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Degradation of Polyhydroxyalkanoates for the Development of Sustainable Polyurethanes

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Abstract

Polyhydroxyalkanoates (PHAs) are biodegradable, biocompatible polyesters produced by microorganisms and considered promising alternatives to petrochemical plastics. Within this family, poly(3-hydroxybutyfrate) (PHB) stands out for its high crystallinity and bio-origin, but its inherent brittleness and processing window limit advanced applications. This work explores a route to value added PHB-based polyurethanes by first generating hydroxyl terminated PHB oligomers via acid-catalyzed alcoholysis and then using these oligomers as soft segments in polyurethane synthesis.

PHB was depolymerized with ethylene glycol (EG) in chloroform using p-toluenesulfonic acid (p-TsOH) (24–72h), and the evolution of molar mass was tracked by Size Exclusion Chromatography (SEC) with RI/RALS/LALS detection, which showed progressive peak shifts to higher elution volumes, indicating chain scission and formation of oligomers. Fourier Transform Infrared Spectroscopy (FTIR) of the oligomers confirmed preservation of ester carbonyls and the emergence of terminal O–H groups.

Polyurethanes were then synthesized using either Hexamethylene Diisocyanate (HDI) (aliphatic) or Methylene Diphenyl Diisocyanate (MDI) (Aromatic) with triethylamine (TEA) as catalyst, from both ethylene glycol (EG) and PHB- oligomers polyols, with and without chloroform. FTIR of the polymers consistently showed diagnostic urethane bands (N–H, C=O, C–N/C–O–C); HDI-based systems exhibited no residual –NCO, whereas some MDI/PHB-oligomers formulations retained a weak –NCO band, indicating incomplete conversion. Size Exclusion Chromatography (SEC) confirmed the formation of high-molecular-weight fractions with minor low-MW by-products, the latter more evident for PHB-oligomer/MDI systems.

Overall, the results demonstrate the feasibility of PHB-oligomers soft segments for polyurethane formation, clarify the impact of diisocyanate type (HDI vs MDI), polyol intensity (EG vs PHB-oligomers), and solvent, and suggests process adjustments (catalyst load, stoichiometry, drying) to minimize residual isocyanate and narrow dispersity.

Keywords: Polyhydroxyalkanoates; poly(3-hydroxybutirate; alcoholysis; hydroxyl-terminated oligomers; Polyurethane; FTIR; Size Exclusion Chromatography.

Resumo

Polihidroxialcanoatos (PHA) são poliésteres biodegradáveis e biocompatíveis produzidos por microorganismos e vitos como alternativas promissoras aos plásticos petroquímicos. Dentro desta família, o poli(3-hidroxibutirato) (PHB) destacta-se pela elevada cristalinidade e origem biológica, embora a fragilidade e a janela de processamento limitem aplicações avançadas. Este trabalho investiga uma rota para poliuretanos à base de PHB, gerando inicialmente oligómeros de PHB terminados em hidroxilo por alcoólise catalisada por p-toluenossulfonico (p-TsOH) e empregando esses oligómeros como segmentos "soft" na síntese de poliuretanos.

O PHB foi depolimerizado com etilenoglicol (EG) em clorofórmio (24-72 h). A evolção da massa molar foi acompanhada por Cromatografia por Exclusão de Tamanho com deteção RI/RALS/LALS, evidenciado desclocamentos do pico para maiores volumes de eluição (menor massa molar), consistentes com cisão de cadeia e formação de oligómeros. Espectroscopia de Infravermelho por Transformada de Fourier (FTIR) dos oligómeros confirmou a preservação das carbonilas éster e o aparecimento de grupos terminais O–H. Os poliuretanos foram sintetzados usando Diisocianato de hexametileno (HDI) (alifático) ou Diisocianato de difenilmetano (MDI) (aromático) com trietilamina (TEA) como catalisador, a partir de EG e de oligómeros de PHB, com e sem clorofórmio. As bandas características de uretano (N–H, C=O, C–N/C–O–C) foram observadas em todos os polímeros; formulações com HDI não apresentaram –NCO residual, enquanto alguns sistemas MDI/oligómero de PHB§ mantiveram um fraco sinal de –NCO, indicando conversão incompleta. A Cromatografia por Exclusão de Tamanho confirmou a formação de frações de elevada massa molar com subfrações oligomericas, mais evidentes em MDI/oligómero de PHB.

Em conjunto, os resultados demonstram a viabilidade dos oligómeros de PHB como segmentos "soft" em poliuretanos, esclercem o efeito do tipo de diisocianato (HDI vs MDI), da natureza do poliol (EG vs oligómero de PHB) e do solvente, e indicam ajustes de processo (carga catalítica, estequiometria, secagem) para reduzir –NCO residual e a dispersidade.

Palavras-chave: Polihidroxialcanoatos; poli(3-hidroxibutirato); alcoólise; oligómeros terminados em hidroxilo; poliuretano; FTIR; Cromatografia por Exclusão de Tamanho.

List of abbreviations

FTIR: Fourier Transform Infrared Spctroscopy.

SEC: Size exclusion Chromatography.

GPC: Gel Permuation Chromatography

RI: Refractive index detector.

UV: Ultraviolet Detector.

RALS: Right Angle Light Scattering (~90°).

LALS: Low Angle Light Scattering (~14°).

MALS: Multi Angle Light Scattering.

PU/Pus: Polyurethane/Polyurethanses.

PHAs : Polyhydroxialkanoates.

PHB/P3HB: Poly(3-hudroxybutirate).

PHB-oligomers: Hydroxyl terminated oligomers obtained by PHB depolymerization.

MDI: 4,4'-Methylene diphenyl Diisocyanate (Aromatic diisocyanate).

HDI: Hexamethylene Diisocyanate (aliphatic diisocyanate).

EG: Ethylene Glycol.

TEA: Triethylamine (Catalyst).

p-TsOH : P-Toluene-4-Sulfonic acid (acid catalyst).

$CHCl_3$: Chloroform.

THF: Tetrahydrofuran.

DMF: N,N-Dimethylformamide.

EtOH: Ethanol

NCO: Isocyanate group.

C=O: Carbonyl group.

N–H: Amide/Urethane N–H stretch.

C–O–C: Ether/Ester stretch.

PU1, PU2…PU7: Polyurethane batches as defined in the experiment section.

DBTDL: Dibutyltin Dilaurate (organotin catalyst).

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Chapter 1 Introduction

Driven by the urgent need to reduce reliance on fossil fuels and mitigate environmental issues associated with petroleum-derived plastics, innovative approaches to polymer synthesis are appearing faster than ever before. In this work, the focus is on sustainable polymer synthesis via ring opening polymerization (ROP) and the integration of bio-based monomers into advanced materials. The development of renewable, biodegradable, and biocompatible polymers is increasingly critical as society shifts towards greener alternatives. Polyhydroxyalkanoates (PHAs), microbial polyesters valued for their inherent biodegradability and renewability, represent promising substitutes for conventional plastics. However, their high crystallinity and brittleness have historically limited industrial adoption.

Recent advances in polymer chemistry enable the conversion of high molar mass PHAs, such as poly(3-hydroxybutyrate) (P3HB), into low-molar-mass oligomers with reactive end groups. These oligomers serve as versatile building blocks for further modification through ring-opening polymerization (ROP) and isocyanate reaction, forming hybrid polyurethanes with tailored properties.

Ring-opening polymerization (ROP), a precise chain-growth polymerization method, allows controlled molecular architecture by opening cyclic monomers (often bio-based) and assembling them into well-defined polymer chains. Incorporating bio-based monomers not only reduces dependence on petrochemicals but also enables customization of mechanical strength, thermal stability, and degradation behaviour. Such tunability is particularly advantageous for high-value applications in biomedicines and eco-friendly packaging[1], [2].

Moreover, converting polyhydroxybutyrate P3HB into reactive oligomers via controlled degradation methods (e.g., alcoholysis and transesterification) enhances its processability. By reducing molecular weight and introducing functional end groups, these techniques address P3HB's brittleness and crystallinity.

The resulting oligomers undergo ROP to form block copolymers with finally tuned properties. Subsequent reactions with diisocyanates, such as hexamethylene diisocyanate (HDI) and methylene diphenyl diisocyanates (MDI), yield hybrid polyurethanes. These materials exhibit superior mechanical, thermal, and degradation profiles, broadening their utility in biomedical implants and advanced packaging.

Chapter 2 Theoretical Background

2.1 Polyhydroxyalkanoates (PHAs) and Their Degradation

2.1.1 Structure, Properties, and Applications

Polyhydroxyalkanoates (PHAs) are a diverse family of naturally occurring biodegradable polyesters synthesized by a wide range of microorganisms under nutrient-limited conditions. Many bacteria, such as *Cupriavidus necator, Alcaligenes eutrophus, Bacillus megaterium, and Pseudomonas putida,* accumulate PHAs as intracellular granules that serve as reservoirs of carbon and energy. This biosynthetic pathway highlights the renewable nature of these polymers and provides a sustainable alternative to petroleum-derived plastics[1], [2].

The natural diversity of PHAs is one of their most significant attributes. Their polymer chains are composed of various hydroxyalkanoate monomers that can be arranged in different sequences and proportions, which in turn determine key properties such as crystallinity, flexibility, and thermal stability. For example, poly(3-hydroxybutirate) (P3HB) is composed exclusively of 3-hydroxybutirate monomer units linked via ester bonds, resulting in the highly linear and crystalline structure. This high degree of crystallinity imparts excellent tensile strength and barrier properties, yet it also leads to inherent brittleness and low elongation at break, limitations that restrict the processability and application range of native P3HB[1].

Other PHAs, such as Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and medium-chain-length PHAs like Poly(3-hydroxyhexanoate) (PHHx), illustrate the versatility within this polymer family. PHBV, a copolymer of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV), typically exhibits reduced crystallinity and enhanced flexibility compared to pure P3HB, making it more suitable for applications where ductility is critical[1], [2]. Meanwhile, medium-chain-length PHAs, such as PHHx, offer improved elasticity and are often preferred in applications that demand soft, rubber-like materials.

The biosynthesis of PHAs is highly influenced by the microbial species, the carbon source available (which can range from simple sugars to complex vegetable oils), and specific cultivation conditions such as nutrient limitation and oxygen availability. These factors not only affect the overall yield of PHAs but also their molecular weight, polydispersity, and monomer composition. High molecular weight PHAs typically exhibit a higher degree of crystallinity, which, while beneficial for tensile strength and barrier properties, also results in reduced processability and increased brittleness[1], [2].

From a physical standpoint, native P3HB typically has a melting temperature (T_m) of around 173°C and a degradation temperature (T_d) near 270°C, underscoring its thermal stability.

However, these thermal properties come with trade-offs; the same high crystallinity that contributes to its strength also limits its elongation and impact resistance, thereby posing challenges for applications that require flexibility, such as in certain biomedical devices and flexible packaging materials[1].

PHAs have been explored in a wide array of applications due to their biodegradability and biocompatibility. In the biomedical field, PHAs are used to manufacture medical implants, tissues engineering scaffolds, and drug delivery systems. Their ability to degrade safely within biological environments makes them ideal candidates for temporary implants and regenerative therapies[1]. In packaging, PHAs offer excellent barrier properties that can protect food products while ensuring that the material biodegrades, thus reducing environmental impact[2]. Additionally, PHAs are promising for agricultural films and disposable items, where their rapid degradation can significantly reduce long-term waste, as well as for various environmental applications such as in waste treatment processes[1].

Recent literature also emphasize the integration of renewable feedstocks into the production of PHAs. Chemical routes that convert plant biomass into monomers and precursors have been developed to complement microbial fermentation. For instance, plant biomass can be processed to yield sugars, which are then fermented to produce lactic acid, succinic acid, or furfural, a platform chemical that can be polymerized into bio-based polymers. This integration of biotechnological and chemical synthesis routes expands the array of available renewable monomers, enhances sustainability, and potentially lowers production costs[3], [4].

While the broader range of PHAs offers a rich diversity of polymers with varying properties, Poly(3-hydroxybutirate) (P3HB) remains a key focus because of its widespread availability and notable biodegradability.

However, the high cristalinity and brittleness of native P3HB limit its utility in high performance applications. To address these limitations, researchers have developed chemical degradation strategies, such as alcoholysis and transesterification, to convert P3HB into low-molecular-weight oligomers with reactive end groups. These oligomers can then be further functionalized through techniques like ring opening polymerization (ROP) and isocyanate coupling to produce advanced hybrid polymers with tailored properties. Such modifications allow for the fine tuning of mechanical strength, thermal stability, and biodegradation behaviour, making these materials more suitable for demanding applications in biomedicine, packaging, and environmental remediation[1], [2], [3], [4].

2.1.2 Comprehensive Overview of PHB Degradation Mechanisms: Conventional Routes and Controlled Chemical Approaches

2.1.2.1 Chemical Structure and Physicochemical Properties of P3HB

Poly(3hydroxybutyrate) (P3HB) is among the most extensively studied members of the polyhydroxyalkanoate (PHA) family due to its promising combination of biodegradability and mechanical strength[1], [2]. Synthesised by a variety of bacteria under nutrient-limited conditions, P3HB accumulates as intracellular granules, serving as a carbon and energy reserve for the producing microorganisms[5], [6]. From a structural standpoint, P3HB consists of repeating 3-hydroxybutirate monomers unit linked by ester bonds, resulting in a highly linear and crystalline polymer backbone[1]. This crystallinity imparts advantageous properties such as high tensile strength and notable barrier performance, which are critical in packaging and biomedical applications[5], [7]. However, the same crystallinity also contributes to its inherent brittleness and narrow processing window, limiting its direct industrial use[1], [7].

Recent research has focused on addressing these limitations by converting high molecular-weight P3HB into lower-molecular-weight oligomers through methods such as alcoholysis and transesterification[6], [8]. These controlled degradation techniques introduce reactive end groups and reduce crystallinity, thereby improving processability and expanding P3HB's application potential. Additionally, studies have explored blending P3HB with other biopolymers (e.g., Polylactide, Polycaprolactone) or introducing soft segments like polyethylene glycol (PEG) to enhance flexibility and tailor degradation behaviour[7], [9]. As a result, P3HB based materials are increasingly viewed as viable candidates for high-value applications, ranging from medical implants to eco-friendly packaging, where both sustainability and performance are paramount[2], [9]. The table 1 summarize the key chemical and physical properties of poly(3-hydroxybutirate) (P3HB).

Table 1: Physicochemical and mechanical properties of poly(3-hydroxybutyrate) (P3HB), including thermal, structural, and biodegradability characteristics, as reported in the literature

Property	Description / Value	Reference
Chemical Formula (Repeat Unit)	–[O–CH(CH_3)–CH_2–C(=O)]–	[1], [2]
Molar Mass (Repeat Unit)	86.09 g/mol	[1]
Polymer Structure	Linear polyester of 3-hydroxybutyrate monomers	[1], [2]
Degree of Crystallinity	~60–80%	[1], [7]
Melting Temperature (Tm)	~173 °C	[6], [7]
Glass Transition Temperature (Tg)	~4 °C	[6], [9]
Thermal Degradation Temperature (Td)	~270 °C	[6], [8]
Solubility	Insoluble in water; soluble in chloroform, dichloromethane	[2], [6]
Density	~1.25 g/cm^3	[1], [7]
Tensile Strength	~40 MPa	[2], [9]
Elongation at Break	~5–10% (unmodified P3HB)	[7]
Biodegradability	Highly biodegradable under aerobic and anaerobic conditions	[2], [5], [10]
Production Method	Microbial fermentation using bacteria (e.g., Cupriavidus necator)	[2], [5]

2.1.2.2 Thermal and Chemical Degradation

Thermal and chemical degradation of PHB occurs when the polymer is exposed to reactive chemical agents, often presents as contaminants or additives, while under moderate heating. In

this process, residual catalysts, metal salts, surfactants, or other impurities incorporated during polymerization or processing act as internal chemical agents. They can promote an anionic degradation mechanism (often following an E1cB pathway) by deprotonating PHB's ester groups. Factors such as the nature and concentration of these chemical species (with larger cations typically accelerating the chain fission) directly affect the degradation rate. The outcome is the cleavage of polymer chains into smaller fragments, evidenced by a loss in mass and modifications in the chemical structure, even under relatively mild temperature conditions[11].

2.1.2.3 Thermodegradation

Thermodegradation refers to the degradation that occurs solely as a result of thermal energy. When PHB is heated, particularly in the presence of oxygen, its long polymer chains undergo random scission of the ester bonds. This results in a progressive reduction in molecular weight over time. At elevated temperature (typically above the polymer's melting point), the chain cleavage is more pronounced, and a drop in mechanical strength and ductility is observed. Interestingly, some transient increases in molar mass may occur due to secondary polycondensation reactions between hydroxyl and carboxylic end groups, although the overall trend remains a decrease in chain length. The degradation rate is a strong function of temperature and exposure time[11].

2.1.2.4 Thermo-Mechanical Degradation

Thermos-mechanical degradation is the synergetic effect of heat and mechanical stresses on PHB, most notably during industrial processing techniques like extrusion, injection molding, and calendaring. In the softened state above the melting temperature, PHB become more susceptible to shearing forces. These forces can significantly accelerate the breakage of polymer chains beyond what would be expected from thermal degradation alone. As a result, there is a marked reduction in molecular weight, along with alterations in melt flow properties (evidenced by an increased melt flow index) and changes in crystallization behaviour (such as a lower cold crystallization temperature). This mechanism is critical to consider during processing because it directly influence the final product's performance and stability [11].

2.1.2.5 Abiotic (Hydrolytic) Degradation

Abiotic degradation of PHB primarily occurs through hydrolysis, where water molecules cleaves the ester bonds along the polymer chain. This process is significantly influenced by the polymer's physical structure, particularly its degree of crystallinity. Amorphous regions, being less densely packed, are more accessible to water, thus leading to a faster hydrolytic reaction. Under environmental conditions that are mild (neutral PH and moderate temperature), hydrolysis proceeds slowly, causing only slight weight losses and surface changes over extended periods.

The hydrolytic process also induces changes in the material's surface properties, such as an increased hydrophilicity (evidenced by a lower water contact angle and a reduction in the glass transition temperature, while sometimes even leading to an increase in crystallinity as the amorphous regions are preferentially degraded[11].

2.1.2.6 Phtodegradation

Photodegradation is driven by the absorption of high energy photons, predominantly in the ultraviolet (UV) range. When PHB absorbs UV or high-intensity visible light, its chromophoric groups become exited, initiating photochemical reactions. These reactions result in the formation of free radicals, which subsequently lead to chain scission and oxidation of the polymer. Photodegradation is primarly a surface phenomenon, as the penetration depth of UV light is limited, causing degradation to be most pronounced on the outer layers of the material. Commonly observed effects include discoloration (often a browning or increased opacity), increased surface roughness, and sometimes a rise in crystallinity on the surface layer, which may even delay subsequent biodegradation processes by reducing enzyme accessibility[11].

To overcome the intrinsic limitations of high-molecular-weight P3HB, researchers employ controlled degradation methods to produce low-molecular-weight oligomers with reactive end groups. Tow widely degradation methods are used.

2.1.2.7 Alcoholysis (Transesterification)

Transesterification is a widely used chemical degradation method for poly(3-hydroxybutirate) (P3HB), enabling the production of low molecular weight oligomers with functional end groups through the cleavage of ester bonds by alcohols. In this process, alcohols such as ethylene glycol and glycerol act as nucleophiles, attacking the electrophilic carbonyl carbon atoms of the ester bonds along the P3HB backbone. The reaction proceeds via a nucleophilic acyl substitution mechanism, wherein the hydroxyl group of the alcohol forms a tetrahedral intermediate with the ester linkage, which subsequently collapses to yield a new ester bond and release a shorter polymer chain. This mechanism results in oligomers terminated with hydroxyl groups, commonly referred to as telechelic diols, making them highly suitable as reactive intermediates for further polymerization processes, including polyurethane synthesis and ring opening polymerization (ROP). The reaction is typically catalyzed by strong acid catalysts, such as p-toluenesulfonic acid (PTSA), which enhances the electrophilicity of the carbonyl group and accelerate the rate of ester exchange.

Solvents like chloroform or toluene are commonly used to dissolve P3HB and ensure a homogeneous reaction medium, while reflux conditions facilitate polymer chain mobility and

promote efficient degradation. Among the alcohols employed, ethylene glycol is particularly effective due to its linear molecular structure and low steric hindrance, which contribute to faster reaction kinetics and a more uniform molecular weight distribution in the resulting oligomers. In contrast, glycerol with its branched structure and higher steric hindrance, reacts more slowly and produces a broader range of oligomer sizes. This difference in reactivity allows researchers to selectively tailor the properties of the degradation products by choosing appropriate alcohols and adjusting reaction parameters such as time, temperature, and alcohol-to-polymer ratio. Oligomers obtained through ethylene glycol mediated transesterification typically exhibit molecular weights in the range of 2 to 4 kDa, with narrow distributions and well defined terminal functionalities. These characteristics make them ideal building blocks for synthesizing hybrid materials with customized mechanical, thermal, and degradation properties. Overall, the use of ethylene glycol and glycerol in the transesterification of P3HB represents a powerful strategy for modifying biobased polymers, offering versatility in molecular design and promoting the development of sustainable, high-performance materials.

