BIODEGRADABLE POLYMERS FROM CASTOR OIL: AN ENZYMATIC DEGRADATION STUDY

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ABSTRACT

Polymers derived from petroleum are widely used due to their abundance and low costs. However, concerns about petroleum depletion and the poor biodegradability of petroleum-derived polymers underscore the need for developing new materials, either wholly or partially sourced from renewable sources, that are also susceptible to degradation. This would help to reduce the adverse environmental impact associated with their disposal. This work aims to evaluate the biodegradation, via enzymatic hydrolysis, of cross-linked polymers containing ester groups. Specifically, these polymers were synthesized from the polymerization of the monomer 2-(methacryloyloxy)ethyl undec-10-enoate obtained through the enzymatic esterification of 10-undecenoic acid, derived from the renewable source, castor oil. Different polymers were obtained from this monomer via thiol-ene photopolymerization, varying the amounts of 2,2′-(ethylenedioxy)diethanethiol (dithiol) and/or pentaerythritol tetrakis(3-mercaptopropionate) (tetrathiol). The Eversa® Transform 2.0 enzyme was used in the degradation assays, conducted under mild conditions at 27°C and pH 7. Due to the formation of a crosslinked matrix, degradation was hindered, with the extent of hydrolysis being inversely correlated with the gel content of the samples. Thus, polymers derived from castor oil-derived monomers containing ester groups and synthesized through enzymatic processes were also partially degraded by enzymatic hydrolysis, aligning with the principles of green chemistry.

Keywords: Enzymatic biodegradation. Renewable source polymers. Green chemistry. Sustainability.

1 INTRODUCTION

Currently, the polymer industry heavily relies on petroleum-derived raw materials, and polymers produced thereof are widely utilized due to their durability, non-toxicity, low cost, and versatility of application, leading to their application to substitute materials such as paper, wood, glass, and metal. However, such polymers are rarely degradable, and their disposal is becoming increasingly critical, causing adverse impacts on ecosystem health and necessitating urgent sustainable solutions. One of the pressing issues today is undoubtedly waste generation. Thus, responsible consumption and production have not only become an important topic of discussion but also the 12th Sustainable Development Goal (SDG) of the United Nations.[1] In this regard, numerous studies are focusing on the utilization of monomers derived from renewable sources for polymer production[2-5] which may become biodegradable.[6]

Among renewable compounds, vegetable oils stand out, being applicable in the synthesis of various types of monomers and polymers.[3,7] Castor oil-derived 10-undecenoic acid was utilized in the synthesis of 2-(acryloyloxy)ethyl undec-10-enoate (AEU) and 2-(methacryloyloxy)ethyl undec-10-enoate (MEU) monomers via enzymatic esterification.[4-5] These monomers were subsequently polymerized via thiol-ene photopolymerization to produce degradable materials, given that polymers containing ester groups in their main backbone are susceptible to hydrolytic degradation.[5] Thiol-ene addition reactions are categorized as click-chemistry, capable of occurring under mild conditions, rapidly, and with high product yields and mostly harmless byproducts.[8]

Degradation is marked by a significant structural change in the material, typically characterized by property loss (color, molecular weight, structural composition, and mechanical performance) and/or fragmentation, influenced by environmental conditions over a specific period, and composed of one or more stages.[9] In the enzymatic degradation of polymers, enzymes are utilized to catalyze the hydrolytic cleavage of polymer chains into smaller components. Hydrolytic enzymes, such as lipases and esterases, catalyze the hydrolysis of ester bonds, enabling the degradation of polyester-type polymers.[6,10] This process can be regarded as a sustainable approach to waste management, reducing the environmental impact of non-degradable polymers. Tokiwa and Suzuki[10] pioneered the enzymatic degradation of synthetic aliphatic polyesters, observing that commercial lipases and esterases are capable of degrading, for example, polycaprolactone. More recently, Hoelscher[6] assessed the enzymatic hydrolysis (*Candida antarctica* lipase B) of poly(thioether-ester) nanoparticles and films derived from renewable sources (castor oil and starch) synthesized via thiol-ene polymerization reactions. Nevertheless, research on the degradation of renewable materials obtained through thiol-ene reactions remains limited.

