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MECHANISMS AND KINETICS OF POLY(3-HYDROXYBUTYRATE) REACTIONS

MECHANISMY A KINETIKA REAKCÍ POLY(3-HYDROXYBUTYRÁTU)

SUMMARY OF PH.D. THESIS

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INTRODUCTION

Everybody of us has heard about the issue of plastic contamination of the planet Earth. The efforts to solve the problem of plastic pollution include replacing all disposable plastic products with a seemingly ecological variant of bioplastics. However, some crucial issues regarding bioplastics arose, such as:

- Their true biodegradability – according to the definition of the term, not all bioplastic, are biodegradable. Moreover, when they are, it is usually under special conditions, which may not be fulfilled in the environment leading to more pollution/microplastic creation.
- End of life – The fate of bioplastics in the waste system is currently neglected in the legislation. This causes confusion and protests of consumers and recycling and composting institutions.
- Resources - In many cases, bioplastics need agricultural resources for their production. This is a problem in terms of greenhouse gas emissions and the overall lack of land for food growing.

In this thesis, I work with poly(3-hydroxybutyrate), which can be produced from variable renewable resources including third generation feedstock – waste. Therefore, the problem of resources will not be addressed. I have no ambitions to solve the problem with the legislation either. In this thesis, I will focus on overcoming the processing difficulties connected to the work with poly(3-hydroxybutyrate), namely its thermal degradation. At the same time, I aim to preserve its original property – biodegradability.

1. THEORY

1.1. Poly(3-hydroxybutyrate)]

Poly(3-hydroxybutyrate) (abbreviation PHB) is the most discussed polymer from the group of microbial polyesters poly(hydroxyalkanoates).¹ Chemically, it is a linear aliphatic polyester comprised of *R* stereoisomers of 3-hydroxybutanoic acid monomeric units, as can be seen in Figure 1.

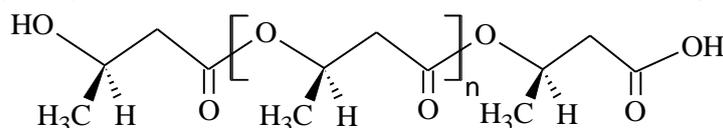


Figure 1 Poly(3-hydroxybutyrate) structure¹

PHB usually possesses 55-90% crystallinity with melting temperature (T_m) between 170–180 °C. Glass transition temperature (T_g) lies between –5 and 5 °C depending on the molecular weight and crystallinity of PHB and the method used.^{1,2} The most appealing attribute of PHB is undoubtedly its biodegradability, while its major drawbacks as a possible material for commercial use are its brittleness and susceptibility to thermal degradation.

1.1.1. Biodegradability

Biodegradation (biotic or biological degradation) is a special type of degradation where material is completely decomposed through a biological process by enzymes produced by living organisms into small molecules like carbon dioxide or water, biomass and/or mineral salts in a defined environment

and timescale.³ In the real environment, both non-enzymatic (abiotic) and enzymatic processes can take place. In the case of enzyme absence, PHB can degrade by hydrolysis, in which the water permeates the polymer bulk and causes a random cleavage of polyester bonds. This leads to a decrease in MW, while the weight remains unchanged up to a great extent of the degradation.⁴⁻⁶ The kinetics is also proportional to the water and ester bond concentration, which are considered constants hence the relationship:

$$M_n^t = M_n^0 e^{-kt}, \quad \text{Eq. 1}$$

where M_n^0 is the number average molecular weight at time 0 and k is the rate constant, and M_n^t is the number average molecular weight at time t . If the chain scission is completely random, the average number of bond cleavage per molecule is linearly dependent on time.⁵

Degradation through the action of enzymes originates at the surface of a material, where the enzyme adsorbs. Selective degradation leads to weight loss, while MW does not change as opposed to non-enzymatic hydrolysis. In the initial stages, preferentially amorphous fraction is consumed.⁴ PHB is degraded by a number of bacteria and fungi. For example, lipase, an enzyme that catalyses the hydrolysis of an ester bond between glycerol and fatty acids, is capable of cleavage of PHB bonds. Konamni et al. studied the degradation of PHB water suspension with lipase from *B. subtilis* (pH 8, 40 °C). After 72 h of the test, a notable decrease of both molecular weight (21%) and weight (28%) was measured.⁷

1.1.1. Thermal degradation

The degradation of PHB was extensively studied by Grassie et al.⁸⁻¹⁰ Upon heating PHB to temperatures between 170 and 200 °C, which are the typical processing temperatures for PHB, the degradation is first manifested by the change in MW and viscosity of the system. Random scission is carried out by the cis-elimination mechanism, also called McLafferty rearrangement. Unstable α -hydrogen of PHB monomeric unit forms a six-membered ring ester intermediate with adjacent unit resulting in the formation of two shorter molecules. One of the formed molecules has an unsaturated crotonate end group, and the other has a carboxylate end group, as depicted in Figure 2.⁹

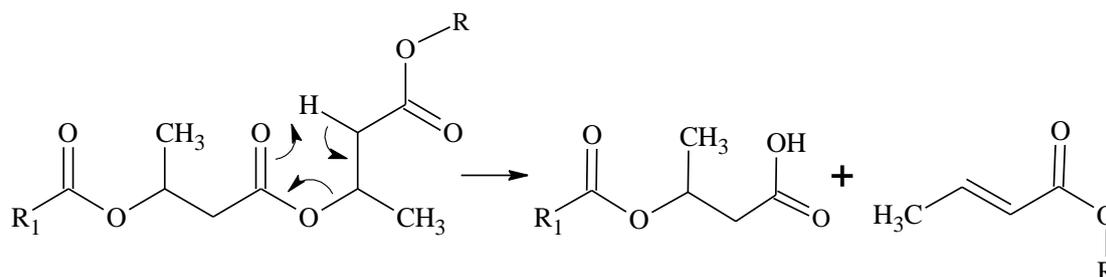


Figure 2 PHB decomposition by cis-elimination⁹

The kinetics of the reaction was described using the number average chain length during the course of random scission at time t , CL_t , and the initial chain length, CL_0 :

$$\frac{1}{CL_t} - \frac{1}{CL_0} = kt \quad \text{Eq. 2}$$

where k is the rate constant of the reaction. Indeed, the linear relationship was confirmed for temperatures 170–200 °C and activation energy of chain scission was estimated as 247 ± 19 kJ/mol.⁹