The reaction of PHB alcoholysis with ethylene glycol or glycerol in presence of p-toluenesulfonic acid is presented in figure 1.

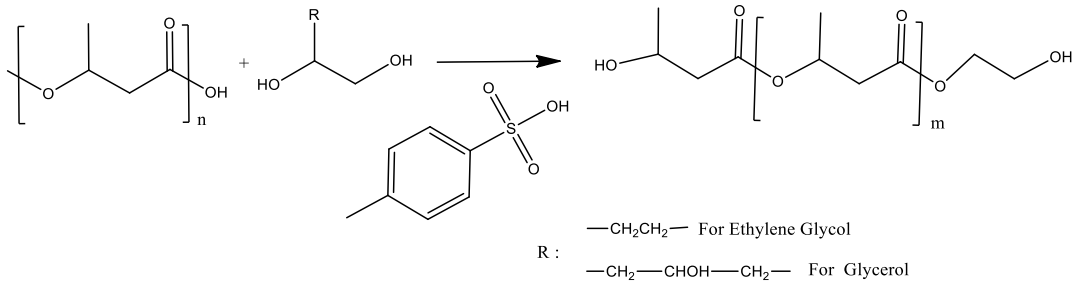

Figure 1: Reaction scheme of PHB alcoholysis with ethylene glycol or glycerol catalysed by p-toluenesulfonic acid, yielding hydroxyl-terminated PHB oligomers (telechelic diols)

2.2 Ring-opening Polymerization (ROP) for Biobased Monomers

2.2.1 ROP Mechanisms and Catalysts

Ring Opening Polymerization (ROP) is a versatile chain-growth process that converts cyclic monomers into linear or block copolymers. The mechanism typically involves initiation (activation of an initiator or catalyst), propagation (sequential addition of monomer units), and termination or chain transfer steps. In the case of biobased monomers, the use of cyclic esters derived from hydroxy acids, allows for precise control over the molecular architecture of the

resulting polymer. Catalytic systems employed in ROP range from metal-based catalysts (e.g., organotin compounds, zinc complexes) to emerging greener alternatives such as organocatalysts and enzyme-based systems. While traditional catalysts like stannous octoate ($Sn(Oct)_2$) have been widely used, concerns over toxicity and environmental impact have spurred research into alternative catalytic methods[2], [5].

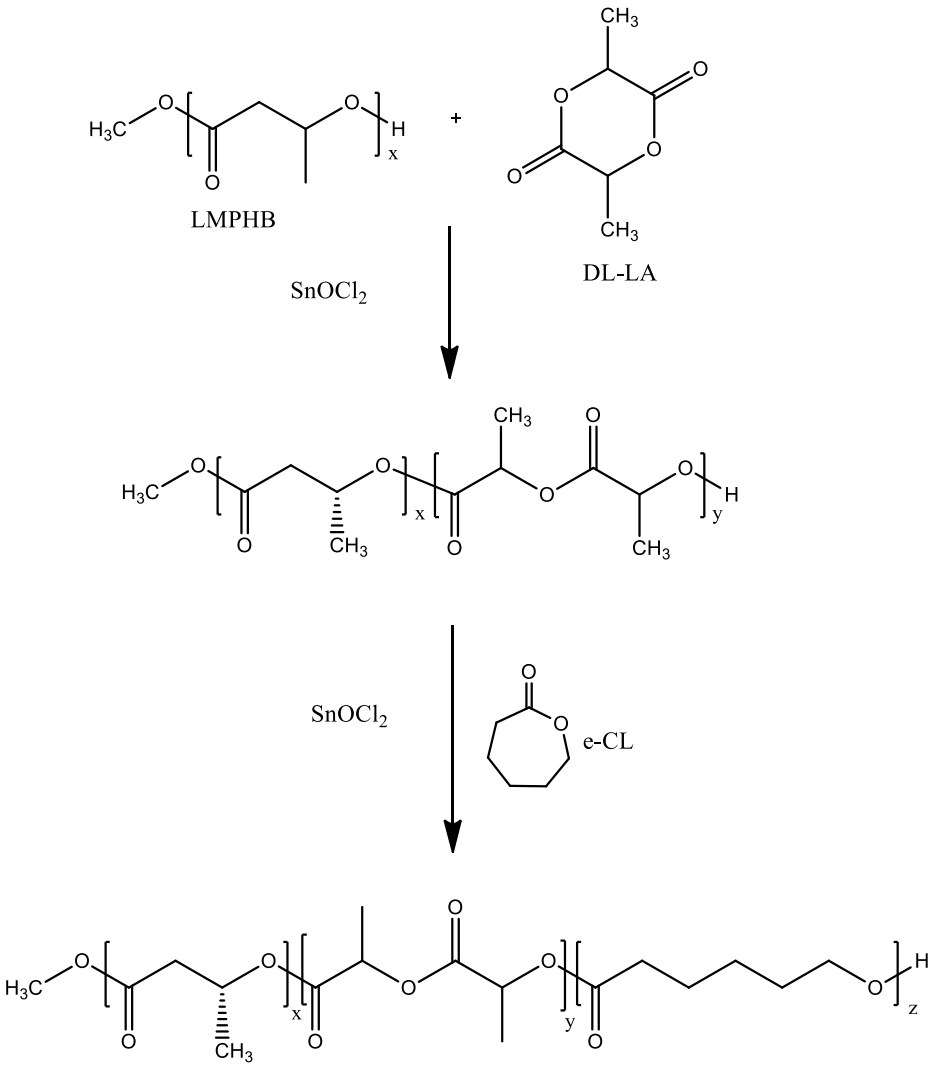

Figure 2: Scheme of the two-step synthesis of the PHB-PLA-PCL triblock copolymer through ring opening polymerization [2]

2.2.2 Recent advances in ROP with Bio-Based Monomers

Recent studies have demonstrated that ROP can be successfully applied to bio-based cyclic monomers to produce polymers with tailored properties.

For instance, anionic ROP has been utilized to synthesize amphiphilic block copolymers (e.g., PHB-b-PCL) with controlled hydrophilicity and biodegradability for applications such as drug

delivery systems. Advances in controlled coordination, insertion ROP techniqus have also enabled the synthesis of triblock copolymers like PHB-PLA-PCL, which combine the biodegradability of PHB with the enhanced flexibility and toughness of PLA and polycaprolactone (PCL)[2], [5]. However, challenges persist in achieving high polymerization efficiency and scalability. Low polymerization rates, the toxicity of conventional catalysts, and difficulties in achieving consistent product quantity at scale remain significant barriers to industrial adoption.

2.2.3 Challenges in Scalability, Efficiency, and Sustainability

Despite promising laboratory results, the scalability of ROP for bio-based polymers is hampered by several factors. First, the catalytic systems used must not only be efficient but also environmentally benign. Second, the multi-step synthesis processes, which often involve initial monomer production, purification, and subsequent polymerization, can be complex and cost intensive. As such, further research into continuous flow reactors, integrated process schemes, and alternative catalytic systems is required to enhance both the efficiency and the sustainability of ROP for industrial applications.

2.3 Hybrid polyurethane synthesis using isocyanates (HDI &MDI)

2.3.1 Chemistry and reactivity of HDI and MDI

Hybrid polyurethane synthesis relies on the reaction between hydroxyl-terminated oligomers, derived from the controlled degradation of poly(3-hydroxybutyrate) (P3HB), and diisocyanates. Two diisocyanates commonly used in this context are **Hexamethylene Diisocyanate (HDI)** and **Methylene Diphenyl Diisocyanate (MDI)**. Their chemical structures and reactivity profiles play a crucial role in dictating the properties of the resulting polyurethanes.

2.3.1.1 Hexamethylene Diisocyanate (HDI)

HDI is a linear aliphatic diisocyanate featuring a flexible hexamethylene spacer between its two isocyanate groups. This structural arrangement imparts several advantages:

- **Flexibility and lower viscosity:** The aliphatic nature and flexibility of HDI result in polymer networks with lower crosslink density and enhanced chain mobility. Consequently, HDI-based polyurethanes tend to exhibit greater flexibility and improved processability, which is particularly valuable in applications requiring materials that can withstand dynamic mechanical stresses.
- **Reduced yellowing and enhanced UV stability:** Unlike aromatic diisocyanates, HDI does not absorb UV light to the same extent, minimizing yellowing during long-term exposure to sunlight, a desirable trait for applications in coatings and packaging.

- **Rapid reaction kinetics:** The low steric hindrance around the isocyanate groups in HDI facilitates a more rapid nucleophilic attack by the hydroxyl groups on the P3HB derived oligomer. This results in efficient formation of urethane linkages, which constitute the backbone of the polyurethane network[7], [10].

2.3.1.2 Methylene Diphenyl Diisocyanate (MDI)

MDI, in contrast, is an aromatic diisocyanate composed of two isocyanate groups attached to methylene diphenyl structure. Its distinct chemical feature impart different properties to resulting polymers:

- **Higher thermal stability and rigidity:** The aromatic rings in MDI provide a rigid structure and promote stronger intermolecular interactions (including π-π stacking). These characteristics contribute to a more thermally stable and mechanically robust polymer network. MDI based polyurethanes often display enhanced tensile strength and dimensional stability, making them well-suited for high-performance applications in areas such as biomedical devices where mechanical durability is critical.
- **Slower Reaction Rates**: The aromatic structure of MDI, while beneficial for stability, introduces a degree of steric hindrance around the isocyanate groups. This can lead to slower reaction kinetics when compared to HDI. However, this slower reactivity can be advantageous for controlling the polymerization process and achieving a uniform crosslinked network when reaction conditions are carefully optimized[7], [10].

2.3.2 Mechanism of Polyurethane Formation

Polyurethane formation proceed through the nucleophilic addition of hydroxyl groups to isocyanate groups. Both HDI and MDI follow this fundamental mechanism. As illustrated in figure 3, the lone pair of electrons on the oxygen atom of the hydroxyl group attacks the electrophilic carbon atom in the isocyanate group[10], [12].

Urethene Linkage

Figure 3:The Illustration of urethane linkage*[13]*

Polyurethane formation occurs through the nucleophilic addition of hydroxyl groups to isocyanate groups. Both HDI and MDI react with hydroxyl groups via this mechanism. In this process, the lone pair of electrons on the oxygen atom of the hydroxyl group attacks the electrophilic carbon

atom in the isocyanate group [10], [12]. As illustrated in Figure 4, this nucleophilic attack generates a tetrahedral intermediate that subsequently undergoes proton transfer and rearrangement to form the stable urethane (carbomate) linkage. In the case of aliphatic diisocyanate such as HDI, the absence of aromatic stabilization resuls in lower intrinsic reactivity, leading to slower chain extention and more flexible polymer segments. Conversely, aromatic diisocyanates such as MDI exhibit higher electrophilicity due to the electron withdrawing effect of the aromatic ring, promoting faster urethane formation and producing stiffer, more rigid polymer domains.

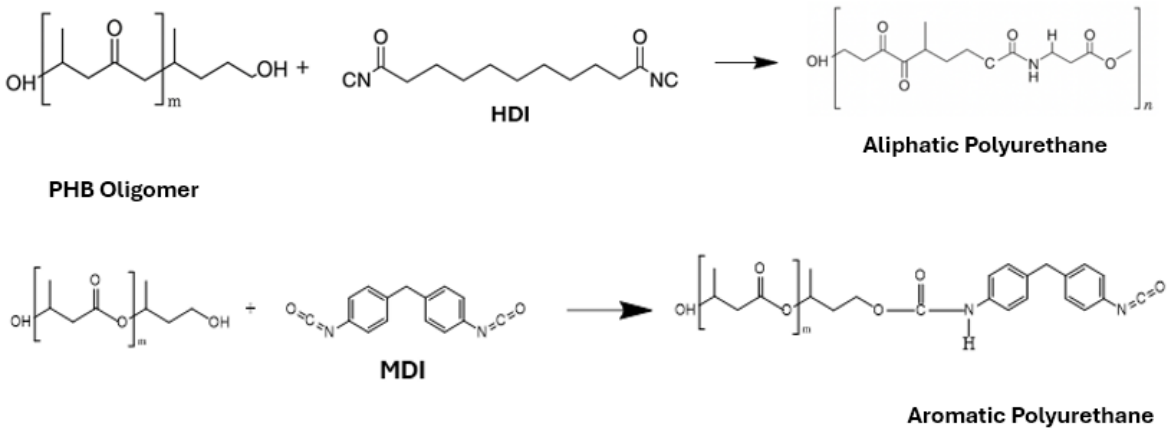

Figure 4: Reaction schemes showing the formation of aliphatic and aromatic polyurethanes from PHB oligomers using HDI and MDI, respectively.

This addition forms an unstable intermediate that subsequently rearranges to yield a stable urethane linkage. The efficiency of this reaction is highly dependent on factors such as the steric environment around the isocyanate groups and the nature of the catalyst used. The choice of catalyst (acidic, basic, or organocatalytic) can significantly influence the reaction rate and the final crosslink density of the polyurethane network[14].

2.3.3 Tailoring Polymer Properties Through HDI and MDI.

The selection between HDI and MDI (or even their combination) allows for fine-tuning of the final polymer properties. For instance, using HDI predominantly can yield a more flexible, low-viscosity system that is ideal for applications requiring soft, elastomeric materials. On the other hand, employing MDI can enhance the rigidity and thermal stability of the polymer, which is desirable for applications demanding high mechanical strength and durability. By adjusting the NCO/OH ratio, as well as the choice and concentration of the diisocyanate, researchers can achieve a balanced hybrid polyurethane system that meets specific performance criteria.

Moreover, the advanced chemical routes described by Gomes et al.[3], [4]

Highlight emerging methods for deriving diisocyanate precursors from renewable plant biomass, further enhancing the sustainability of the process. Integrating such biobased precursors can contributes to the overall "green" profile of the polymer synthesis, aligning with the broader goals of environmental sustainability and circular economy.

In summary, the distinct chemical structures and reactivity of HDI and MDI are central to the design and synthesis of hybrid polyurethanes. HDI's flexible aliphatic nature promotes rapid reaction kinetics and improved flexibility, while MDI's aromatic structure confers enhanced thermal stability and rigidity. Together, these diisocyanates enable the formation of urethane linkages that serve as the backbone of advanced hybrid polyurethanes, whose properties can be meticulously tailored to meet the demands of high-value applications in biomedicine, packaging, and beyond[7], [10].

2.3.4 Advances in Biobased Polyurethane Synthesis

Recent studies have focused on developing biobased polyurethanes that combine the inherent biodegradability of polyhydroxyalkanoates (PHAs) with significantly enhanced mechanical and thermal properties. One notable approach involves the use of hydroxyl terminated oligomers derived from poly(3-hydroxybutirate) (P3HB) as precursors for polyurethane synthesis. For example, research has demonstrated that blending PHB-diols with a small percentage (e.g., 10 wt.%) of PEG-based polyurethanes (PU1000) can yield materials exhibiting up to a 70% increase in impact strength and an improvement in thermal stability by approximately 30°C compared to pure P3HB[7]. Such enhancements are attributed to the improved chain mobility and reduced crystallinity impaired by the PEG segments.

Additionally, chain extension reactions using polyethylene glycol PEG) have led to the development of amphiphilic polyurethanes that demonstrate tensile strength around 20 MPa and elongation at break of approximately 210%. These properties are particularly promising for biomedical applications where both mechanical resilience and controlled degradation are essential. Moreover, catalyst-free synthesis routes have been explored to generate non toxic hydrogels with an elastic modulus of 1.6 MPa, thereby broadening the potential application spectrum of these materials and reducing environmental and health-related concerns[10]. Overall, advances in biobased polyurethanes synthesis underscore the potential of combining tailored P3HB oligomers with diisocyanates to create high-performance materials.

2.3.5 Factors Affecting Performance

The final properties of hybrid polyurethanes are governed by a range of critical factors. Understanding these parameters is essential for tailoring materials with desired mechanical, thermal, and biodegradation properties.

2.3.5.1 NCO/OH Ratio and Crosslinking

Adjusting the isocyanate-to-polyol ratio dramatically alters network structure. In general, higher NCO/OH ratios drive the formation of more urethane/urea links, increasing phase separation and hard-segments crystallinity[14]. This raises the glass transition and tensile strength but can embrittle the material. For example, Huang and Zhang report that increasing NCO/OH in lignin-PU films increased transition temperature (Tg), stiffness and tensile strength[14]. Similarly, Fuensanta et al. found that raising the NCO/OH ratio in segmented TPUs improved hard -soft segment miscibility and significantly increased modulus[15] [10].

2.3.5.2 Molecular Weight of PHB Diols

The molecular weight and distribution of the PHB- derived oligomers directly influence the mechanical properties of the final polymer.

Oligomers with controlled, lower molecular weights generally allow for better processability and more uniform reactivity during subsequent polymerization steps. In contrast [10].

2.3.5.3 Diisocyanate Structure

The chemical nature of the diisocyanate significantly affects the resulting polyurethane properties [10]. For instance, the symmetry and functionality of the diisocyanate (aromatic vs aliphatic) govern hydrogen bonding and phase morphology. Wang et al. showed that an aromatic diisocyanate (MDI) produced the highest hydrogen-bonding index and greatest tensile strength (23.4 MPa), along with very distinct phase separation[16]. In contrast less symmetric aliphatic isocyanates (HMDI, IPDI) yielded more elastic networks with different adhesion or self-healing properties. Thus, choosing an appropriate diisocyanate can tune hardness vs flexibility[16].

2.3.5.4 Additional Soft Segments

The incorporation of additional soft segments such as PEG or polycaprolactone (PCL) can promote phase separation, which in turn enhances the material's toughness and impact resistance while also influencing its biodegradation behaviour [10].

The length and type of soft polyols (e.g. high-Mn PEG or PCL) tend to increase crystallinity and modulus, while very short diols reduce flexibility. Olszewski et al. found that increasing the polyol molecular weight in rigid PU foams produced up to a 40% higher compressive strength due to

increased cross-link density[17]. In P3HB based Polyurethanes Krzykowska et al. observed that using a mid-range PEG (Mn ≈ 1000) as the soft segment gave the best mechanical toughness[18]. In contrast, very long soft chains lowered modulus, and very short chains made the Polyurethane brittle.

2.3.5.5 Reaction and Formulation Conditions

Variables such as temperature, solvent type, and reaction time play pivotal roles in ensuring a uniform morphology and optimal interfacial adhesion within the polymer network [10].

Catalyst choice, temperature, and mixing order also affect performance. For instance, one-step, solvent-free polymerizations have been used to improve mixing of rigid and soft segments (as in TPU adhesives synthesized from PLA, PCL, PTMEG polyols)[15]. Similarly, controlling reaction temperature and time can optimize chain extention: e.g. longer transesterification of PHB into diol yields higher Mn PHB-diols with different reactivity. In summary, optimizing NCO/OH ratio, hard segment symmetry, polyol length, and processing variables is key to tailoring biobased polyurethane properties[14], [17]

2.3.6 Optimization of Biobased Copolymers

2.3.6.1 Segment Design (Copolymer Architecture)

Rationally choosing block lengths and soft/hard compositions can optimize performance. For example, Cai et al. synthesized block polyurethanes from polyhydroxyvalerate (PHBV) copolymer and PEG via MDI; these materials were fully degradable and exhibited excellent mechanical properties and hemocompatibility[19]. This shows that incorporating biobased ester blocks (PHBV) with PEG in blocks copolymers yields promising biodegradable elastomers.

2.3.6.2 Blend/Composition Tuning

Adjusting the ratio of biopolymers to polyurethane modifiers can dramatically improve toughness. Krzykowska et al. found that adding just 10 wt.% of an aromatic polyurethane modifiers (MDI-based, PEG1000 soft segments) to P3HB raised its degradation temperature by ~30°C and markedly increased impact strength[18]. Similarly, Zarzyka et al. reported that P3HB blended with 5-15% of linear HDI/PPG PU significantly increased strain-at-break and impact strength (over 60% higher than neat P3HB[20]. Thus, a small fraction of copolymer or plasticizer can be optimized to balance strength and ductility.

2.3.6.3 Additives and Fillers

Combining biobased polyurethanes with nanoscale fillers or co-polyesters enables further tuning. For instance, introducing organo-modified montmorillonite (nanoclay) together with a

polyurethane modifier into P3HB created a nanobiocomposite with synergistic effects: even 1% clay plus 10% polyurethane raised thermal stability and toughness simultaneously[21]. Optimization can also include reactive chain extenders (e.g., glycerol, trimethylpropane) or coupling agents to enhance interfacial bonding. In all cases, iterative adjustment (e.g. via design-of-experiments) of monomer ratios, catalysts and processing conditions is used to maximize biobased content while meeting target properties[18][20].

2.4 Key Factors Influencing Polymer Properties

For the successful development of biobased copolymers, several parameters must be meticulously optimized.