This study aims to assess the enzymatic degradation of polymers containing ester groups, partially sourced from renewable materials, specifically synthesized through thiol-ene photopolymerization of MEU using varying amounts of dithiol and/or tetrathiol.

2 MATERIAL & METHODS

The monomer 2-(methacryloyloxy)ethyl undec-10-enoate (MEU), previously synthesized via enzymatic esterification of a castor oil derivative, 10-undecenoic acid (Sigma-Aldrich, 98%) (Figure 1, reaction a),[4-5] was mixed in different proportions with the thiols 2,2′-(ethylenedioxy)diethanethiol (EDDET, Sigma-Aldrich, 95%) and pentaerythritol tetrakis(3-mercaptopropionate) (PETMP,

Sigma-Aldrich, 95%), according to Table 1, and photopolymerized using the initiator 2,2-dimethoxy-2-phenylacetophenone (DMPA, iGM Resins), at 1 wt% or 4 wt% relative to MEU (Figure 1, reaction b).[5]

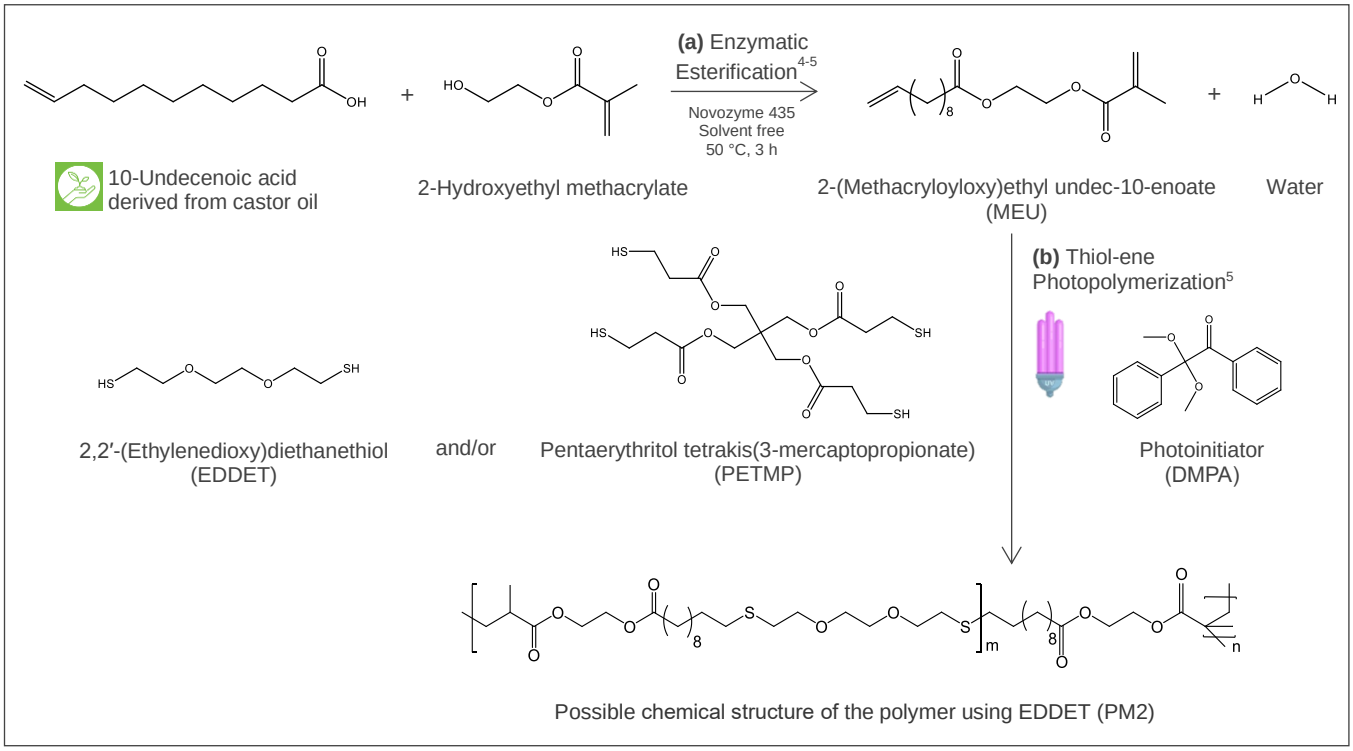

Figure 1 Synthesis of monomer MEU via enzymatic esterification[4-5] (reaction a) and its thiol-ene photopolymerization[5] (reaction b).

Following the weighing of the reagents, the mixture was homogenized using a vortex mixer for 90 s, also facilitating the complete dissolution of DMPA. The mixture was then pipetted into transparent molds arranged in a chamber containing two UV lamps, where the temperature was approximately 40 °C. These molds enable the production of polymer samples with uniform dimensions (8 mm in diameter and around 1.3 mm in thickness) and a mass of approximately 100 mg.

The polymer's gel content was assessed by quantifying the fraction of insoluble polymer. Each polymer sample (20 mg) was dissolved in chloroform for 48 h and then filtered using a 0.45 µm nylon filter. The beaker and filter retaining the gel were dried at 60 ºC until a constant mass was achieved. Gel content was determined by comparing the final dry mass to the original sample mass.

