operational stability with the gradual reduction of catalytic polymerization efficiency.

The synthesis of 5OH2FA_ECL copolyesters in a continuous process has been studied for the best batch lipase results (GF-CalB-IM) at different temperatures, and it has been demonstrated for the first time the possibility of polymerization under such conditions.

Enzymatic synthesis and characterization of terpolymers obtained from renewable sources

In this chapter is presented the enzymatic polymerization of three bio-based monomers: dimethyl 2,5-furandicarboxylate (DMFDC), dimethyl itaconate (DMI) and 1,6-hexanediol (HDO) in organic solvent in order to obtain new terpolymers.

Two immobilized enzymes, commercially available, were tested. The GF-CalB-IM enzyme (*Candida antarctica* B lipase immobilized on microporous ion exchange resin) demonstrated greater efficacy for the polyestersynthesis than Novozyme 435, an immobilized enzyme widely used in polymerization reactions.

The optimal molar ratio of monomers, biocatalyst concentration and reaction time were determined, demonstrating that both the degree of polymerization and the relative content of the reaction products can be controlled by fine tuning of these parameters.

The stability of the GF-CalB-IM biocatalyst was investigated over 5 cycles of reaction, showing a decrease of average molecular weights after three re-use. Polyesters containing more than 90% terpolymer were synthesized over a longer reaction time, the results being calculated based on the MALDI TOF-MS spectra.

NMR analysis demonstrated the structures of copolyesters and has provided relevant information on the reactivity and regioselectivity of the reactions. Thermal analysis has confirmed the high stability of the terpolymer, making it available for various applications.

Chemical synthesis and characterization of copolymers obtained from ε-caprolactone and hydroxy-fatty acids

New copolyesters of ε-caprolactone with three different hydroxy-fatty acids (16-hydroxyhexadecanoic acid, 12-hydroxystearic acid and ricinoleic acid) were chemically synthesized, using $SnOct_2$ as the catalyst. These syntheses have been performed in addition to previous studies regarding the synthesis of these polymers in enzymatic catalysis, studies not covered by this thesis [18]. The MALDI TOF-MS mass spectrometry determined the average molecular weight and the degree of polymerization of the synthesized copolymers.

In order to synthesize copolymers with high molecular weight and high copolymer content, the optimal molar ratio of comonomers and the influence of temperature were investigated. The best results, molecular weights above 2200 Da and polymerization degrees greater than 30, were obtained at ECL: RCA and ECL: 16HHDA molar ratio of 5: 1 at 90°C.

The synthesized copolymers were characterized by FT-IR and NMR, confirming the desired polymeric structures, and the thermal properties were evaluated by TG and DSC.

Enzymatic and microbial degradation of selected biopolymers

Two methods of degradation were selected to determine the biodegradability of synthesized copolyesters. In the first method, copolyesters were incubated at 37°C in phosphate buffer (pH = 7.4) in the presence of lipase from porcine pancreas and the second method used a mixture of microorganisms collected from a natural aqueous medium (Bega River, Timişoara, Timiş County).

Evaluation of polyester degradability was performed by quantifying the weight loss over time and complemented by MALDI TOF-MS analysis of organic extracts. The 5OH2FA_ECL copolymers degraded most rapidly, whereas the terpolymers had a high resistance to degradation. It has been shown that, depending on the heterocyclic or aliphatic structure introduced into the PCL chain, copolyesters with different degrees of biodegradability have been obtained, depending on the degradation method used.

Molecular modeling of synthesized oligoesters

Molecular modeling is a method by which certain correlations can be established between the structure of molecules and their properties [19]. The purpose of the theoretical calculations was to determine which of the possible oligomeric or polymeric structures, having a certain structural sequence of monomeric units, is more likely to be synthesized,based on its stability.

3.The experimental part include the materials and methods used in the synthesis of oligoesters or in other procedures and the methods of analysis used (MALDI TOF-MS, NMR, ATR FT-IR, TG, DSC, HPLC, and the HyperChem software package by methods MM +, molecular mechanics, and PM3, semi-empirical).

4. Final conclusions and original contributions, resulting from the experimental and theoretical studies, in accordance with the proposed objectives.

The objectives of the doctoral dissertation were fulfilled, resulting in the following conclusions:

1. The literature study, presented in the first part of the doctoral thesis, showed the importance of biocatalysis in the synthesis of oligo and polyesters, in the following aspects:

- Synthesis of new polymeric materials with specific properties is required for medical and food applications;
- Renewable raw materials can function as monomers in polymer synthesis;
- The use of enzymes is an ecological alternative to chemical catalysts for polymer synthesis;
- Polymers containing renewable monomers have been used in areas such as biomedicine, food industry, elastomers and resins.