2.4.1 Molecular weight and Polymerization Degree

The degree of polymerization and the molecular weight distribution critically impact both the mechanical performance and biodegradation rate of the polymers. Achieving a controlled molecular weight distribution ensures reproducible material properties [10]. Higher molecular weight segments generally enhance mechanical strength in segmented polyurethanes, increasing soft-segment Mn or hard-segment polymerization raises crystallinity and modulus. For example, Olszewski et al. showed that higher molecular weight bio-polyols increased foam compressive strength by ~40% (via more crosslinks and crystals)[17]. Conversely, too low Mn yields a more amorphous, soft material.

2.4.2 Crosslinking Density

An optimal balance between rigidity and flexibility is essential. Over-crosslinking may lead to brittle materials, whereas under-crosslinking could result in insufficient mechanical strength [10]. The density of chemical crosslinks (from multifunctional polyols or excess NCO) is a primary determinant of rigidity. More crosslinking raises transition temperature and stiffness but reduces elongation. Studies consistently report that raising the isocyanate index boosts hardness. Huang and Zhang noted that higher NCO/OH increased Transition temperature, stiffness, and tensile strength of polyurethane films[17]. In microphase-separated polyurethanes, additional crosslinks also augment inter-domain H-bonding, further reinforcing the network[17].

2.4.3 Phase Separation (Morphology)

Controlled microphase separation in block copolymers contributes significantly to enhancing toughness and barrier properties, making it a key design parameter [10].

The degree of hard/soft segment segregation strongly affects elasticity and toughness. Strong hard-segment interactions (e.g., from symmetric aromatics like MDI) lead to well-defined rigid domains, which increase strength but can reduce toughness[22]. In contrast, better soft-hard miscibility (achieved by using mixed soft polyols or by raising NCO/OH) can blur domain boundaries and yield more elastic behaviour[15].

Fuensanta et al. found that increasing the NCO/OH ratio actually improved miscibility of mixed soft segments (PPG/Pad) and decreased phase separation, altering melting temperature and raising TPU modulus [15]. Thus molecular weight and chemistry of both soft and hard blocks must be balanced to achieve the desired phase morphology and final properties[17], [22].

2.5 Techniques for Enhancing Biodegradability, Strength, and Stability

Various strategies have been implemented to tailor the properties of biobased copolymers.

2.5.1 Catalyst Optimization

Transitioning to green catalysts, such as enzyme-based systems or organocatalysts, helps minimize toxicity while ensuring efficient polymerization reactions.

Traditional organotin catalysts (e.g., DBTDL) can be replaced by biocompatible metals (bismuth, zinc, iron carboxylates). These new catalysts promote urethane formation efficiency without cytotoxicity[22]. Recent reviews highlight that that ZN-Or biobased catalysts enable high reactivity with much lower human/environmental toxicity.)

2.5.2 Biobased Monomers Selection

Integrating monomers derived from plant biomass (e.g., lactic acid, furfural) can improve the sustainability of the copolymers and add new functional properties. Studies illustrate how renewable feedstocks can serve as effective precursors for high-performance polymers[3], [4].

Incorporating hydrolysable ester segments greatly boosts biodegradability. For instance, Saha et al. used bacterial PHB-diol (from transesterified P3HB) alongside castor oil in polyurethane synthesis[9]. They observed that increasing PHB-diol content (especially using shorter-chain PHB-diol) tripled tensile strength and increased crystallinity, while polyurethanes made with long-chain PHB-diol degraded much faster than those with short chains[9]. In general, combining rigid PHB blocks with flexible PEG/PCL diols produces materials that are both though and hydrolytically labile.

2.5.3 Chemical Functionalization

Advanced techniques such as click chemistry and post-polymerization modifications for instance, Pylation) have been employed to enhance solubility, processability, and biocompatibility. Chemical Or nano-scale additives can synergize degradability and strength. Bialkowska et al. demonstrated that adding just 1% organo-modified montmorillonite (with 10% polyurethane modifier to P3HB increased thermal decomposition temperature by ~30°C and raised impact strength by ~15%[21]. The clay and polyurethane act as a plasticizing adduct that both toughens the polymer and hinders crack propagation. Similarly, introducing flexible polyester segments (PEG, PCL) into the backbone is known to reduce brittleness: Saha et al. note that incorporating soft segments like PEG, PCL or polyglycerol-based polyester can compensate for PHB's inherent brittleness[9]

2.5.4 Process Integration

The development of continuous flow reactors and the establishment of standardized protocols for P3HB-diol synthesis promise improved scalability, reproducibility, and cost-effectiveness in producing these biobased copolymers.

Techniques such as reactive extrusion, one-shot polymerizations, and melt blending can improve the integration of components. For example, solvent-free one-step syntheses of TPU pressure-sensitive adhesives (using mixtures of PLA, PCL, and PTMEG polyols) yielded uniform networks with excellent adhesion[15]. In polyurethane foams, using bio-polyols of varying PEG chain lengths allowed control over foaming and cell structure. Overall, combining biobased polyols with optimized catalysts and processing (e.g. appropriate mixing times and temperature) is key to maximizing both the biodegradability and performance of the final polyurethane.

2.6 Case Studies in Biobased Polyurethane Development

Several case studies underscore the progress in this field.

The first demonstrated that incorporating aromatic rings via MDI can yield polyurethanes with superior thermal and mechanical properties, rendering them suitable for high-performance biomedical applications [7].

The second showed that optimizing blend ratios and reaction conditions significantly enhances the impact strength and flexibility, producing robust hybrid materials [10].

Another case study illustrated the integration of biobased monomers from plant biomass to produce renewable and cost-effective polymer precursors, thereby facilitating the synthesis of high-performance, sustainable biobased copolymers [3], [4].

Chapter 3 Materials and Equipements

3.1 Introduction

This chapter outlines the materials, equipment and instruments employed during the experimental procedures of this research. The study involved the synthesis of polyurethanes using Polyhydroxybutyrate (PHB) oligomers and ethylene glycol as a polyol component, along with diisocyanates such as methylene diphenyl diisocyanate (MDI). Additionally, degradation reactions of PHB were performed using acidic catalysts in organic solvents to obtain hydroxyl-terminated oligomers suitable for further polymerization.

To ensure reproducibility and accuracy, all reagents, solvents, and catalysts were sourced from recognized suppliers and used as received unless otherwise stated. The experimental work required a combination of chemical reagents, controlled reaction setups, and advanced characterization tools such as FTIR and GPC. This chapter is organized into tables that summarize the chemicals used, solvents, laboratory equipment, and analytical instrumentation, accompanied by brief descriptions of their roles in the study.

3.2 Materials

3.2.1 Chemicals and Reagents

The chemicals used in this study played essential roles in both the degradation of PHB and the subsequent polyurethane synthesis reactions. All reagents were of analytical grade and were used without further purification unless otherwise noted. Poly(3-hydroxybutirate) (PHB) served as the main biopolymer for the transesterification reactions, while diols such as ethylene glycol were used as chain extenders. Diisocyanates including hexamethylene diisocyanate (HDI) and methylene diphenyl diisocyanate (MDI) were employed to form the urethane linkages. Triethylamine (TEA) acted as a catalyst in several polyurethane synthesis reactions. The following table lists all the key chemicals and reagents used throughout the experimental work, including their sources and purity levels where applicable.

The detailed list of the chemicals used in the experimental procedures is presented in table 2.

Table 2: List of chemicals, their purity, suppliers, and functional roles in the study

Chemical Name	Formula	Purity
Poly(3-hydroxybutyrate) (PHB)	$(C_4H_6O_2)n$	≥98%, bio-based
Ethylene glycol	$C_2H_6O_2$	≥99%
p-Toluenesulfonic acid (PTSA) (Catalyst)	$C_7H_8O_3S$	≥98.5%
Hexamethylene diisocyanate (HDI)	$C_8H_{14}N_2O_2$	≥98%
Poly(3-hydroxybutyrate) (PHB)	$(C_4H_6O_2)n$	≥98%, bio-based

3.2.2 Solvents

The solvents used in this study were essential for both the chemical degradation of PHB and the synthesis of polyurethane materials. They provided homogeneous reaction media, assisted in solubilizing reagents, and facilitated polymer precipitation and purification. All solvents were of high purity, procured from recognized suppliers, and used without further purification unless otherwise stated. Chloroform was particularly critical for PHB dissolution during alcoholysis reactions, while tetrahydrofuran (THF) was employed as the mobile phase in GPC analysis. Cold ethanol was used for product precipitation and washing steps. The following table lists the solvents used, along with their sources, catalog number, purity grades, and any relevant pretreatment

Table 3: List of solvents used in this work, including their chemical formula, supplier, catalog information, purity/grade, and pretreatment conditions

Solvent	Chemical Formula	Purity/Grade	Pretreatment
Chloroform	$CHCl_3$	≥99%, analytical	Used as received
Tetrahydrofuran (THF)	C_4H_8O	HPLC grade	Filtered before GPC use
Dimethylformamide (DMF)	C_3H_7NO	≥99%, analytical	Used as received
Ethanol	C_2H_5OH	95%, technical	Used cold for polymer precipitation
Distilled Water	H_2O	Laboratory grade	Used for washing steps

3.3 Equipement

3.3.1 Laboratory Equipment and Glassware

The chemical reactions, PHB decomposition, and subsequent polyurethane synthesis were performed using standard borosilicate glassware to ensure chemical resistance and thermal stability. Three-necks round-bottom flasks allowed for simultaneous addition of reagents, temperature monitoring, and reflux. Reflux condensers maintained solvent volumes during heating. Syringes and PTFE-stopcock funnels enabled precise addition and phase separation . All joints were ground-glass (24/40) to ensure airtight seals, and magnetic stirring bars facilitated homogeneous mixing.

The key pieces of glassware and general labware used throughout the depolymerization and polyurethane synthesis procedures are listed in table 4.

Table 4: Glassware and General Labware

Item	Volume/Size	Model or Feature	Use in Experiment
Three-neck round-bottom flask	250 mL	24/40 ground-glass joints	PHB depolymerization; PU synthesis
Reflux condenser	250 mm length	Liebig type	Maintain solvent reflux during reactions
Separatory funnel	100 mL	PTFE stopcock	Phase separation after reaction
Syringes	5 mL, 10 mL	Luer-lock	Addition of catalyst and diisocyanate
Beakers	100, 250 mL	Griffin low-form	Solution transfers; precipitation steps
Graduated cylinder	50 mL	Class A	Accurate solvent measurement
Magnetic stir bars	20 mm × 6 mm	PTFE-coated	Ensuring uniform stirring

3.3.2 Analytical Instruments

3.3.2.1 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy was employed to identify functional groups and confirm the chemical structure of the synthessized oligomers and polyurethanes. Spectra were recorded using a JASCO V-730 spectrophotometer (Figure 5), equipped with an ATR (Attenued Total Reflectance) accessory for direct analysis of solid and liquid samples without prior preparation. The spectral range was typically 400–4000 cm^{-1}, with a resolution of 4 cm^{-1} and averaging 32 scans per spectrum to ensure a high signal-to-noise ratio.

This analytical technique was fundamental to monitor the progress of depolymerization and polymerization reactions by detecting the disappearance or appearance of characteristic Vibrational bands, such as –NCO stretching (~2250 cm^{-1}), C=O urethane (~1700 cm^{-1}), and N–H stretching (~3300 cm^{-1}). The FTIR spectra provided direct evidence of chemical transformations occurring during the synthesis of PHB oligomers and the formation of urethane linkages in the polyurethane materials.

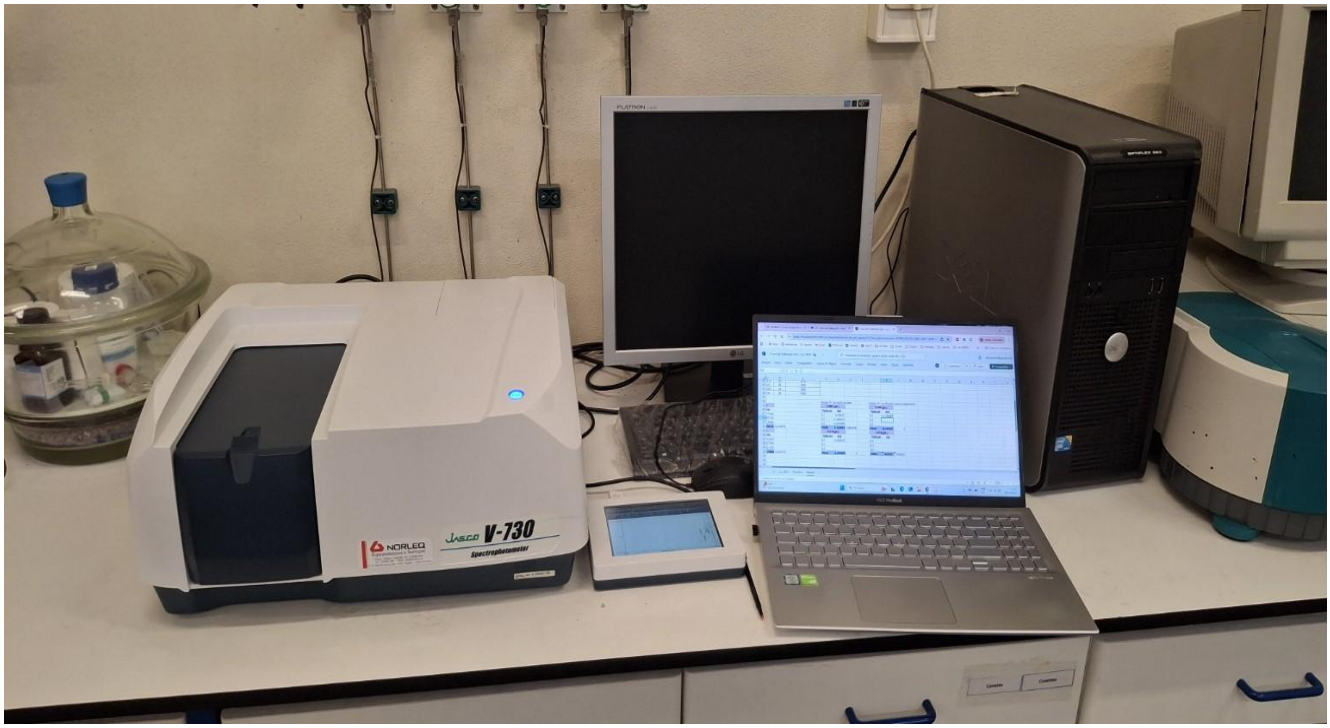

Figure 5: JASCO V-730 FTIR spectrophotometer used for structural characterization of PHB oligomers and polyurethane samples. Measurements were performed in the 4000–400 cm^{-1} range using ATR mode with a resolution of 4 cm^{-1}.

3.3.2.2 Size Exclusion Chromatography (SEC) in DMF

To characterize the molecular structure of the soluble polymer, a size exclusion chromatography (SEC) system (figure 6) equipped with refractive index (RI), Low Angle Light Scattering (LALS), and Right Angle Light Scattering (RALS) detectors was used. The system consisted of a viscoteck GPCmax VE 2001 pumping unit and two analytical columns (PLgel 5 µm Mixed-D and PLgel 3 µm Mixed-E; 300 × 7.5mm).

Dimethylformamide (DMF) with 20 mM LiBr was used as the eluent at a constant temperature of 50°C and a flow rate of 0.5mL/min. this system was applied to determine the molecular weight distributions of PHB oligomers and polyurethane synthesized in this work.

Figure 6: GPC system with RI/LALS/RALS detection (DMF-based) used for oligomer analysis

3.3.2.3 Size Exclusion Chromatography in Chloroform

A second GPC/SEC system (Figure 7) was used to analyse samples diluted in chloroform ($CHCl_3$). This apparatus was composed of a polymer Laboratories PL-GPC-50 integrated system coupled with a differential refractometer (950 ± 30 nm) and a multi-angle light scattering detector (Wyatt DAWN8+HELEOS at 658 nm). Separation was performed on a set of three PLgel columns (300 × 7.5 mm, 10 µm particle size, Mixed-B LS pore type) operated at 30°C with a flow rate of 1.0 mL/min. This configuration enabled the determination of weight-average molecular weights as well as the z-average radius of gyration. It was particularly useful in assessing the chain architecture of synthesized polyurethane.

Figure 7 : GPC system with RI/LALS/RALS detection chloroform-based) used for PHB oligomers and polyurethane analysis

3.3.2.3.1 Procedure

Gel Permeation Chromatography (GPC) was employed to determine the molecular weight distribution of different samples, including pure diisocyanates (MDI and HDI) and polyurethane polymers synthesized using MDI with either PHB oligomers (Experiment 4) or ethylene glycol (Experiment 5). This analysis allows for the evaluation of polymer chain length, molecular weight averages (Mn, Mw), and dispersity.

3.3.2.3.1.1 Materials and Sample Preparation

- **Samples analysed**

- Pure 4,4'-methylenediphenyl diisocyanate (MDI)
- Pure hexamethylene diisocyanate (HDI)
- Polyurethane from PHB oligomers + MDI (Experiment 4)
- Polyurethane from ethylene glycol + MDI (Experiment 5)

- **Solvent**

- Tetrahydrofuran (THF, HPLC grade)

- **Sample preparation procedure:**

Each sample was dissolved in THF to a concentration of 2 mg/mL.

Solutions were filtered using 0.45 µm PTFE syringe filters.

Approximately 100 µL of each solution was injected into the GPC system.

3.3.2.4 Centrifuges

Tow centrifuges were used throughout the experimental procedures to aid in phase separation and solid-liquid separation steps during sample preparation and polymer purification. The first was small, analogy benchtop centrifuge, suitable for quick separations in small-volume vials. The second was a large-capacity, digitally controlled centrifuge, offering programmable speed and time setting for processing larger sample volumes with higher precision. Figure 8 shows the numerical centrifuge used for large-scale separation during PHB oligomer and polyurethane purification. and Both centrifuges played essential roles in post-reaction separation steps, particularly during the purification of PHB oligomers and polyurethane precipitates prior to during and analysis.

Figure 8: Large-capacity digital centrifuge (Eppendorf 5810 R) used for phase separation and purification of PHB oligomers and polyurethane precipitates during sample processing.

Chapter 4 Results and Discussion

4.1 Polyhydroxyalkanoates Experiments

4.1.1 Experimental Methodology

4.1.1.1 Solubility Assessment

The initial step in this study involved assessing the solubility of poly(3-hydroxybutirate) (PHB) in various solvents to identify suitable conditions for subsequent degradation experiments. PHB, was tested for solubility in three organic solvents: Tetrahydrofuran (THF), Dimethylformamide,

(DMF), and Chloroform ($CHCl_3$). The experiments was conducted at room temperature (approximately 25°C) to evaluate solubility under ambient conditions.

For each solvent, 12.7 mg of PHB was added to the respective solvent in sealed containers. The volumes of the solvent used were 7 mL of DMF, 5mL of THF, and 5 mL of Chloroform. The mixture were stirred for 30 minutes using a magnetic stirrer, and the solubility was assessed visually. A solvent was deemed suitable if the PHB completely dissolved, forming a clear solution.

Table 5: Checking solubility of P3HB with DMF, THF, and Chloroform.

	DMF			**THF**			**$CHCl_3$**		
Boiling point (°C)	**~153**			**~66**			**~58**		
T (°c)	**PHB (mg)**	**V (ml)**	**solubility**	**PHB (mg)**	**V (ml)**	**solubility**	**PHB (mg)**	**V (ml)**	**solubility**
25	12.7	7	No	11	5	No	10.4	5	No
40	–	–	–	–	–	–	10.4	5	Yes

The results indicated that PHB is not soluble in DMF, THF, or chloroform at room temperature. However, upon heating chloroform to 40°C, PHB dissolved completely, forming a clear solution. This finding established chloroform as a suitable solvent for subsequent experiments under elevated temperature conditions.

4.1.2 Depolymerization of Poly(3-hydroxybutyrate) (PHB) for 6 Hours

The depolymerization of PHB was conducted in a three-neck round-bottom flask (figure 9) equipped with a reflux condenser, magnetic stirrer, and thermometer. Initially, 3g of PHB was dissolved in 30 mL of Chloroform under heating at 58°C, the boiling point of Chloroform, and stirred until a clear solution was formed, separately, a solution of ethylene glycol (6g, 5.41 mL, 1.11mg/mL) and 1.44g of p-toluenesulfonic acid (48% of the PHB mass) was prepared, with 2.5 mL of Chloroform added to ensure complete solubility. Once the PHB-chloroform solution reached a steady boil, the prepared catalyst solution was injected into the boiling mixture using a syringe, ensuring gradual addition for uniform mixing. The reaction was maintained at 58° under reflux with continuous stirring for 6 hours. After the reaction, the mixture was cooled to room temperature and washed three times with distilled water to remove residual catalyst and by-

products. The organic layer was then concentrated by evaporating the Chloroform under reduced pressure, yielding the depolymerized product.

Table 6: Experimental conditions: reagent amounts used in PHB degradation.