Polymer degradation was induced by hydrolyzing the ester bonds within the polymer chains. For this purpose, commercial enzyme Eversa® Transform 2.0, generously provided by Novozymes Latin America Ltda (Araucária, PR, Brazil), was utilized. The hydrolytic activity was spectrophotometrically measured at 348 nm using 0.05 M p-nitrophenyl butyrate (p-NPB) in 25 mM sodium phosphate at pH 7 and 25 °C. Enzymatic activity (U) was defined as µmol of hydrolyzed p-NPB per minute per mg of enzyme under the described conditions.[11] The activity was calculated using $\varepsilon = 5.150\ M^{-1}\ cm^{-1}$,[11-12] and the determined value was $772.3 \pm 3.4\ U\ ml^{-1}$. Polymer samples were placed in individual vials with the Eversa enzyme solution (in sodium phosphate buffer, pH 7) at 27 °C, with an enzymatic activity of 11 U. After predetermined periods, the samples were removed, washed with distilled water, and dried with absorbent paper. Subsequently, samples were dried at 40 °C and under vacuum for 2 h. Finally, samples were weighed and returned to the same vial containing the enzyme solution. Enzymatic degradation was assessed by observation of the mass loss of polymer samples over 92 days. The pH of enzymatic solutions was monitored throughout the period.

3 RESULTS & DISCUSSION

The use of either the dithiol or the tetrathiol results in different cross-linked matrixes, and the gel content analysis, shown in Table 1, is valuable for assessing this effect, considering that the cross-linked fractions of polymers are insoluble and expected to reduce enzymatic degradation as well.

Table 1 Formulations and experimental conditions of MEU polymerizations, and gel content of polymers.

Entry	MEU: EDDET: PETMP mol	Double bond: Thiol	DMPA (wt%)	Time (min)	Light intensity ($mW\ cm^{-2}$)	Gel Content (%)
PM1	1: 0.33: 0.33	1:1	1	120	2.92	72 ± 4
PM2	1: 0.50: 0	2:1	4	10	4.13	84 ± 2
PM3	1: 0: 0.25	2:1	4	10	4.13	95 ± 2

All polymers exhibited high gel content, including PM2, confirming the methacrylate propagation as otherwise the polymerization using a dithiol could only lead to linear (non-crosslinked polymer). PM3, which has both, a higher ratio of double bonds to thiol

groups, and uses PETMP, acting as a crosslinker, achieved the highest gel content. As for PM1, the higher thiol ratio, along with the use of EDDET lowers the probability of propagation, and therefore, crosslinking.