2. Linear and cyclic copolyesters were obtained using ε-caprolactone and 5-hydroxymethyl-2-furoic acid in enzymatic catalysis.

2.1. MALDI TOF-MS assays demonstrated the formation of the ε-caprolactone oligoesters in which one or more 5-hydroxymethyl-2-furoic acid units were inserted.

2.2. Three immobilized lipases, commercially available, were tested, the most effective being GF-CalB-IM lipase.

2.3. The optimum temperature for the formation of polyesters with ε-caprolactone and 5-hydroxymethyl-2-furoic acid with high molecular weights was determined to be 80°C.

2.4. The presence of water in the system has a negative effect on the formation of polyesters with high molecular weights.

2.5. 2-D HMBC-NMR spectroscopy confirmed the formation of the ester linkage between 6-hydroxyhexanoic acid (derived from ε-caprolactone) and 5-hydroxymethyl-2-furoic acid.

2.6. Thermal stability of the oligoesters was studied by thermogravimetry to obtain a less stable compound than poly (ε-caprolactone), with major weight losses in the temperature range of 200-300°C.

2.7. The re-use of the biocatalyst in 4 reaction cycles led to polyesters with lower molecular weights, but with highcontentsof copolyester.

2.8. Synthesis of the copolyester in a continuous process was successfully carried out in tert-butanol, being a suitable solvent for the solubilization of 5-hydroxymethyl-2-furoic acid, at the same time compatible with the lipases as the reaction medium.

3. The enzymatic synthesis of the terpolymers from dimethyl 2,5-furandicarboxylate, dimethyl itaconate and 1,6-hexanediol was performed for the first time.

3.1. Two immobilized enzymes, commercially available,were tested: Novozyme 435 and GF-CalB-IM, better results were obtained for the GF-CalB-IM biocatalyst.

3.2. Enzymatic synthesis of terpolymers using immobilized lipases was much more effective than synthesis of terpolymers using a metal catalyst (tin (II) 2-ethylhexanoate).

3.3. The optimization of the reactions was carried out by investigating two parameters: the molar ratio of the reactants and the biocatalyst concentration. 5% of the biocatalyst (% weight based on the total weight of monomers) and the molar ratio DMFDC: DMI = 2:1 were chosen as ideal for the synthesis of terpolymers.

3.4. The optimum reaction time to obtain terpolymers with high molecular weights and a high content of terpolymers has been shown to be 24 h.

3.5. It has been demonstrated the possibility of reusing the immobilized GF-CalB-IM enzyme in several reaction cycles, but the average molecular weights of terpolymers have decreased.

3.6. The formation of terpolymers was demonstrated by MALDI TOF-MSspectrometry and NMR spectroscopy.

3.7. The thermal properties of the terpolymers were studied by TG and DSC, the thermal stability of the terpolymers being well above the stability of the monomers.

4. The synthesis of oligoesterscontaining hydroxy-fatty acids and ε-caprolactone was carried out in chemical catalysis, using tin (II) 2-ethylhexanoate.

4.1. The formation of linear and cyclic oligoesters was demonstrated by MALDI TOF-MS and NMR spectroscopy.

4.2. The influence of temperature and ECL: HFA molar ratio on the average molecular weights of the oligoesters obtained was investigated.

4.3. The structure of the polyesters was elucidated by the two-dimensional HMBC-NMR spectrum.

4.4. The thermal stability of copolyesters was demonstrated by thermogravimetry, compared to PCL homopolymer.

5. The biodegradability of the synthesized polyesters has been investigated in the presence of lipase from porcine pancreas and in the presence of a culture of microorganisms.

5.1. Among the synthesizedpolyesters, the best degradation capacity in the presence of lipase was the oligoester obtained from ε-caprolactone and 5-hydroxymethyl-2-furoic acid. The biodegradability of PCL homopolymer and terpolymer was very low.

5.2. Microorganisms have proven effective in the degradation study of synthesized polyesters, and less effective for PCL and terpolymer.

6. The stability of the polyesters was determined by molecular modeling.

6.1. According to theoretical calculations, the most stable structure of polyesterswith 5-hydroxymethyl-2-furoic acid and ε-caprolactone consists of 8 units (4 units of ECL and 4 units of 5OH2FA).

6.2. The quantum chemical calculations made on the possible structures of the terpolymers correlate with the results obtained from MALDI TOF-MS and NMR, itaconatebeing most likely a terminal unit.

6.3. Molecular modeling of homopolymers containing hydroxy-fatty acids and ECL showed increased stability of 12HSA homopolymer, while PCL homopolymer is the least stable.

6.4. The stability of the linear and cyclic polyesters of ECL with hydroxy-fatty acids increases in the order: RCA_ECL <16HHDA_ECL <12HSA_ECL.

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