Reagent	Mass (g)	Volume (mL)	Density (g/mL)
PHB	3	–	–
Chloroform ($CHCl_3$)	–	30	–
Ethylene Glycol (EG)	6	5.41	1.11
p-Toluenesulfonic Acid	1.44	–	–

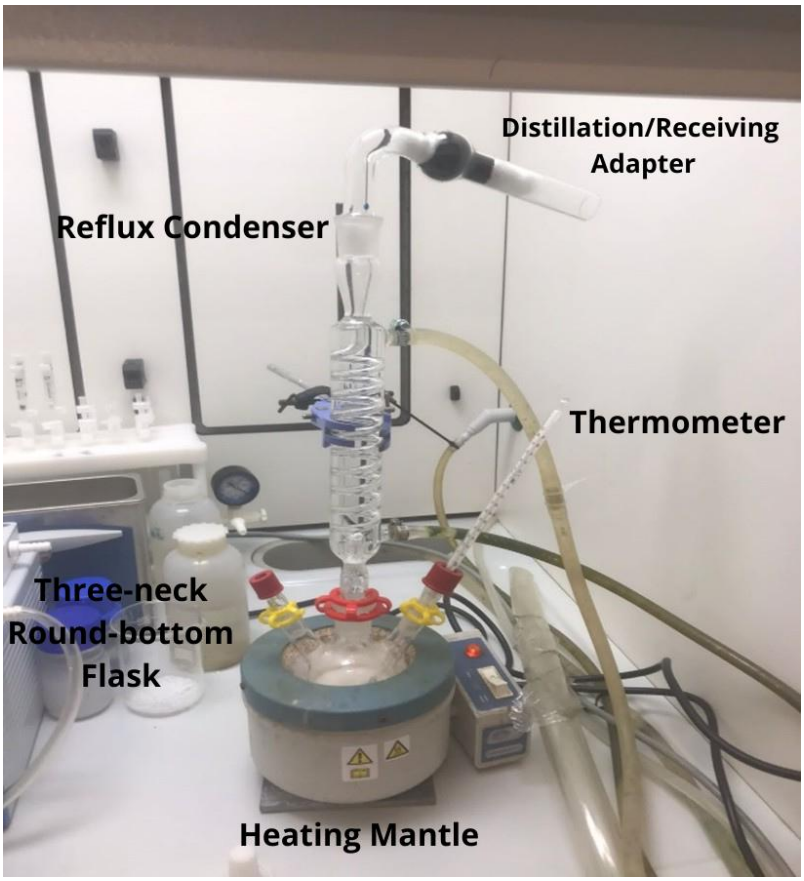

Figure 9: Apparatus for depolymerization of PHB

4.1.2.1 Observations

The product obtained from the depolymerization reaction was precipitated in the cold ethanol to separate it from the reaction mixture. After precipitation, the product was dried in an oven to

remove any residual solvents. The final product exhibited a powder-like morphology, indicating successful depolymerization and recovery of the material.

4.1.2.2 FTIR Analysis of Depolymerized PHB

Fourier transform infrared (FTIR) spectroscopy was employed to analyse the structural changes in PHB before and after depolymerization. The goal was to detect the appearance of hydroxyl (-OH) groups in the depolymerized product, which would indicate successful cleavage of the polymer chains. The analysis was performed on three samples: (1) the original PHB, (2) the depolymerized product not fully dried from ethanol, and (3) the depolymerized product fully dried from ethanol.

FTIR spectra were recorded in the range of 400–4000 cm^{-1}. Each sample was prepared as a thin film and analyzed under identical conditions to ensure consistency.

The FTIR spectrum of the original PHB (figure 10) exhibited characteristic absorption bands, including: A strong band at ~1720 cm^{-1} corresponding to the ester carbonyl (C=O) group. Bands in the region of ~1180-1270 cm^{-1} associated with the C-O stretching vibrations of the ester group. The spectrum of the depolymerized product, both not fully dried and fully dried, showed similar characteristic bands. However, an additional broad band around ~3300 – 3500 cm-1, indicative of hydroxyl (-OH) groups, was observed in the spectrum of the not fully dried product. This band diminished significantly in the spectrum of the fully dried product, suggesting that it was primarily due to residual ethanol rather than the formation of hydroxyl groups in the depolymerized product.

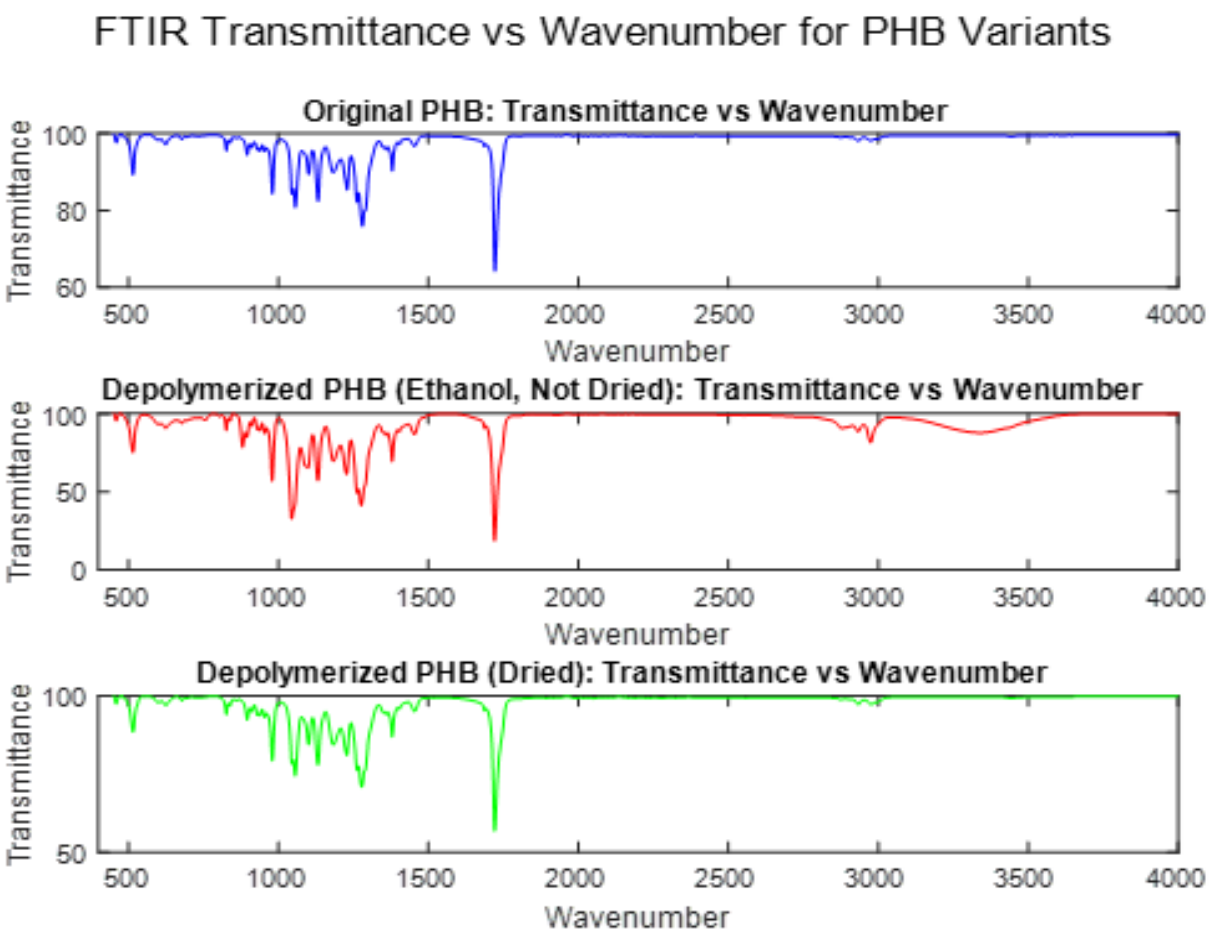

Figure 10: A comparison of the different FTIR spectra of original PHB, not fully dried degraded PHB, and well died degraded PHB

No significant differences were observed between the original PHB and the fully dried depolymerized product. This indicates that the expected hydroxyl groups from depolymerization were not detectable under these conditions, potentially due to the low concentration of hydroxyl-terminated oligomers or overlapping signals from the ester groups.

FTIR analysis revealed that the band corresponding to the hydroxyl group (-OH) in the depolymerized product was attributed to the presence of residual ethanol rather than structural changes in the polymer. This highlights the importance of thoroughly drying the product to avoid interference from residual solvents.

4.1.3 Transesterification of P3HB for 24 Hours: First Attempt

Following the initial 6-hour depolymerization experiment, a second depolymerization experiment was conducted under identical conditions but extended over a total duration of 24 hours. The reaction was performed in four intervals of 6 hours each, using the same apparatus setup. The

purpose of this extended reaction was to assess the impact of prolonged depolymerization on the final product, particularly the formation of hydroxyl-terminated oligomers.

4.1.3.1 Experimental Procedure

In this experiment, 3.0197 g of PHB was dissolved in 30 mL of chloroform under heating at 58°C in a three-neck round-bottom flask equipped with a reflux condenser, magnetic stirrer, and thermometer. Separately, a solution of ethylene glycol (6 g, 5.41 mL, 1.11 mg/mL) and 1.4481g of p-toluenesulfonic acid (48% of the PHB mass) was prepared, with 2 mL of chloroform added to ensure full solubility.

The PHB-Chloroform solution was heated to boiling, and the prepared ethylene glycol and catalyst solution was injected into the boiling mixture using a syringe. The reaction was maintained at 58°C under reflux with continuous stirring. After each 6-hour interval, a sample of the reaction mixture was taken, precipitated in cold ethanol, and dried in an oven to remove residual solvents.

This process was repeated for three additional intervals of 6 hours each, with the total reaction time amounting to 24 hours.

4.1.3.2 Post-Reaction Treatment

At the end of the 24-hour reaction, the product was precipitated in cold ethanol and then dried in an oven at 100°C to remove residual solvents. While the product from the previous 6-hour depolymerization experiment, dried at 70°C, was well-dried and presented as a fine powder, the product obtained after 24 hours exhibited a viscous texture despite being dried at a higher temperature

This unexpected viscosity could be attributed to several factors related to the extended reaction time:

- Increased molecular weight distribution.
- The prolonged depolymerization may have led to the formation of oligomers with a higher degree of polymerization, resulting in a more viscous product.
- Partial crosslinking.
- Extended reaction times and higher temperatures might have facilitated secondary reactions, such as crosslinking or branching, which can increase the product's viscosity.
- Residual ethylene glycol or catalyst interaction.

Even with drying at 100°C, traces of ethylene glycol or p-toluenesulfonic acid might remain, potentially contributing to the viscous nature of the product.

- Thermal degradation or side reactions.

At 100°C, some thermal degradation of PHB or its oligomers might have occurred, leading to changes in the product's physical properties.

4.1.4 Transesterification OF P3HB for 24 hours: Second Attempt with Reduced Catalyst

Building upon the previous 24-hour depolymerization experiment, a second trial was conducted under similar conditions but with a significantly lower catalyst loading (reduced from approximately 50% to 2% relative to PHB). In this experiment, 6.0394 g of PHB was dissolved in 60 mL of chloroform at 58°C in the same three-neck round-bottom flask setup described earlier. Ethylene glycol (12 g, 10.81 mL, 1.11 mg/mL) was used as the depolymerizing agent, while the mass of p-toluenesulfonic acid was lowered to 0.1208 g. Unlike the previous experiment, no additional chloroform was required to dissolve the catalyst.

The reaction proceeded for a total of 24 hours, with samples taken at 6-hours intervals to monitor the progress of depolymerization. Each sample was immediately quenched by precipitation in cold ethanol and subsequently dried. The final product at 24 hours was also recovered by the same method. Table 7 summarizes the reagent quantities used in this second 24-hour experiment.

Table 7: Reagent Quantities for the Second 24-Hour Depolymerization with Reduced Catalyst

Reagent	Mass (g)	Volume (mL)	Density (mg/mL)
PHB	6.0394	-	-
Chloroform ($CHCl_3$)	-	60	-
Ethylene Glycol (EG)	12	10.81	1.11
p-Toluenesulfonic Acid	0.1208	-	-
Additional Chloroform	-	Not needed	-

As with the previous depolymerization runs, the mixture was stirred continuously under reflux at 58 °C. samples were analysed to determine the extent of depolymerization and to compare any variations in product morphology or composition resulting from the reduced catalyst concentration. The final precipitated product was dried in an oven and evaluated for any difference in physical properties, such as viscosity or powder formation, relative to the products obtained from earlier experiments.

4.1.4.1 Mass Balance Determination

To monitor the progress of depolymerization and quantify the amount of recovered polymer, mass measurements were taken at 6-hour intervals (6, 12, 18, and 24 hours). At each interval, the

reaction mixture was sampled, the polymer was precipitated in cold ethanol, and then dried before weighing. The dried product was weighed along with the empty flask, allowing for the calculation of polymer mass by difference. Multiple measurements were performed for each time point to improve accuracy. The specific data collected are shown in the Table 8 adapted from the "mass balance" sheet).

Table 8: : Mass balance determination during the depolymerization of PHB, showing flask mass, flask plus polymer mass, calculated polymer mass at different reaction times (6, 12, 18, and 24 h), and corresponding averages.

Time (h)	Mass of flask (g)	Mass of the flask with the polymer (g)	Mass of polymer (g)
6	10.7206	11.4936	0.7730
	10.7206	11.0913	0.3707
	10.7206	11.0706	0.3500
	10.7206	11.0735	0.3529
		Average	0.3579
12	10.8000	11.6474	0.8474
	10.8000	11.1948	0.3948
	10.8000	11.1756	0.3756
		Average	0.3756
18	10.7668	11.6038	0.8370
	10.7668	11.1579	0.3911
		Average	0.3911
24	10.7796	11.1666	0.3871
		Average	0.3871
Total			**7.0926**
Mass Balance			**0.393 (39.3%)**

➔The mass of the recovered polymer $m_{polymer}$ was calculated as:

$$m_{polymer} = m_{(flask + polymer)} - m_{flask}$$

Where m_{flask} is the mass of the empty flask and $m_{(flask + polymer)}$ is the mass of the flask containing the dried polymer. Each measurement was performed in triplicate at every time point, and the ariythmetic mean was reported.

4.1.4.2 Cumulative Mass and Final Mass Balance

The total mass of polymer recovered over all sampling intervals (6, 12, and 24 hours) was summed to give a final value of 7.0926 g.

The overall mass balance was then calculated as 0.393 (i.e., 39.3%), including the fraction of the initial polymer that was recovered under the specific reaction and work-up procedure.

4.1.4.3 Interpretation and Possible Factors

- A mass balance of 39.3% suggests that a significant portion of the polymer or its oligomers may remain dissolved in the reaction medium, may be lost during precipitation and washing, or may have undergone side reactions leading to volatile by-products.
- Further investigation (e.g., analyzing the filtrate or washing solutions) would help clarify the fate of the remaining mass.
- Optimizing reaction conditions, precipitation steps, and drying procedures could improve the overall recovery.

4.1.4.4 FTIR Analysis of the PHB Oligomers Obtained After Depolymerizing PHB for 24 Hours (Second Attempt)

The FTIR spectrum of the PHB oligomers obtained after 24 hours of depolymerization (second attempt) with reduced catalyst is presented in figure 11. The spectrum provides evidence of ester bond cleavage and the formation of hydroxyl-terminated oligomers.

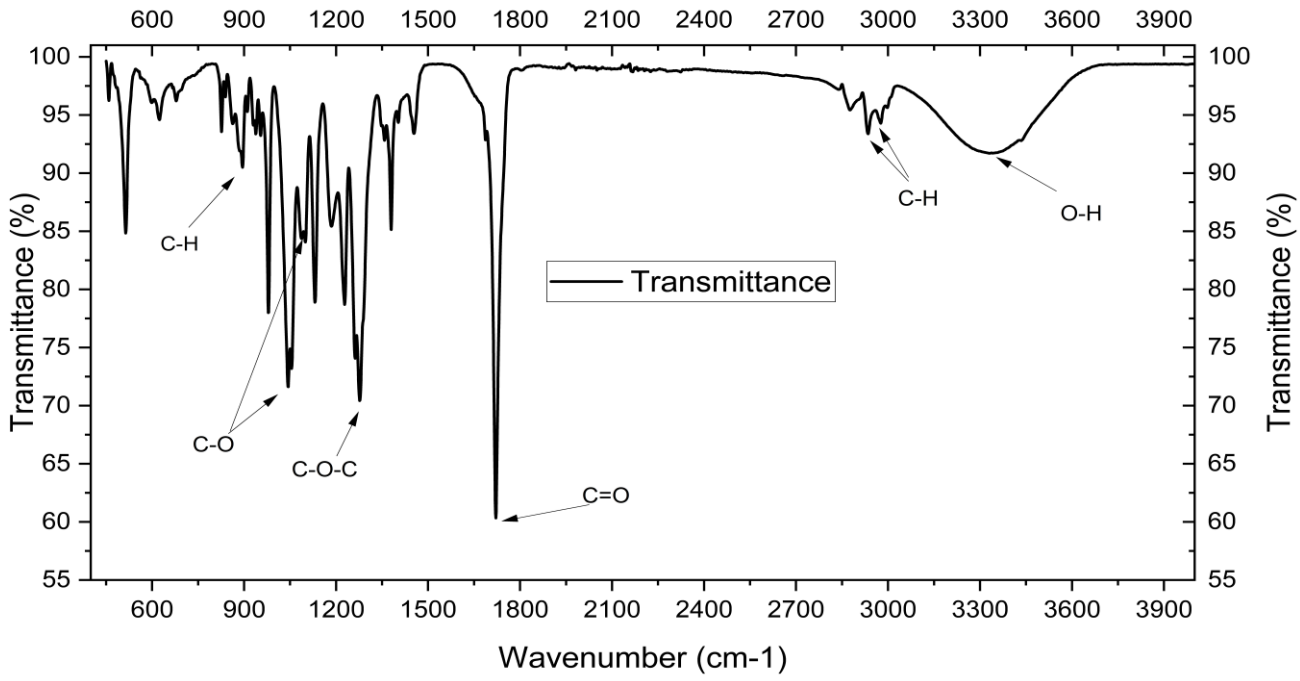

Figure 11: FTIR spectrum of PHB oligomers obtained after 24 h depolymerization (second attempt with reduced catalyst). Characteristic absorptions of polyester backbones and terminal hydroxyl groups are identified, confirming the successful generation of hydroxyl-terminated oligomers

Table 9 summarizes the peak by peak FTIR analysis of the PHB oligomers obtained after 24 hours of depolymerization (second attempt with reduced catalyst).

Table 9: Peak-by-peak FTIR band assignments for PHB oligomers obtained after 24 h depolymerization (second try), highlighting the preservation of ester functionalities and the emergence of terminal hydroxyl groups

Wavenumber (cm^{-1})	Assignment	Interpretation
~3340	O-H stretching	Confirms formation of hydroxyl terminated oligomers
~2935 and 2975	C-H stretching (CH_2 and CH_3 groups)	Aliphatic vibration from PHB backbone
~1720	C=O stretching for ester carbonyl	Characteristic of PHB ester group. The persistence of the band confirms polyester nature is preserved.
~1450	CH_2 - CH_3 bending mode	Vibrations of aliphatic groups in PHB backbone
~1277	C-O-C asymmetric stretching	Confirms ester linkages remain in oligomers
~1050 and 1100	C-O stretching (Alcohols/esters)	Overlap between ester C-O and terminal hydroxyl vibrations.
~890	C-H bending backbone stretching	Fingerprint region for PHB

4.1.4.5 Size Exclusion Chromatography (SEC) Analysis of PHB Depolymerization Products

To evaluate the molecular weight evolution during the alcoholysis reaction of poly(3-hydroxybutyrate) (PHB) with ethylene glycol (EG), size exclusion chromatography (SEC) coupled with refractive index (RI) and multi-angle laser light scattering (MALLS) detectors was performed. The resulting chromatograms are shown in figure 11.