Figure 2 (a) illustrates the mass loss of the polymer samples resulting from enzymatic degradation with the Eversa® Transform 2.0 enzyme over time. To varying extents, all polymers experienced mass loss. Polymer PM1, with the highest degradation rate, exhibited a 23% mass loss (remaining mass of 77%) after 92 days, while in polymer PM2 it was 19%, and in PM3, which demonstrated the lowest degradation, it was 5%. The difference in degradation could be linked to the observed gel content, with polymer PM1 displaying the highest degradation and the lowest gel content (72%), while PM3 exhibited the highest gel content (95%) and the lowest mass loss. During the degradation process, the polymeric samples, characterized by translucency and a smooth surface (Figure 2(b)), manifested erosion and crack formation (Figure 2(c-e)). These phenomena were more pronounced with higher mass loss. In some instances, fragmentation of the edges was observed (Figure 2(c, d)). Conversely, this phenomenon was not observed in the PM3 polymer sample, which maintained its smooth surface and well-defined edges. The pH of the incubation medium was regularly assessed over time, and although there were slight fluctuations in the value, as expected, remained around 7.

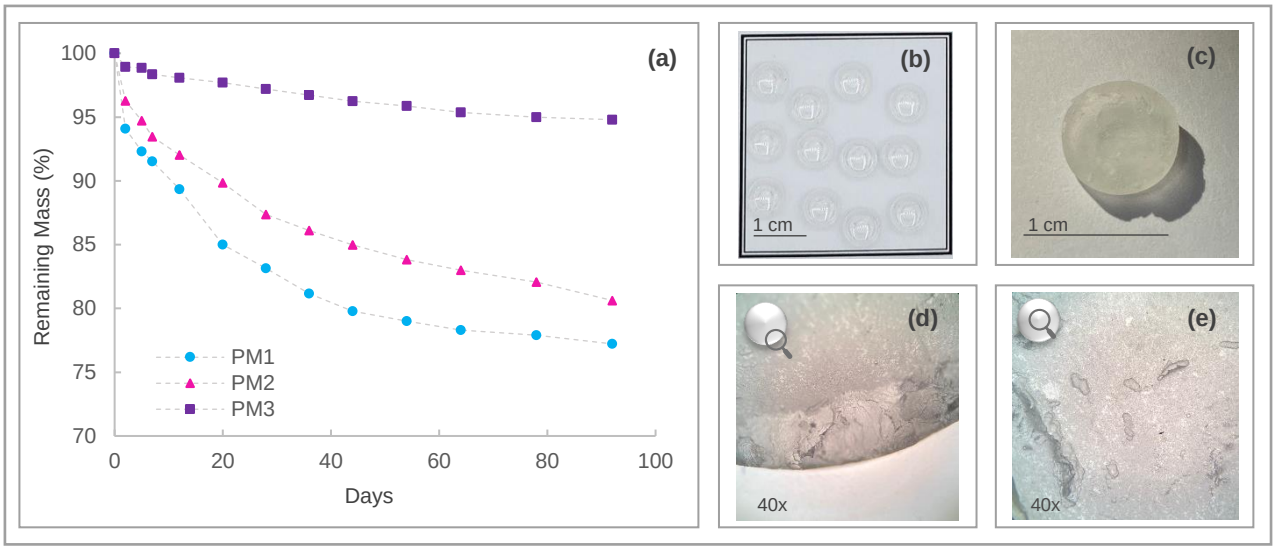

Figure 2 (a) Enzymatic degradation of polymers using Eversa® Transform 2.0; dash-dotted lines serve solely as visual aids. Samples PM1: (b) before degradation, and (c-e) after 92 days of incubation in the enzymatic solution.

4 CONCLUSION

The polymers synthesized in this study from monomers derived from castor oil were degraded by enzymatic hydrolysis, and the extent of degradation is correlated with the formation of a crosslinked matrix. Polymers with higher gel content exhibited less degradation, whereas polymers with lower gel content degraded more.

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