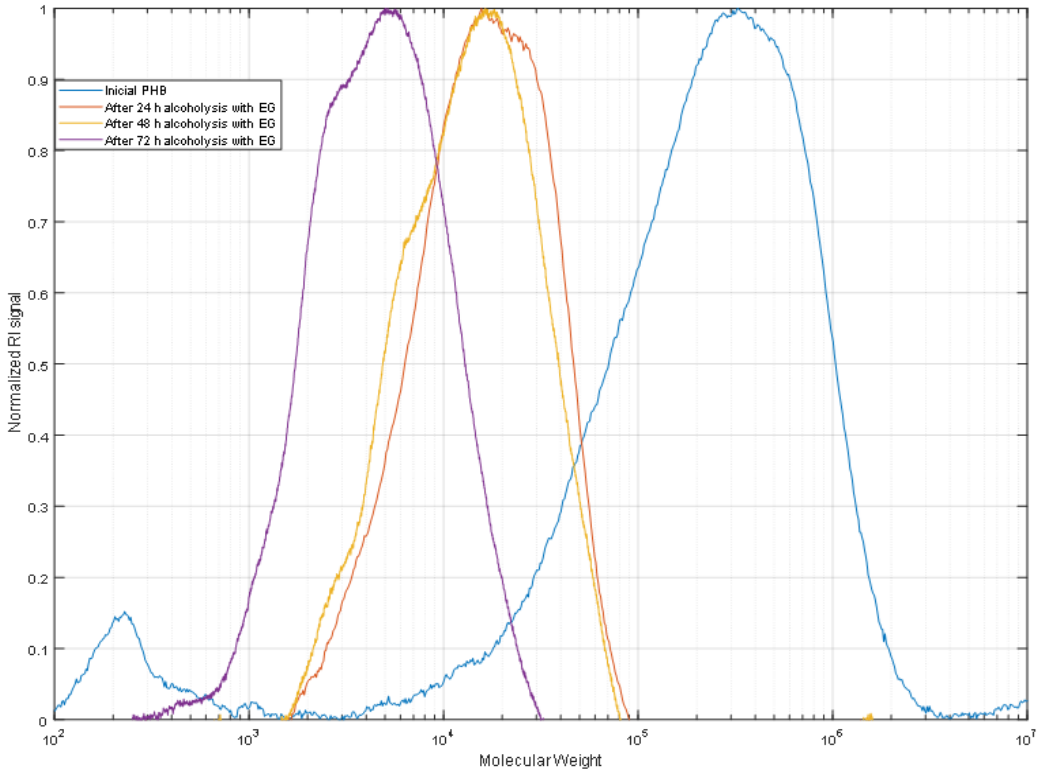

Figure 12: SEC/RI/MALLS analysis of the PHB sample and corresponding products obtained by depolymerization with ethylene glycol (EG) considering different reaction times. A clear depolymerization of the initial PHB is observed. SEC/RI/MALLS analysis performed with chloroform at a flow rate of 1 ml/min and temperature of 30 °C

The size exclusion chromatography (SEC) profiles in figure 11 clearly demonstrate the progressive depolymerization of the initial PHB as reaction time increases.

The original PHB (blue curve) exhibits a single, narrow peak at lower elution volumes, corresponding to its high molar mass (~10^6 Da). After 24 hours of alcoholysis with ethylene glycol (EG)(orange curve), a significant shift of the peak toward higher elution volumes (lower molecular weight) is observed, confirming extensive chain scission.

After 48 and 72 hours (red and purple curves, respectively), the main peak continues to move toward even higher elution volumes, with a noticeable narrowing of the distribution, indicating the formation of shorter, more homogeneous oligomeric species. This evolution demonstrates that longer reaction times promote deeper degradation of PHB chains into oligomers, consistent with the cleavage of ester linkages and formation of hydroxyl-terminated species.

The overall trend confirms that the depolymerization process effectively converts PHB into oligomers suitable for further polyurethane synthesis. These findings align with FTIR results, in which the appearance of broad O–H stretching bands and reduced C=O ester intensity also indicate partial chain cleavage and hydroxyl formation.

4.1.5 Polyurethane Synthesis From PHB Oligomers (Second Attempt) Via Isocyanate Reactions

This section presents a series of experiments aimed at synthesizing polyurethane materials from depolymerized PHB oligomers obtained from the recent depolymerization of PHB for 24 hours (second try). The overall objective was to explore the reactivity of PHB oligomers in polyurethane formation using different isocyanates (HDI and MDI) and polyols (PHB oligomers vs ethylene glycol), with triethylamine (TEA) as the catalyst. All reactions were performed at 70°C for 24 hours, and in selected experiments, additional chloroform ($CHCl_3$) was used to enhance solubility. Below is a detailed account of the experimental design and formulations.

4.1.5.1 Synthesis of the Polyurethane (PU1) With PHB Oligomers, HDI, and TEA

In the first experiment, PHB oligomers obtained from 24-hour depolymerization (second attempt) were used as the polyol component. HDI was employed as the isocyanate, and TEA acted as the catalyst. The formulation was designed to test the feasibility of forming polyurethane directly from PHB oligomers under the set reaction conditions.

Table 10: Formulation of reagents used for the synthesis of polyurethane (PU1) from PHB oligomers (24 h depolymerization), HDI, and TEA.

Component	m (mg)	Density (g/mL)	m (g)	V (mL)	V (µL)
PHB (EXP3_24H)	10.4	–	–	–	–
HDI	100	1.047	0.1	0.09551	95.5
TEA	2	0.728	0.002	0.00275	2.7

4.1.5.2 Synthesis of the Polyurethane (PU2) With Ethylene Glycol (EG), HDI, and TEA

Experiment 2 served as a baseline reaction using a well-characterized polyol (ethylene glycol) in place of PHB oligomers. This experiment was performed under identical conditions (70°C for 24 hours) to compare the reactivity of Ethylene Glycol with that of PHB oligomers.

Table 11: Reagent composition for the synthesis of polyurethane (PU2) using Ethylene Glycol, HDI, TEA

Component	m (mg)	Density (g/mL)	m (g)	V (mL)	V (µL)
EG	36	1.10	0.036	0.03273	32.7
HDI	100	1.047	0.1	0.09551	95.5
TEA	2	0.728	0.002	0.00275	2.7

4.1.5.3 Synthesis of the Polyurethane (PU3) With PHB Oligomers, HDI, TEA, and Chloroform ($CHCl_3$)

In Experiment 3, PHB oligomers were again employed as the polyol, however, this formulation included the addition of 2 mL of chloroform (CHCl3) to enhance solubility. The reaction components (HDI and TEA) were used in the same amounts as in experiment 1, and the experiment was repeated several times to ensure reproducibility.

Table 12: Reagent composition for the synthesis of polyurethane (PU3) using PHB oligomers, HDI, TEA, and chloroform ($CHCl_3$) as a co-solvent

Component	m (mg)	Density (g/mL)	m (g)	V (mL)	V (µL)
PHB (EXP3_24H)	10.4	–	–	–	–
HDI	100	1.047	0.1	0.09551	95.5
TEA	2	0.728	0.002	0.00275	2.7
$CHCl_3$	–	–	–	2	–

4.1.5.4 Synthesis of the Polyurethane (PU4) with Ethylene Glycol (EG), MDI, and TEA

To evaluate the effect of a different isocyanate, Experiment 4 replaced HDI with methylene diphenyl diisocyanate (MDI) while using ethylene glycol as the polyol. This allowed a direct comparison of isocyanate reactivity on the formation and properties of the resulting polyurethane.

Table 13: Reagent composition for the synthesis of Polyurethane (PU4) using Ethylene Glycol (EG), Methylene Diphenyl Diisocyanate (MDI), and TEA

Component	m (mg)	Density (g/mL)	m (g)	V (mL)	V (µL)
EG	36.00	1.10	0.04	0.033	32.73
MDI	145.14	1.05	0.145	0.10	–
TEA	2.00	0.728	0.002	0.00275	2.75

4.1.5.5 Synthesis of the Polyurethane (PU5) With PHB Oligomers, MDI, and TEA

In Experiment 5, PHB oligomers (EXP3 (24h)) were reacted with MDI and TEA. This experiment was intended to assess whether PHB oligomers could successfully form polyurethane when paired with MDI, and to compare the resulting product properties with those obtained using HDI.

Table 14: Polyurethane Synthesis with PHB Oligomers, MDI, and TEA

Component	m (mg)	Density (g/mL)	m (g)	V (mL)	V (µL)
PHB (EXP3_24H)	10.4	–	–	–	–
MDI	148.78	1.047	0.149	0.142	142.1
TEA	2.00	0.728	0.002	0.003	2.75

For all experiments, the reactions were carried out at 70 °C for 24 hours with continuous stirring to ensure uniformity.

4.1.6 Spectroscopic and Chromatographic Analysis of the Polyurethanes Obtained Using HDI Diisocyanate

4.1.6.1 FTIR Analysis of Hexamethylene Diisocyanate (HDI)

To provide a reference for interpreting polyurethane spectra, the FTIR profile of the neat aliphatic diisocyanate HDI was recorded and is showen in the figure 13.

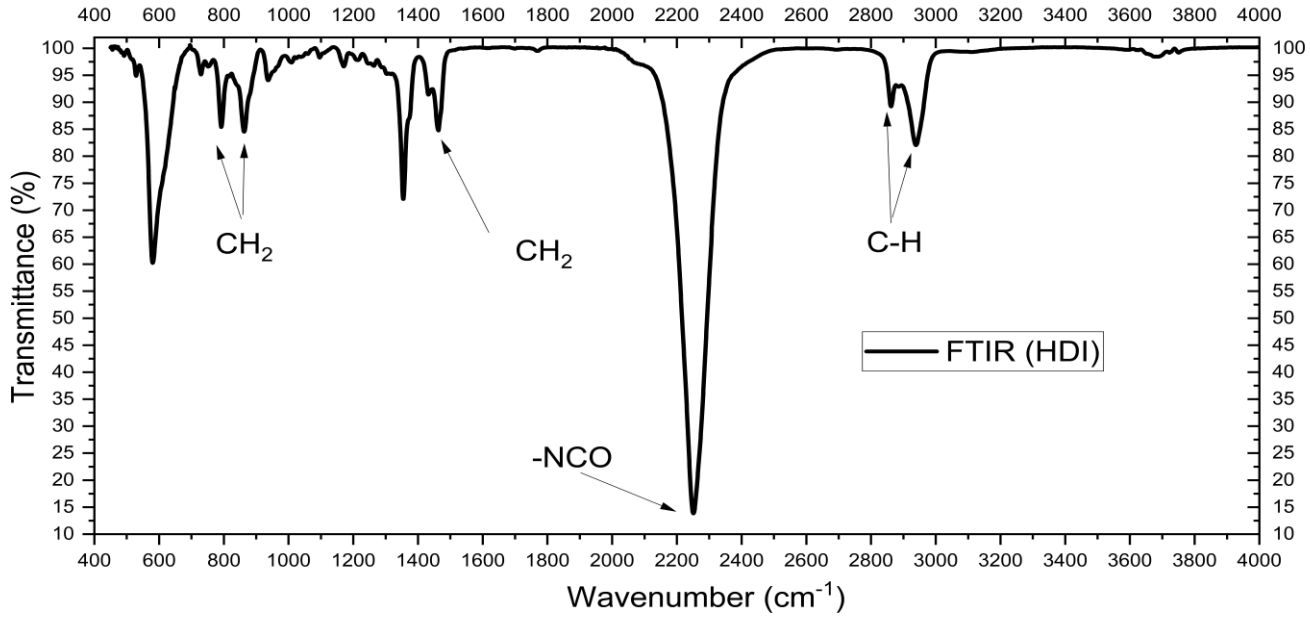

Figure 13: FTIR spectrum of neat hexamethylene diisocyanate (HDI)

Table 15 presents the peak by peak FTIR assignment for hexamethylene diisocianate (HDI).

Table 15: Peak-by-peak FTIR assignments for hexamethylene diisocyanate (HDI)

Wavenumber cm^{-1}	Assignment	Interpretation
~2252	–NCO stretch	Primary marker of free HDI, must disappear after complete conversion
~2935	C–H Asymmetric stretching	Confirms aliphatic backbone, distinguish HDI Pus from MDI PUs
~2862	C–H symmetric	Complementary CH_2 vibration
~1464	CH_2 bending	Confirms aliphatic methylene groups
~790 and 861	CH_2	Fingerprint bands, typical of linear aliphatic chains, absent in aromatic isocyanates (MDI).

The FTIR spectrum of hexamethylene diisocyanate (HDI) shows a strong and sharp band at ~2252 cm^{-1}, which corresponds to the stretching vibration of the isocyanate group (–NCO). This band is highly diagnostic and will vanish after reaction with hydroxyl groups during polyurethane synthesis, making it a crucial reference for confirming complete polymerization.

The aliphatic nature of HDI is confirmed by the symmetric and asymmetric CH_2 stretching bands at ~2935 and 2862 cm^{-1}, as well as the bending vibration at ~1464 cm^{-1}, additionally, the rocking vibrations at ~861 and 790 cm^{-1} further confirm the presence of long aliphatic chains. Importantly,

no aromatic C=C or out-of-plane C–H bending bands are detected, which distinguishes HDI from aromatic diisocyanates such as MDI.

Overall, the FTIR profile of HDI provides the baseline reference for identifying urethane bond formation in later polyurethane spectra, where the disappearance of the 2252 cm^{-1} (–NCO) band and the emergence of new C=O (~1700 cm^{-1}) and N–H (~3300 cm^{-1}) bands are expected.

4.1.6.2 FTIR Analysis of Polyurethane (PU1) Formed With PHB Oligomers, HDI, and TEA

The FTIR of the polyurethane PU1 obtained using PHB oligomers, HDI, and TEA is shown in the figure 14.

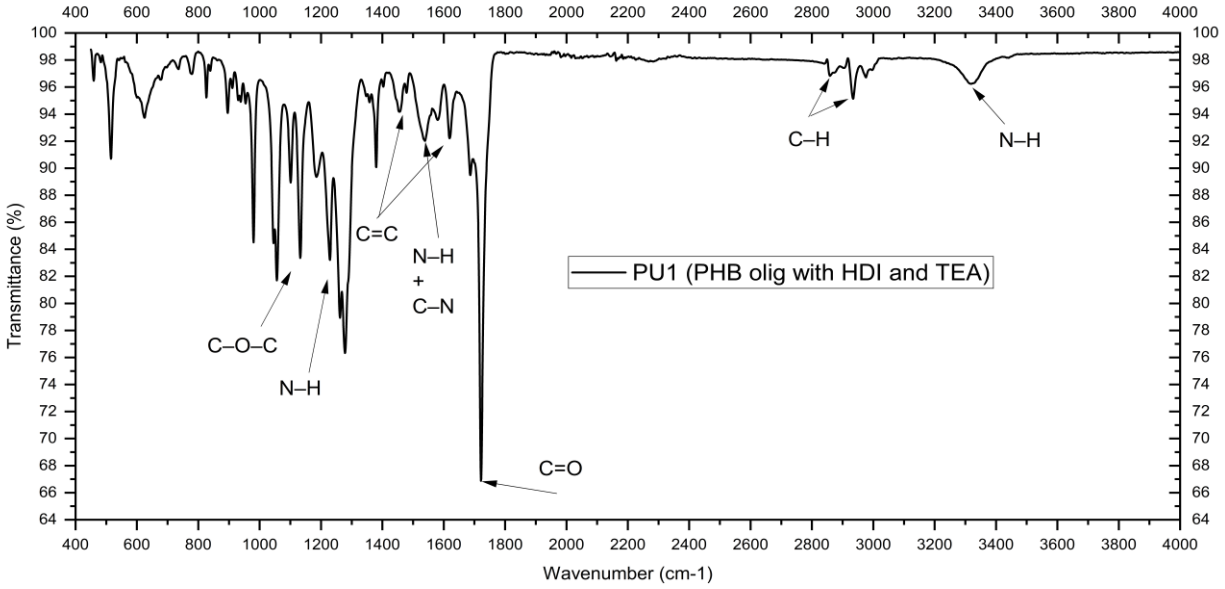

Figure 14: FTIR spectrum of the Polyurethane (PU1) synthesized from PHB oligomers, HDI, and TEA.

Table 16 Shows the Peak by Peak Assignment of the FTIR Spectrum of the Polyurethane PU1 Synthesized from PHB Oligomers, HDI, and TEA.

Table 16: Peak-by-peak FTIR assignments for polyurethane (PU1) synthesized from PHB oligomers, HDI, and TEA

Wavenumber (cm^{-1})	Band assignment	Interpretation
~3323	N-H stretching of Urethane -NH-COO-	Confirms urethane linkages
~2858 and 2935	Aliphatic C-H stretching (CH_2 of HDI and PHB segments	Confirms the presence of long aliphatic chains from HDI/PHB
~1722	Carbonyl stretching mainly urethane C=O	Strong proof of Urethane formation
~1617 and 1456	Aromatic C=C stretching	Not present /very weak, consistent with aliphatic HDI (distinguishes from MDI-based polyurethane
~1532	Amide II (N-H bending + C-N stretching of urethane)	Secondary confirmation of urethane linkage
~1227	C-N stretching of urethane	Fingerprint of urethane bond -NH-COO-
~1133	C-O-C stretching (Urethane/ester; EG/PHB segments)	Confirms ether/ester vibrations within soft segment and urethane C-O
~770	Aliphatic and backbone fingerprint modes.	Typical for polyester/PU backbone; no aromatic out of plane C-H (again consistent with HDI).
~2265-2285	Absence of -NCO stretching (unreacted isocyanates)	Indicate near complete consumption of HDI essential quality check

The FTIR spectrum of the polyurethane synthesized from PHB oligomers and HDI in the presence of TEA displays all diagnostic absorptions expected for urethane formation. A broad band at ~3323 cm^{-1} corresponds to N-H stretching vibrations of urethane linkages (-NH-COO-), confirming successful incorporation of isocyanate groups into the polymer backbone. Strong absorptions at ~2935 and 2858 cm^{-1} are assigned to aliphatic C–H stretching of CH_2 units both HDI and PHB segments consistent with the aliphatic nature of diisocyanate employed. The intense carbonyl band at ~1722 cm^{-1} provides strong evidence of urethane C=O formation, partially overlapping with residual ester groups of PHB. Additional confirmation of urethane linkages is provided by the amide II band at ~1532 cm^{-1} (N-H bending and C–H stretching) and the C–N stretching vibration at ~1227 cm^{-1}, a characteristic fingerprint of urethane bonds. The C–O–C

stretching region at ~1133 cm^{-1} further supports the coexistence of ester and urethane groups in the polymer. Fingerprint vibrations observed at ~770 cm^{-1} are typical of aliphatic polyurethane backbone, with no aromatic out of plane C–H absorptions, consistent with the use of aliphatic HDI rather than aromatic MDI importantly, no absorption is detected In the region ~2265–2285 cm^{-1}, confirming the complete consumption of free isocyanate groups validating the efficiency of the polymerization process.

4.1.6.3 FTIR Analysis of the Polyurethane (PU2) Synthesized Using Ethylene Glycol (EG), HDI, and TEA

The FTIR spectrum of the polyurethane obtained from ethylene glycol (EG), hexamethylene diisocyanate (HDI), and triethylamine (TEA) is presented in figure 15.

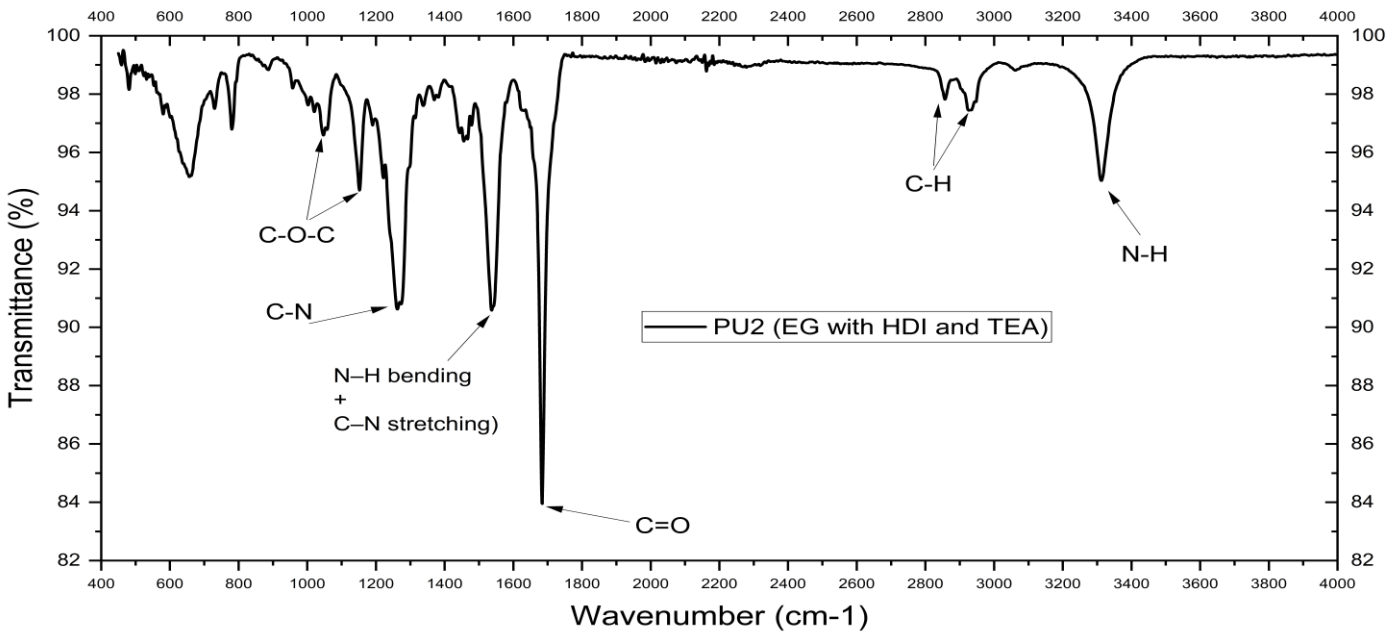

Figure 15: FTIR spectrum of the second polyurethane (PU2) synthesized from EG, HDI, and TEA. The spectrum displays the typical absorptions of urethane linkages, together with aliphatic C–H and carbonyl signals. The absence of the isocyanate band confirms complete reaction of HDI.

Table 17 shows the Peak by Peak Assignment of the FTIR Spectrum of the Polyurethane PU2 Synthesized from EG, HDI, and TEA.

Table 17: Peak-by-peak FTIR assignments for polyurethane (PU2) synthesized from EG, HDI, and TEA.

Wavenumber (cm^{-1})	Band assignment	Interpretation
~ 3315	N–H stretching of Urethane –NH–COO–	Evidence of urethane bond formation, hydrogen bonded
~2925 and 2858	Aliphatic C–H stretching	Vibrations from EG and HDI aliphatic chains
~1685	Carbonyl stretching (Urethane C=O)	Confirms urethane linkage Overlaps with ester groups
~1535	Amide II (N–H bending + C–N stretching)	Diagnostic of Urethane backbone
~1260	C–N stretching (Urethane)	Fingerprint of Urethane bond
~1050 and 1150	C–O–C stretching (Urethane + EG segments)	Confirms EG incorporation and Urethane formation
~770	Backbone fingerprint	Typical of aliphatic polyurethane
~2265 to 2285	Absence of NCO– stretching	Indicate complete consumption of HDI

The FTIR spectrum of the ethylene glycol-based polyurethane shows the characteristic absorptions confirming successful urethane bond formation. The broad band at 3315 cm^{-1} corresponds to N–H stretching of urethane groups (–NH–COO–), indicating hydrogen-bonded linkages. Aliphatic C–H stretching vibrations appear at ~2925 and 2858 cm^{-1}, originating from the methylene groups of both EG and HDI. The strong absorption at ~1685 cm^{-1} is attributed to the urethane carbonyl stretching, with partial overlap from residual ester functionalities. Further evidence of urethane formation is provided by the amide II band at ~1535 cm^{-1} and the C–N stretching at ~1260 cm^{-1}, both of which are diagnostic of the urethane backbone. Additional contributions from the C–O–C stretching bands at ~1150 and 1050 cm^{-1} confirm the incorporation of ethylene glycol segments into the polymer chain. The fingerprint band at ~770 cm^{-1} is consistent with the aliphatic polyurethane structure. Importantly, the absence of the NCO absorption in the 2265 to 2285 cm^{-1} range indicates complete consumption of HDI, demonstrating the efficiency of the polymerization process.

4.1.6.4 FTIR Analysis of the Polyurethane (PU3) Synthesising Using PHB Oligomers, HDI, TEA, and Chloroform ($CHCl_3$)

The FTIR spectrum of the polyurethane (PU3) obtained using PHB oligomers , HDI, TEA, and chloroform is presented in the figure 16.

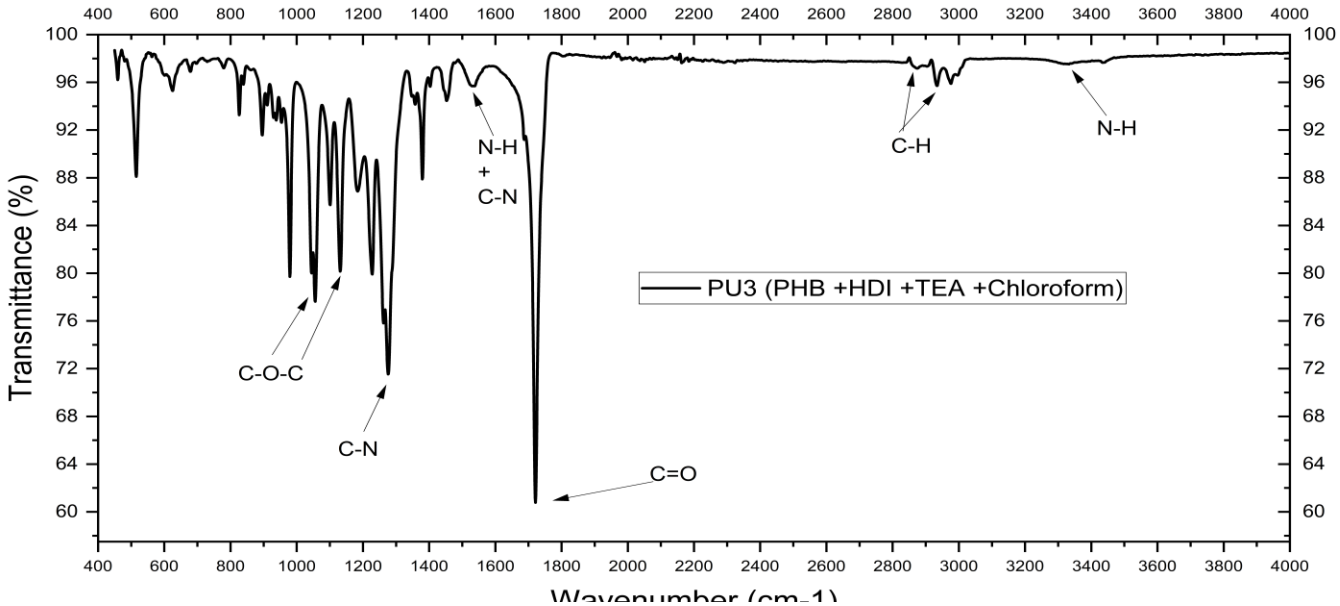

Figure 16: FTIR spectrum of Polyurethane PU3 synthesized from PHB oligomers, hexamethylene diisocyanate (HDI), triethylamine (TEA), and chloroform

Table 18 Shows the peak by peak Assignment of the: FTIR Spectrum of Polyurethane PU3 Synthesized from PHB Oligomers, Hexamethylene Diisocyanate (HDI), Triethylamine (TEA), and Chloroform.

Table 18: Peak-by-peak FTIR assignments for Polyurethane (PU3) synthesized from PHB oligomers, HDI, TEA, and chloroform

Wavenumber (cm^{-1})	Band assignment	Interpretation
~ 3330 (weak band)	N–H stretching (urethane –NH–COO–)	Only a faint signal observed, indicates suppressed hydrogen bonding: (chloroform effect)
~ 2870 and 2930	Aliphatic C–H stretching	Confirming presence of aliphatic CH_2 groups from HDI/PHB
~ 2720	Carbonyl stretching (Urethane C=O, ester overlap)	Strong band confirms urethane linkage formation
~ 1530	Amide II (N–H bending + C–N stretching)	Secondary evidence of urethane bonds
~ 1277	C–N stretching (Urethane)	Fingerprint band of urethane group
~ 1055 and 1130	C–O–C stretching	Confirms ether/ester contribution from PHB segments
~ 675	Backbone fingerprint	Typical of aliphatic PU, no aromatic out of plane band (consistent with HDI)
~ 2265 to 2285	Absence of –NCO stretching	Indicates complete consumption of isocyanate groups

The FTIR spectrum of the polyurethane synthesized from PHB oligomers, HDI, TEA; and chloroform clearly confirms the formation of urethane linkages while also reflecting the influence of the solvent environment.

A weak and broad band ~3330 cm^{-1} was observed, corresponding to NH stretching of the urethane (–NH–COO–) group. Its faint character suggests reduced hydrogen bonding, likely due to the solvation effect of chloroform. The strong absorptions at ~2870 and 2930 cm^{-1} are assigned to aliphatic C–H stretching, confirming the incorporation of HDI-derived methylene units.

The characteristic urethane carbonyl (C=O) band appeared at ~1720 cm^{-1} (overlapping with ester groups), providing direct evidence of successful urethane bond formation. Additional confirmation comes from the amide II band at ~1530 cm^{-1}, attributed to N–H bending coupled with C–N stretching.

The C–N stretching vibration of urethane was detected at ~1277 cm^{-1}, serving as a clear fingerprint of the urethane bond. Moreover, the bands at ~1055 and 1130 cm^{-1} were assigned to C–O–C stretching, consistent with contributions from the PHB-derived ester groups and urethane ether linkage.

In the lower wavenumber region, a distinct band near ~675 cm^{-1} was observed, corresponding to the aliphatic polyurethane backbone fingerprint. Importantly, no signals were detected in the 2265 to 2285 cm^{-1} region, confirming the complete consumption of isocyanate groups (–NCO) during polymerization.

Overall, the spectral profile provides strong evidence for the successful formation of a fully reacted aliphatic polyurethane network. The reduced intensity of the N–H stretch highlights the influence of chloroform on hydrogen-bonding interactions compared to solvent-free syntheses.

4.1.6.5 Comparative FTIR Analysis of Polyurethanes Synthesized Using HDI Diisocyanate

To better highlight the similarities and differences between the three polyurethane systems, the FTIR spectra (Figure 17) of the polyurethane obtained using PHB oligomers with HDI and TEA (PU1), polyurethane obtained using Ethylene Glycol (EG) with HDI and TEA (PU2), and the polyurethane obtained using PHB oligomers with HDI, TEA and chloroform were overlaid for comparison.

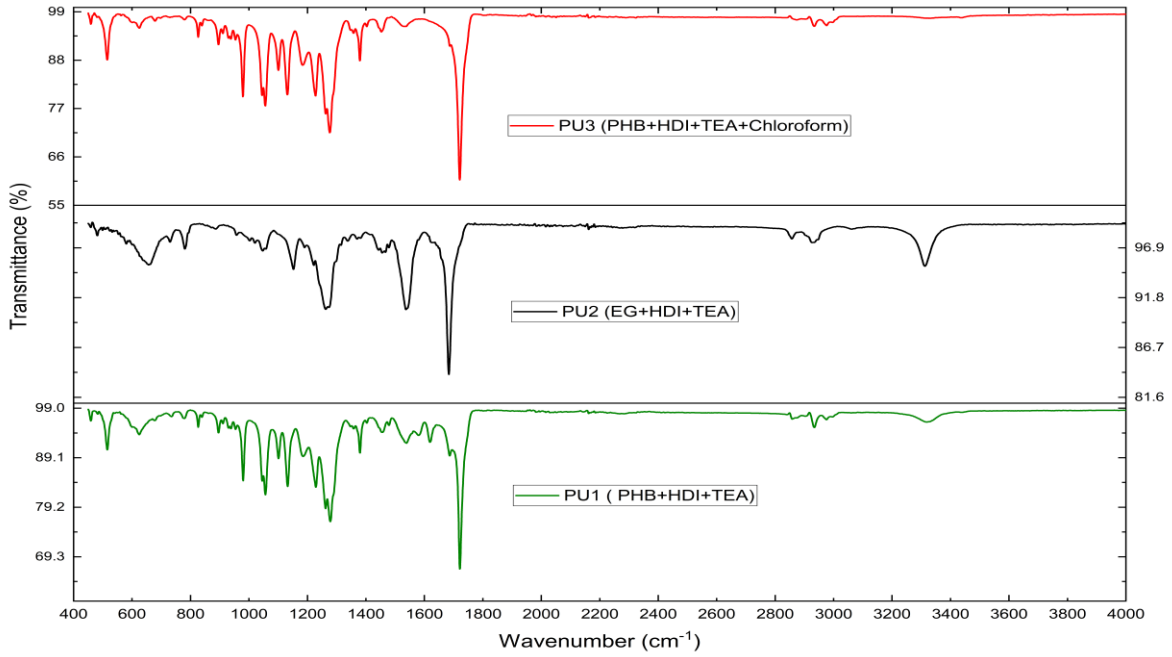

Figure 17: Comparative FTIR spectra of PU1, PU2, and PU3. All samples exhibit the diagnostic absorptions of urethane linkages, with variations in N–H intensity and carbonyl band shape

Across the three polyurethane samples, several consistent features confirm successful urethane formation: **N–H** stretching in the **3300 cm^{-1}** region, strong carbonyl bands near **1680–1725 cm^{-1}**, **C–N** stretching at **1220–1280 cm^{-1}** and **C–O–C** signals around **1050–1150 cm^{-1}.** However, clear differences are observed: PU1 and PU2 both display strong **N–H** and **urethane carbonyl** bands**,** while PU3 shows a weaker **N–H** stretch, attributed to chloroform disrupting hydrogen bonding interactions. The carbonyl peak of PU2 is sharper and more defined, consistent with the simpler structure of ethylene glycol as a diol, whereas PU1 and PU3 exhibit broader carbonyl signals due to overlapping contributions from PHB ester groups. Importantly, no –NCO band is observed in any sample, confirming complete isocyanate conversion. These differences emphasize how both the choice of soft segment (PHB vs. EG) and the use of solvent influence the molecular organization and hydrogen bonding environment of the resulting polyurethanes.

4.1.7 FTIR Analysis of the Polyurethanes Synthesized Using MDI Diisocyanate

4.1.7.1 FTIR Analysis of Methylene Diphenyl Diisocyanate (MDI)

The FTIR spectrum of polyurethane PU4 synthesised from ethylene glycol (EG) and methylene diphenyl diisocyanate (MDI), catalyzed by triethylamine (TEA), is shown in the figure 18.

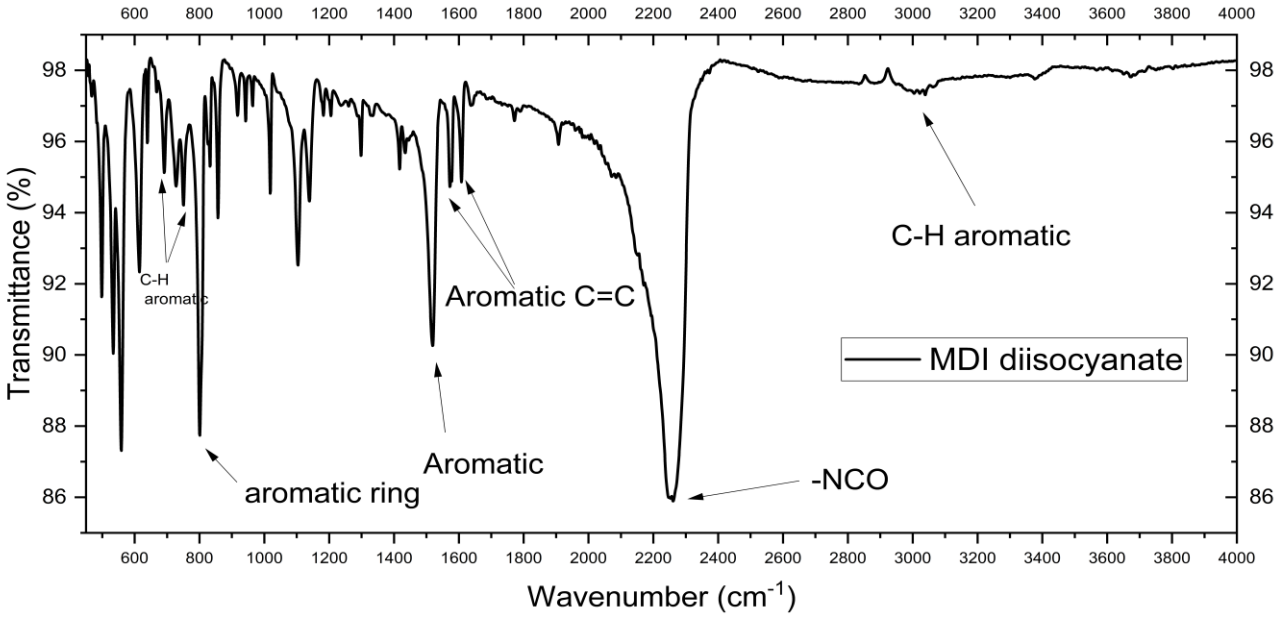

Figure 18: FTIR spectrum of neat MDI showing the diagnostic –NCO band (~2260 cm⁻¹) and aromatic markers (C=C ~1608/1572 cm⁻¹; para C–H ~815/750 cm⁻¹).

Table 19 shows the peak by Peak Assignments of the FTIR Spectrum of Methylene Diphenyl Diisocyanate (MDI).

Table 19: Peak-by-peak FTIR assignments of methylene diphenyl diisocyanate (MDI).

Wavenumber (cm^{-1})	Band assignment	Interpretation
~ 3000	Aromatic C–H stretch	–
~2260	–NCO stretching.	
~1608 and 1572	Aromatic C=C (MDI Ring)	Diagnostic of MDI (distinguishes from HDI Pus)
~1518	➔ Aromatic ring mode	Additional aromatic marker
~1064	C-O-C stretch (ether/ester).	Supports EG/Urethane C-O environment
~815 and 750	Aromatic C-H	MDI fingerprint

The FTIR spectrum of neat MDI is dominated by the sharp NCO stretching band at ~2260 cm^{-1}, used as reference for monitoring isocyanate consumption in polyurethane formation. Aromatic features characteristic of MDI are clearly visible, namely C=C ring vibrations at ~1608 and 1572 cm^{-1}, together with para-substituted out of plane C–H bands at ~815 and 750 cm^{-1}. A weaker aromatic C–H stretch around ~3000 cm^{1} is also observed. Minor bands in the 1100–1400 cm^{-1} region are ring/ C–N modes and are not used diagnostically. These markers are later used to

confirm incorporation of MDI into the polyurethane backbone and to verify the disappearance of free isocyanate in fully reacted samples.

4.1.7.2 FTIR Analysis of the Fourth Polyurethane (PU4) Synthesized Using Ethylene Glycol (EG), MDI, and TEA.

The FTIR spectrum of the polyurethane (PU4) is shown in the figure 19.

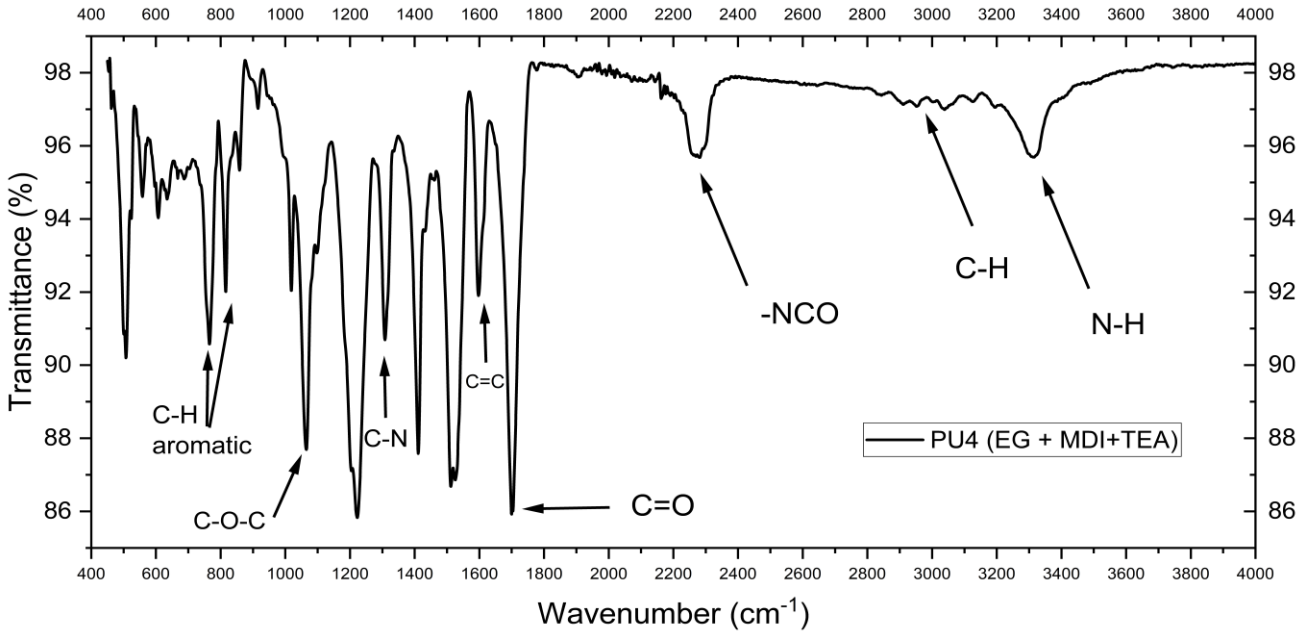

Figure 19: FTIR spectrum of polyurethane PU4 synthesized from ethylene glycol (EG), methylene diphenyl diisocyanate (MDI), and triethylamine (TEA)

Table 20 shows the peak by Peak Assignment of the FTIR Spectrum of Polyurethane PU4 (EG/MDI/TEA).

Table 20: peak by Peak Assignment of the FTIR Spectrum of Polyurethane PU4 (EG/MDI/TEA).

Wavenumber (cm^{-1})	Band assignment	Interpretation
~ 3312	N–H stretching of Urethane –NH–COO–	Confirms urethane linkages. (moderate intensity)
~2936	Aliphatic C–H stretching	Support presence of EG/MDI methylene
~2270	–NCO stretching (unreacted isocyanate from MDI.	Shows incomplete consumption of MDI ➔ Reaction not fully completed.
~1700	Urethane/ester C=O	Primary marker of urethane formation
~1595	Aromatic C=C (MDI Ring)	Diagnostic of MDI (distinguishes from HDI Pus)
~1308	C–N stretching ➔ Urethane fingerprint.	Confirm Urethane bond formation.
~1064	C-O-C stretch (ether/ester).	Supports EG/Urethane C-O environment
~815 and 767	Aromatic C-H	MDI fingerprint

The EG/MDI polyurethane shows clear urethane formation (N–H at ~3312 cm^{-1}, strong C=O at ~1700 cm^{-1}, and the urethane fingerprint ~C–N at ~1308 cm^{-1}). Aromatic MDI incorporation is confirmed by the C=C band at ~1595 cm^{-1} and the para-substituted C–H out of plane bands at ~815 and 767 cm^{-1}.

Crucially, a distinct absorption at ~2270 cm^{-1} is observed, assigned to the NCO stretching of free isocyanate. This indicates that a fraction of MDI remained unreacted, i.e., the polymerization was not fully completed under the applied conditions. The presence of residual –NCO is consistent with the only moderate N–H intensity (less extensive hydrogen bonding than in fully reacted networks).

The polyurethane PU4 contains both urethane linkages and an aromatic MDI hard segment, but the –NCO band at ~2270 cm^{-1} demonstrates incomplete isocyanate conversion. To drive the reaction to completion, consider checking NCO:OH stoichiometry, increasing time/temperature, ensuring dry conditions, or adjusting TEA concentration/mixing efficiency.

4.1.7.3 Size Exclusion Chromatography (SEC) analysis of the Polyurethane (PU4)

The molecular weight distribution of PU4 was investigated by SEC using a triple detection system (LALS, RALS, and UV). The chromatograms are shown in figure 20.

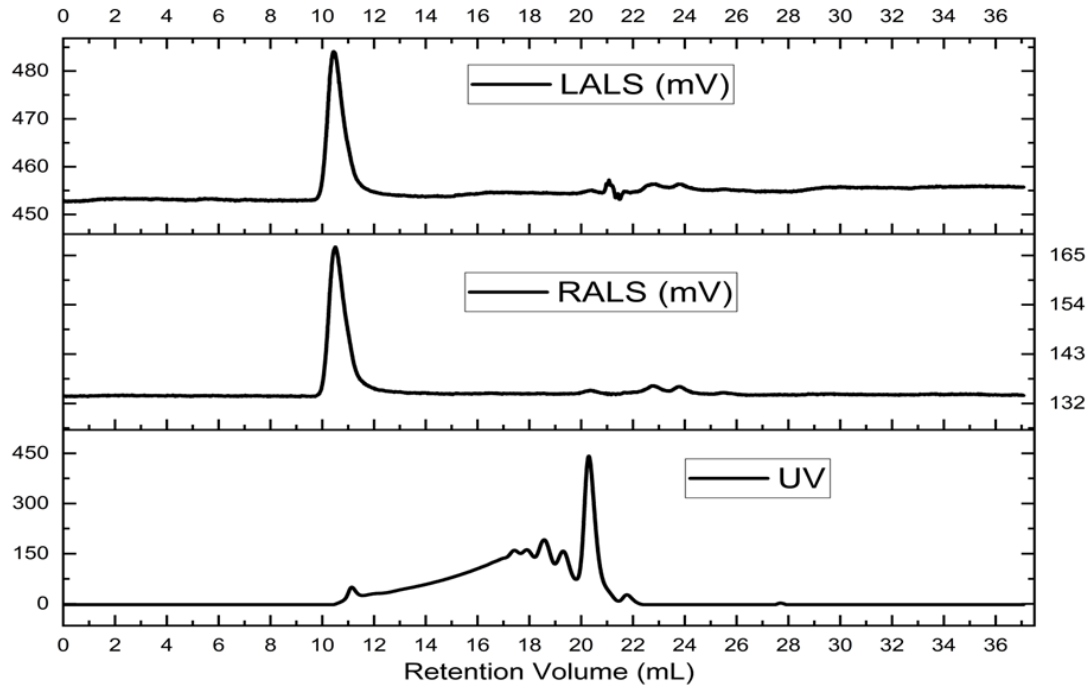

Figure 20: size exclusion chromatography (SEC) chromatograms of PU4 (EG + MDI + TEA) obtained using LALS, RALS, and UV detectors.

Table 21 shows the peak by peak Assignment of the Size Exclusion Chromatography (SEC) Analysis of Polyurethane PU4.

Table 21: Peak assignments from Size Exclusion Chromatography (SEC) analysis of polyurethane PU4

Retention volume (mL)	Detector	Assignment	Interpretation
~11	LALS/RALS	Main polymer peak	High molecular weight polyurethane chains, dominant fraction.
15-18	UV	Aromatic absorption	Presence of MDI derived aromatic species incorporated into polymer chains.
20-24	LALS/RALS/UV	Broad hump	Low molecular weight and possible residual by-products.

The LALS and RALS detectors clearly identify a dominant peak eluting at ~11 mL, which corresponds to the high-molecular-weight polyurethane fraction. This confirms that the reaction between ethylene glycol and MDI, catalyzed by TEA, successfully generated long-chain polymers. In contrast, a secondary broad response in the region of 20-24 mL is visible in both scattering detectors, suggesting the presence of low-molecular-weight oligomeric fractions.

The UV chromatogram shows significant signal intensity between 15 and 23 mL, consistent with the presence of aromatic moieties derived from MDI. This indicates that while the majority of MDI units were incorporated into the polyurethane backbone, some aromatic-containing species remain unreacted or partially reacted

Overall, the SEC analysis of PU4 confirms the synthesis of high-molecular-weight polyurethane chains with good incorporation of MDI. However, the detection of oligomeric fractions and aromatic residues suggests that the polymerization was not fully selective, leaving behind a minor fraction of low-molecular species.

4.1.7.4 FTIR Analysis of the Polyurethane (PU5) Obtained Using PHB Oligomers With MDI and TEA

The FTIR spectrum of polyurethane PU5, synthesised using PHB oligomers and 4,4'-methylenediphenyl diisocyanate (MDI) with TEA as catalyst, is shown in the figure 21.

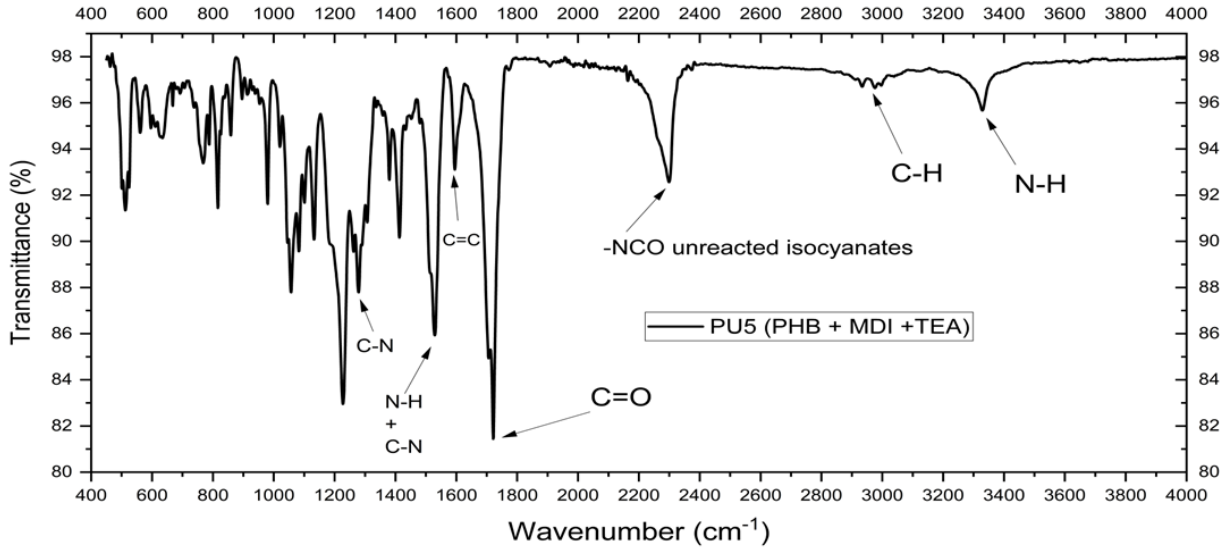

Figure 21: FTIR spectrum of polyurethane (PU5) (PHB oligomers + MDI + TEA)

Table 22 shows the peak be Peak Assignment of the FTIR Spectrum of Polyurethane PU5 (PHB oligomers/MDI/TEA).

Table 22: Peak-by-peak FTIR band assignments for polyurethane PU5 (PHB oligomers/MDI/TEA).

Wavenumber (cm^{-1})	Band assignment	Interpretation
~ 3330	N–H stretching of Urethane –NH–COO–	Confirms urethane linkages. Broadness due to hydrogen bonding
~2973	Aliphatic C–H stretching	CH_2 groups from PHB segments and MDI linkers
~2300	–NCO stretching (unreacted isocyanate from MDI.	Shows incomplete consumption of MDI ➔ Reaction not fully completed.
~1720	Carbonyl stretching C=O.	Strong evidence of urethane bond formation
~1535	Aromatic C=C stretching	Diagnostic of aromatic MDI units
~1530	Amide II (N–H bending + C–N stretching)	Secondary evidence of urethane linkage
~1278	C–N stretching	Fingerprint band of urethane group
~1130	C-O-C stretching	Contribution from ester groups in PHB and urethane ether linkages
~770	Aromatic C–H out of plane bending	Confirms aromatic structure from MDI

The FTIR spectrum shows clear evidence of urethane formation together with unmistakable signatures of the aromatic MDI hard segment. A weak/broad band near 3330 cm^{-1} (N–H stretch) indicates a limited population of hydrogen bonded urethane groups. Aliphatic C–H stretching at ~2973 cm^{-1} confirms incorporation of PHB/MDI methylene units. The urethane carbonyl appears

at ~1720 cm^{-1}, providing primary evidence for urethane linkages (partly overlapping PHB ester). The amide II band at ~1530 cm^{-1} and the C–N (urethane) at ~1278 cm^{-1} constitute the urethane fingerprint region, further corroborating urethane formation, while C–O–C vibrations at ~1130 cm^{-1} reflect ester/ether contributions from PHB soft segment and urethane linkages. Aromatic features diagnostic of MDI are present: C=C stretching in the ~1595 cm^{-1} region and C-H out of plane bending near ~770 cm^{-1}. Critically, a distinct –NCO absorption at ~2270 cm^{-1}, demonstrating residual unreacted isocyanate and, therefore, incomplete conversion under the applied conditions. Overall, PU5 contains both urethane and aromatic MDI signatures, but the persistent –NCO band indicates that reaction conditions (NCO:OH stoichiometry, temperature/time, moisture control or catalyst level) should be optimized to drive the polymerization to full completion.

4.1.7.5 Comparative Discussion of PU4 and PU5

Figure 22 shows the overlaid FTIR spectra of PU4 (EG + MDI + TEA, black) and PU5 (PHB oligomers + MDI +TEA), red), enabling a direct comparison of urethane bands and the –NCO region to assess conversion while highlighting the common aromatic MDI features.

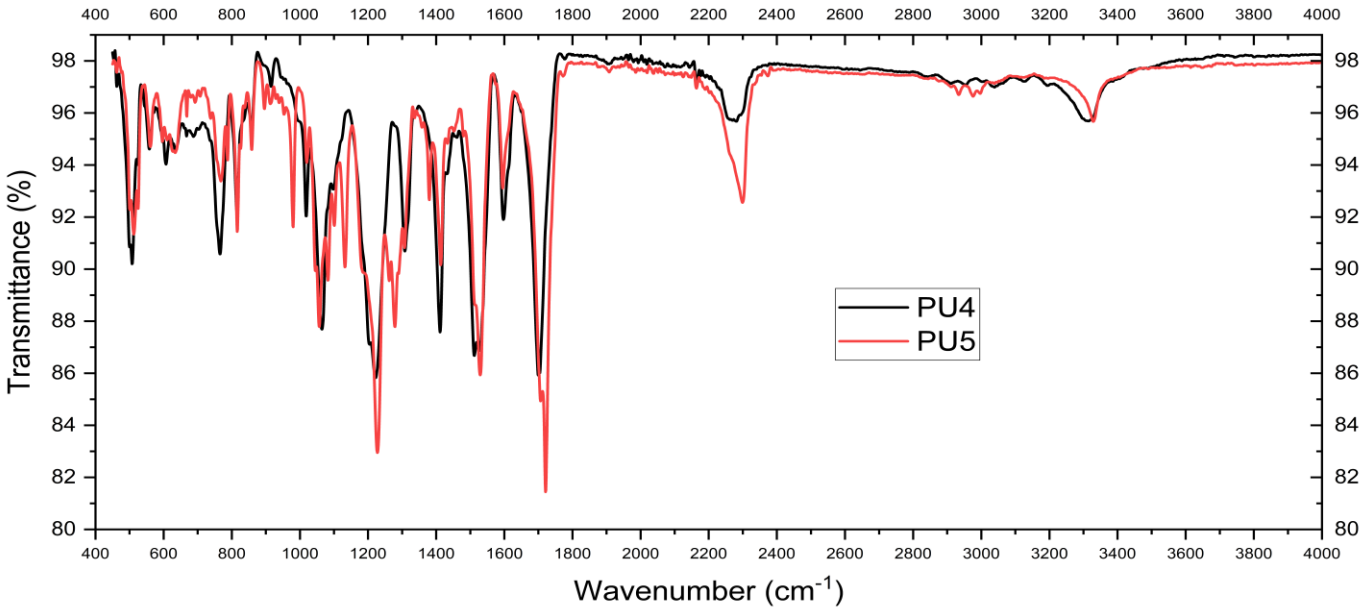

Figure 22: Overlay FTIR spectra of PU4 (EG + MDI + TEA, black) and PU5 (PHB-oligomers + MDI + TEA, red).

The overlaid FTIR spectra shows that both PU4 and PU5 contain the same polyurethane and aromatic markers: a urethane N–H stretch around ~3300 cm^{-1}, a strong carbonyl near ~1700–1720 cm^{-1}, urethane fingerprint bands (C–N in the 1220–1310 cm^{-1} window), and the MDI aromatic

features (C=C ~1595 cm^{-1} and para-subtitled C–H out of plane at ~815/767 cm^{-1}). The key differences lie in conversion and carbonyl shape: the –NCO band at ~2270 cm^{-1} is visibly stronger in PU5 than PU4, indicating more residual isocyanate and thus lower extent of reaction for the PHB-based formulation; PU4 exhibits a weaker –NCO signal. In addition, the C=O region, is broader/more intense in PU5 because the urethane carbonyl overlaps with PHB ester carbonyls, whereas PU4 (EG-based) shows a cleaner urethane C=O. consistent with the stronger –NCO band, the N–H envelope near ~3300 cm^{-1} is slightly weaker in PU5, reflecting fewer hydrogen-bonded urethane linkages. The C–N/C–O–C fingerprint is also richer in PU5 owing to the PHB soft-segment environment, while aliphatic C–H stretches (~2940/2860 cm^{-1}) are comparable in both. In short: both materials are aromatic MDI-based polyurethanes, but PU4 achieves higher isocyanate conversion, whereas PU5 shows lower conversion and stronger ester/urethane carbonyl overlap due to the PHB oligomers.

4.1.7.6 Size Exclusion Chromatography (SEC) of the Polyurethane (PU5)

The molecular weight distribution of the polyurethane PU5 was investigated by size exclusion chromatography (SEC) using triple detection system (low-angle light scattering, right angle light scattering, and UV absorption). The chromatograms are presented in figure 23.

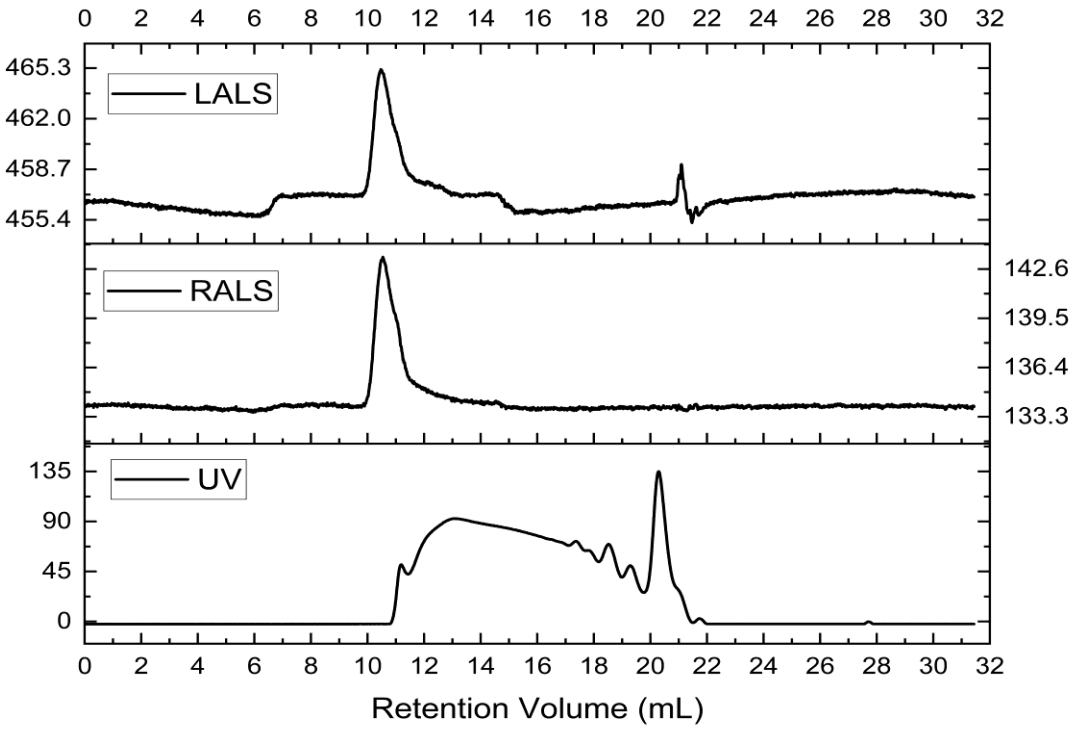

Figure 23: Size exclusion chromatography (SEC) chromatograms of polyurethane PU5 (synthesised from PHB oligomers, MDI, and TEA) obtained with triple detection: low-angle light scattering (LALS), right-angle light scattering (RALS), and UV absorption.

Table 23 shows the peak by Peak Assignment of the SEC Chromatograms of the Polyurethane (PU5)

Table 23: Peak by peak assignment of the SEC chromatograms of the polyurethane PU5

Retention volume (mL)	Detector	Assignment	Interpretation
~11	LALS/RALS	Main polymer peak	High molecular weight polyurethane chains, dominant PU5 fraction.
20-22	UV	Aromatic absorption (MDI)	Presence of MDI derived aromatic species incorporated into polymer chains.
~12–18 (Broad region)	UV	Broad absorption	Evidence of PHB derived ester segment and oligomeric species
>24	LALS/RALS/UV	Low intensity	Low molecular weight and possible residual by-products.

The size exclusion chromatography (SEC) of the polyurethane (PU5) shows a sharp and intense main peak around 11 mL, detected by both LALS and RALS which corresponds to the dominant fraction of high molecular weight polyurethane chains, indicating successful chain growth. Compared to PU4, the UV detector reveals a broader signal between 12-18 mL, attributed to the incorporation of PHB-derived ester oligomers into the polymer network. A secondary UV signal at 20-22 mL confirms the presence of aromatic chromophores from MDI units, further supporting the FTIR evidence of aromatic incorporation. Finally, the weak late-eluting features (>24 mL) are associated with low-molecular weight residues, possibly unreacted species or short oligomers.

4.1.8 Third Attempt at Acid-Catalyzed Depolymerization of Poly(3-hydroxybutirate)

In this experiment, poly(3-hydroxybutirate) (PHB) was subjected to acid-catalyzed transesterification with ethylene glycol (EG) in chloroform, using p-toluenesulfonic acid as catalyst. This trial represents the third attempt at PHB depolymerization, conducted over 24 hours under conditions previously optimized in third Attempt of depolymerization, but with the catalyst loading doubled to accelerate chain cleavage. The reaction was carried out at 58 °C with continuous stirring. Upon completion, the reaction mixture was separated into liquid and solid phases, and the solvent from the liquid phase was evaporated to recover the soluble OH-terminated oligomers.

The quantities of reagents used in this experiment are summarized in the table 24.

Table 24: Reagent quantities used in the third attempt of acid-catalysed depolymerization of poly(3-hydroxybutyrate) (PHB).

Component	Mass (g)	Volume (mL)	Density
PHB	4.5296	–	–
Chloroform ($ChCl_3$)	–	45	–
Ethylene glycol (EG)	12	8.11	1.11
P-toluenesulfonic acid	0.1812	–	–

At the end of the 24 hours reaction, the mixture was cooled and centrifuged to separate the insoluble residue from the liquid phase. The final product was obtained through two complementary recoveries routes:

- Drying of the solid fraction, yielding a first portion of product.
- Evaporation of the separated liquid phase, which provided an additional portion of the same product after solvent and excess ethylene glycol removal.

The recovered quantities are reported in the table 25.

Table 25: Mass of products recovered after 24-hour acid-catalysed depolymerization of PHB (third attempt)

Recovery route	Mass (g)
Product dried from solid fraction	7.166
Product from evaporated liquid fraction	6.382
Total recovered product	**13.550**

The newly obtained PHB oligomers will now be used to prepare polyurethane materials. For clarity, the depolymerization run completed in 24 hours will be referred to as Exp.4_24h, and its recovered fractions will serve as the soft-segment precursors in the following syntheses.

Sixth Synthesis of Polyurethane Using Solid Oligomers Recovered from PHB Depolymerization (24 h, Third attempt).

4.1.9 Synthesis of the Polyurethanes Using Oligomers Recovered from PHB Depolymerization for 24 hours (Third Attempt)

4.1.9.1 Synthesis of the Polyurethane PU6 Using the New PHB Oligomers MDI, and TEA

In this experiment, polyurethane was synthesized using the solid oligomeric fraction obtained from the depolymerization of PHB (3rd attempt_24h). The procedure involved first melting methylene diphenyl diisocyanate (MDI) at 60°C to obtain a homogeneous liquid phase. Once

liquefied, the recovered PHB oligomers were introduced, followed by triethylamine (TEA) as the catalyst. The reaction was maintained at 70°C for 24 hours, allowing chain extension and urethane linkage formation between the hydroxyl groups of the oligomers and the isocyanate groups of MDI.

The quantities of reagents employed are summarized in table below, which details the respective masses, densities, and calculated volumes of each component.

Table 26: Reagents used in the 6th synthesis of polyurethane from PHB oligomers, MDI, and TEA.

reagent	Mass (g)	Volume (mL)	Density (g/mL)
PHB	0.104	–	–
MDI	1.488	1.421	1.047
TEA	0.020	0.027	0.728

4.1.10 Synthesis of the Polyurethane (PU7) With PHB Oligomers (Liquid phase), MDI, and TEA

In this experiment, PHB oligomers obtained from the fourth depolymerization (24h with reduced catalyst) were used as the polyol component. Methylene diphenyl diisocyanate (MDI) was selected as the isocyanate, while Triethylamine (TEA) served as the catalyst. The formulation is summarized in table 27.

Table 27: Reagent composition for the synthesis of polyurethane (PU7) using PHB oligomers (liquid phase), MDI, and TEA.

Components	Mass (g)	Volume (mL)	Density (g/mL)
PHB-oligomers (liquid phase)	0.104	–	–
MDI	1.488	1.421	1.047
TEA	0.020	0.027	0.728

In the polyurethanes PU6 and PU7, the flask containing MDI was first heated to 60°C to ensure melting. Subsequently, the PHB Oligomers and catalyst were added. In PU7, an immediate reaction was observed, indicating high reactivity between the components. To further evaluate this behaviour, the temperature was increased from 60°C to 70°C, confirming that higher thermal input aceelerates the polyurethane formation.

4.1.11 Chromatographic Analysis of The Polyurethanes PU6 and PU7 Obtained Using PHB Oligomer After the Third Attempt of Depolymerization of the Original PHB

4.1.11.1 Size Exclusion Chromatography (SEC) of the Polyurethane PU6

The molecular weight distribution of the polyurethane PU6, synthesized from PHB oligomers, MDI, and TEA, was analysed by Size Exclusion Chromatography (SEC) using a triple detection system consisting of Refractive Index (RI), Low-Angle Light Scattering (LALS), Right-Angle Light Scattering (RALS), and Ultraviolet (UV) detectors. The corresponding chromatograms are presented in figures 24 and 25.

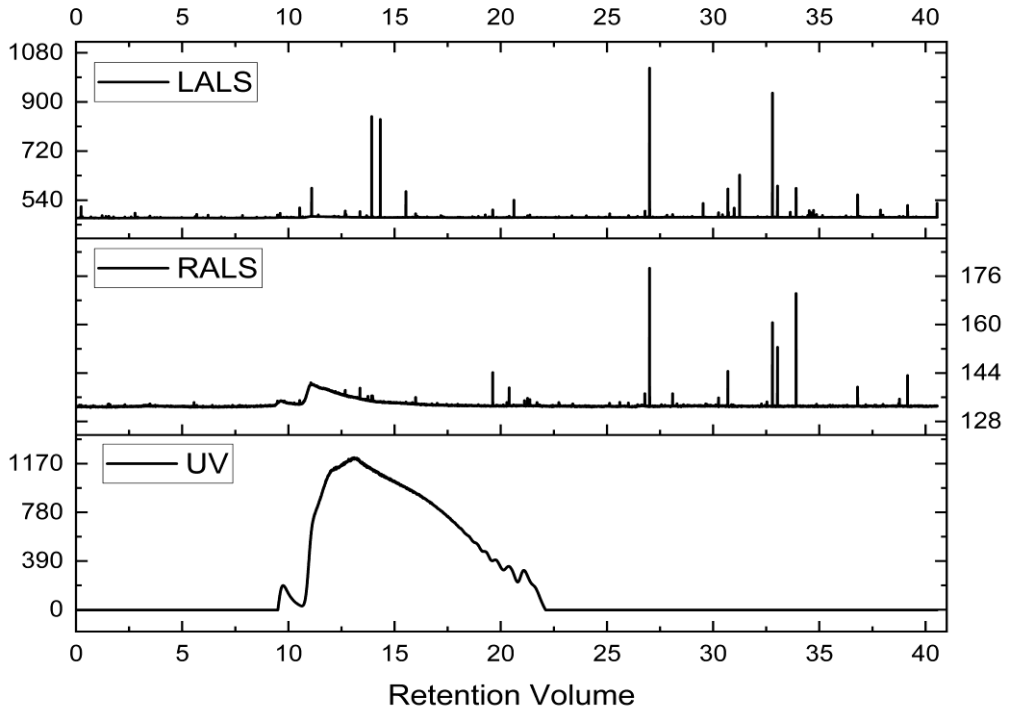

Figure 24: Size Exclusion Chromatography (SEC) chromatograms of polyurethane PU6 obtained with triple detection (LALS, RALS, and UV).

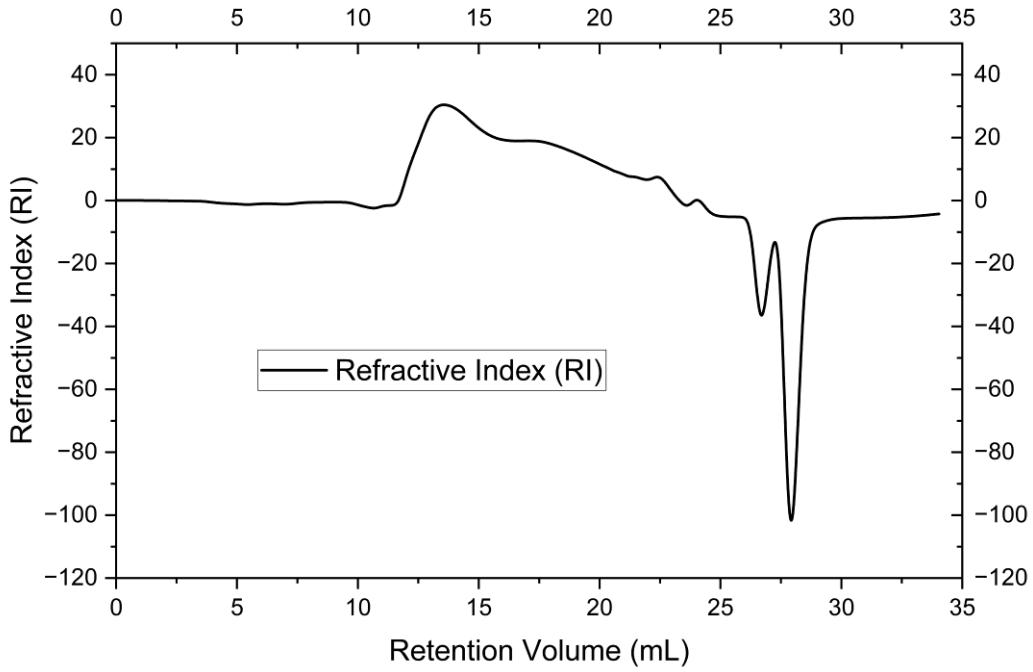

Figure 25: Refractive Index (RI) chromatogram of polyurethane PU6 synthesized from PHB oligomers, MDI, and TEA.

The Refractive Index (RI) chromatogram (Figure 25) of the new polyurethane PU6 displays a dominant elution peak centered around 26-28 mL, corresponding to the main polyurethane fraction. This sharp and intense signal indicates the presence of high-molecular-weight polymer chains as the primary component. The relatively symmetrical peak profile suggests a moderate polydispersity, consistent with a controlled polymerization process.

The initial rise 15-20 mL may correspond to intermediate oligomeric species or partially reacted components, while the minor late-eluting shoulders (>30 mL) can be attributed to low-molecular weight residues or small unreacted molecules, possibly originating from incomplete chain extension or remaining PHB-derived oligomers.

In the triple detection chromatograms (Figure 23), the LALS and RALS detectors confirm the presence of a dominant high-molecular-weight population through their concurrent scattering signals at approximately the same retention volume as the RI peak. The scattered intensity in this region reflects the contribution of long polymer chains with significant molar mass.

The UV detector provides complementary information about the presence of aromatic chromophores from the MDI units. A broad UV absorption between 12–25 mL confirms the incorporation of MDI-derived aromatic structure into the polyurethane backbone. This absorption envelope overlaps with the main polymer signal detected by RI and LALS/RALS, confirming that

the aromatic moieties are chemically bound within the polymer matrix rather than being present as unreacted diisocyanate.

Beyond 25 mL, the UV intensity gradually decreases, coinciding with lower scattering signals and suggesting the presence of minor fractions containing short oligomers or low-aromatic-content residues. These results collectively confirm the successful formation of high-molecular-weight aromatic polyurethane chains derived from PHB-based oligomers.

The comparison of the scattering detector (LALS and RALS) further demonstrates that the PU6 polymer network is well-developed, although some heterogeneity in chain length remains. This behavior may be attributed to small variations in the stoichiometric ratio of isocyanate to hydroxyl groups and the incomplete homogenization of viscous PHB oligomers during synthesis.

In summary, the SEC analysis of the polyurethane PU6 confirms the successful polymerization of PHB oligomers with MDI, yielding polyurethane chains with a predominant high-molecular-weight fraction, incorporated aromatic segments, and a limited amount of low-molecular-weight residues. These findings are consistent with the FTIR results, which also evidenced complete urethane formation and the presence of aromatic MDI signatures.

4.1.11.2 Size Exclusion Chromatography (SEC) of the Polyurethane (PU7)

The polyurethane PU7 was synthesized from PHB oligomers (liquid phase), methylene diphenyl diisocyanate (MDI), and triethylamine (TEA) as catalyst. The polymer's molecular weight distribution was analysed using Size Exclusion Chromatography (SEC) equipped with a triple detection system (Refractive Index RI), Low-Angle Light Scattering (LALS), Right-Angle Light Scattering (RALS), and Ultraviolet(UV). The corresponding chromatograms are displayed in figure 26 and 27.

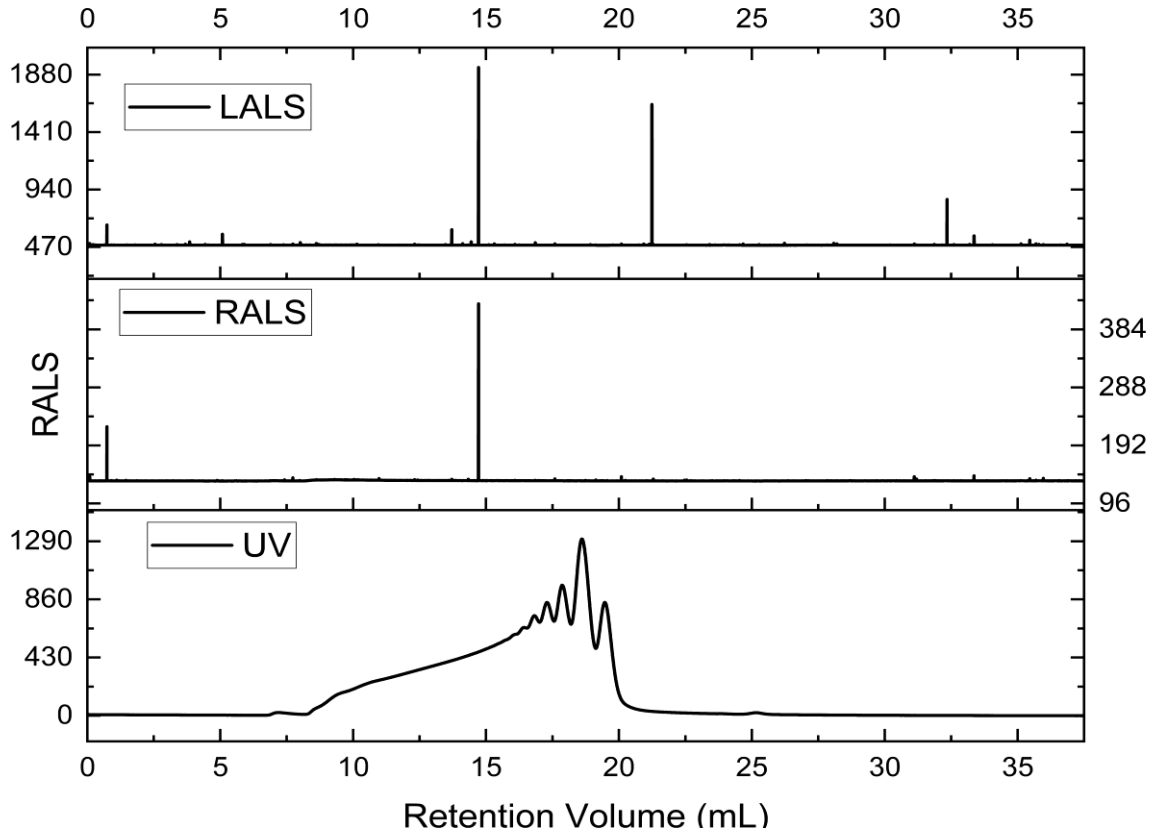

Figure 26: Size Exclusion Chromatography (SEC) chromatograms of polyurethane PU7 obtained with triple detection (LALS, RALS, and UV).

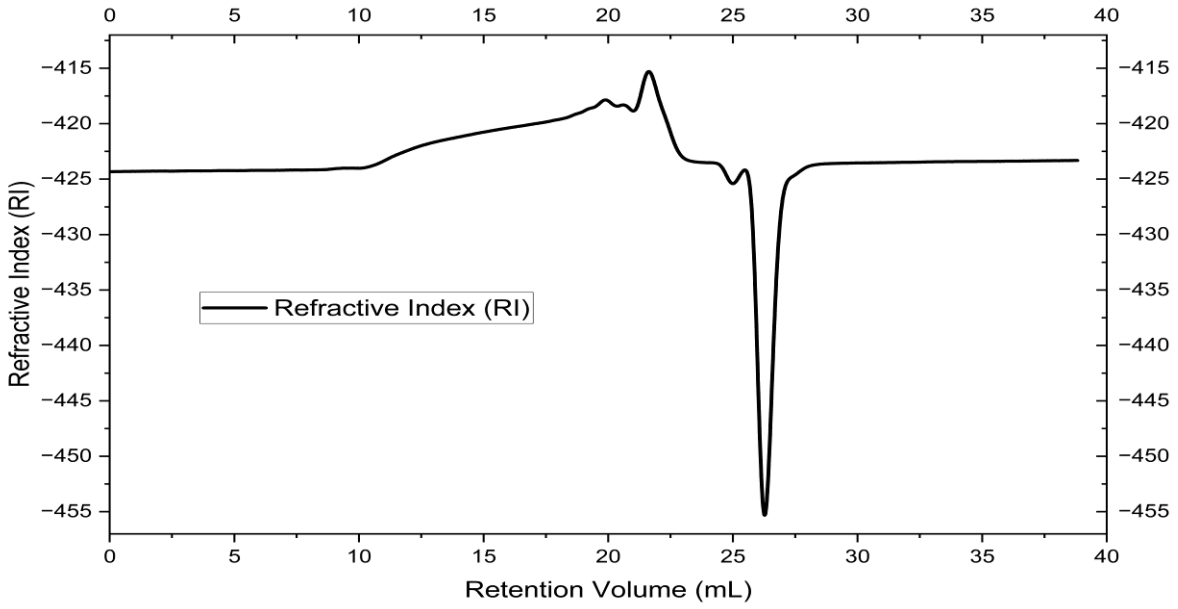

Figure 27: Refractive Index (RI) chromatogram of polyurethane PU7 synthesized from PHB oligomers (liquid phase), MDI, and TEA.

The Refractive Index (RI) chromatogram (figure 27) of the polyurethane PU7 displays a sharp, intense peak centered at approximately 24–26 mL, indicating that the polymerization resulted predominantly in high-molecular-weight polyurethane chains. Compared to PU6, the main elution region of PU7 appears slightly shifted toward higher retention volumes, implying a moderatly lower molecular weight for this formulation. This difference likely arises from variations in the reactivity or structure of the PHB oligomers used liquid phase vs solid phase fractions).

The broad shoulder observed between 18-22 mL may correspond to intermediate chain lengths or partially extended oligomers, while the minor features after 30 mL represent low-molecular-weight residues or unreacted fragments. The relatively narrow distribution suggests that the reaction between PHB oligomers and MDI proceeded in a controlled manner, producing a polymer network with acceptable uniformity.

In the LALS and RALS chromatograms (figure 26), distinct scattering signals are observed near 19–22 mL, confirming the presence of macromolecular species contributing significantly to the overall molar mass. The scattering intensity, though slightly lower than that of PU6, supports the formation of a well developped polyurethane structure, albeit with somewhat shorter average chain length.

The UV detector exhibits a broad and intense absorption band spanning 15–25 mL, reflecting the incorporation of aromatic chromophores from MDI within the polymer backbone. The relatively stronger UV signal compared to PU6 suggests a higher aromatic content in PU7, which is consistent with the higher MDI-to-polyol ratio used in this synthesis. The peak's alignment with the main RI and scattering regions confirms that these aromatic units are chemically bonded within the polyurethane network, not as free isocyanate residues.

Collectively, these results indicate that PU7 is mainly of high-molecular-weight aromatic polyurethane chains, with minor fractions of unreacted or oligomeric species. The slight shift in retention volume compared to PU6 indicates subtle differences in molecular weight distribution, possibly due to different solubility and chain mobility of the liquid-phase PHB oligomers.

Overall, the Size Exclusion Chromatography (SEC) analysis confirms the successful polymerization of the PHB-derived oligomers with MDI, yielding a polymer of good structural homogeneity and strong aromatic character.

Chapter 5 General Conclusion

This work aimed to explore sustainable routes for developing bio-based polyurethanes derived from polyhydroxyalkanoates (PHAs), particularly poly(3-hydroxybutirate) (PHB). The study focused on the controlled degradation of PHB via acid-catalysed alcoholysis, the characterization of the resulting hydroxyl-terminated oligomers, and their subsequent use as soft segments for polyurethane synthesis with aliphatic (HDI) and aromatic (MDI) diisocyanates.

The experimental work demonstrated that the depolymerization of PHB in chloroform using p-toluenesulfonic acid (p-TsOH) and ethylene glycol (EG) is an effective approach to obtain hydroxyl-terminated oligomers (telechelic diols). These oligomers retained the ester backbone and exhibited new terminal –OH groups, confirmed by FTIR analysis. Size Exclusion Chromatography (SEC) revealed a clear decrease in molecular weight with increasing reaction time, confirming chain scission and the formation of oligomeric products.

The synthesized polyurethanes, obtained by reacting these oligomers (and EG) with HDI or MDI in the presence of triethylamine (TEA), displayed characteristic urethane functional groups (N–H,C=O, C–O–C) in their FTIR spectra. HDI-based formulations retained a weak residual band, indicating incomplete reaction. SEC analysis confirmed the formation of polymeric materials with varying molecular weight distributions depending on the formulation and diisocyanate type.

Despite the overall success of the synthetic procedures, several limitations were encountered. The heterogeneous solubility of PHB in some solvents, restricted reaction uniformity, and residual catalyst traces may have influenced product stability. Moreover, incomplete removal of solvent and moisture during synthesis occasionally led to side reactions and lower reproducibility between batches. Additionally, further optimization of stoichiometry, catalyst concentration, and drying conditions is required to achieve consistent polymer quality and narrow dispersity.

For future work, several directions are proposed:

- Conduct kinetic and thermodynamic studies to better understand the degradation mechanism of PHB under different catalysts and reaction conditions
- Explore alternative green solvents and biocompatible catalysts to improve environmental sustainability.
- Optimize reaction parameters (temperature, time, and molar ratios) to enhance molecular control and conversion efficiency.
- Extend the study to include mechanical, thermal, and biodegradation tests of the obtained polyurethanes to assess their potential for biomedical or packaging applications.

- Investigate block copolymerization strategies combining PHB oligomers with other bio-based monomers to further tailor flexibility, strength, and degradability.

In conclusion, this research successfully demonstrated the feasibility of producing PHB*-based polyurethanes through controlled degradation and subsequent polymerization, contributing valuable insights into the design of biodegradable and sustainable polymer systems derived from renewable resources.

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