Harrison and Melik developed a methodology for characterizing the degradation kinetics using simple rheology measurement, without the need of complicated MW analysis.¹¹ The authors proceeded from the expression for the change of the chain length given in Eq. 2 and gave a corresponding expression for the change of MW in time, which follows the kinetics of the first-order reaction:

$$\frac{1}{M_{w0}} - \frac{1}{M_w} = -\frac{k}{2M_0} t, \quad \text{Eq. 3}$$

where M_w is weight average molecular weight at time t , M_{w0} is starting weight average molecular weight, k is thermal degradation rate constant and M_0 is monomer MW. In order to eliminate M_w from the equation, the authors used the relationship between M_w and complex viscosity η^* :

$$\eta^* = KM_w^{\alpha(\omega)}, \quad \text{Eq. 4}$$

where K is a material constant and $\alpha(\omega)$ is a power law exponent for given frequency ω . Then, substituting Eq. 4 into Eq. 3, we obtain:

$$\left(\frac{\eta_0^*(\omega, t=0)}{\eta^*(\omega, t)}\right)^{1/\alpha} = 1 + \left(\frac{kM_{w0}}{M_w}\right) t. \quad \text{Eq. 5}$$

Upon the conditions, where we measure complex viscosity as a function of time and frequency – $\eta^*(\omega, t)$ (frequency sweep) and we want to calculate the complex viscosity as a function of frequency at time 0 prior to any degradation – $\eta^*(\omega, t=0)$. This expression can be expanded as:

$$\log \eta^*(\omega, t) = \log \eta_0^*(\omega, t=0) + \sum_{i=1}^{\infty} (-1)^i (R_{vi} t^i), \quad \text{Eq. 6}$$

where R_{vi} is an i th-order viscosity loss rate defined as:

$$R_{vi} = \frac{\alpha}{i} \left(\frac{kM_{w0}}{2M_w}\right)^i. \quad \text{Eq. 7}$$

1.1.2. Chain extension in the melt

Commercial stabilizers for traditional polymers are ineffective in PHB.¹² Therefore, the reactive carboxyl ends of PHB molecules are exploited for chain extension. Epoxide group opens one such possibility. Melt reaction between PHB and up to 30% of epoxidized polybutadiene (EPB, degree of epoxidation 43%) has been studied by Choi et al. He described that reaction occurs between these two polymers leading to higher thermal stability of reactive blends against PHB.¹³

In addition, another polymer containing epoxy rings capable of reacting with PHB chain ends to form a modified material with higher thermal stability is poly(glycidyl methacrylate) (PGMA). Differential thermal analysis confirmed the existence of an exothermic reaction between these two polymers. Cross-linking reaction takes place both before and after severe degradation of PHB due to the enhanced miscibility of PGMA and degraded PHB. Moreover, the temperature of maximum degradation rate was increased by about 24 °C for PGMA/PHB 30/70 compared to pristine PHB.¹⁴

Another functional group reactive towards PHB is carbodiimide, as investigated by Martelli et al.¹⁵ Commercial polymeric carbodiimide Stabaxol® P200 was compounded with PHB in the amount of 1, 3 and 5 wt%. Analysis showed an increase in MW, however, a decrease in PHB thermal stability and mechanical properties was observed with the increase of the additive amount.¹⁵

2. AIMS

The aim of this dissertation is, firstly, to study poly(3-hydroxybutyrate) degradation during processing with respect to the influence of processing parameters, mainly temperature and mechanical stress and to determine the kinetics of the degradation at processing temperature. Secondly, the goal is to find a compound reactive towards the polymer in a way it will compensate for the negative effects of the degradation reaction on the resulting properties. To accomplish this task, poly(3-hydroxybutyrate) will be processed with selected reagents with different functionality and chemical functional groups. The effect of the additives on the molecular weight, the thermal and thermomechanical properties of the material will be analysed. Moreover, infrared spectroscopy will be utilised to observe changes in the polymer structure. The degradation kinetics of poly(3-hydroxybutyrate) in the presence of these compounds will be measured. And thirdly, the influence of performed reactive modifications of poly(3-hydroxybutyrate) on its biodegradability will be studied.

3. EXPERIMENTAL WORK

3.1. Materials

Poly(3-hydroxybutyrate)] (PHB) ENMAT batch numbered 2252 was purchased from TianAn Biologic Materials Co., Ltd. Prior to use, the polymer was purified by washing in acetone, filtering and final drying. The basic material properties of used PHB are listed in Table 1. The reagents used in this work are organized in Table 2 according to the functionalities and chemical functional group.

Table 1 Material properties of used PHB Enmat 2252

Molecular weight		Thermal properties	
Number average MW, M_n (kDa)	150 ± 23	Glass transition temperature, T_g (°C)	$-0.8 \pm 0.2^*$
Weight average MW, M_w (kDa)	533 ± 56	Melting temperature, T_m (°C)	$172.8 \pm 0.5^*$
Polydispersity (-)	3.6 ± 0.5	Crystallinity (%)	$62.2 \pm 0.5^*$
		Degradation temperature, T_d (°C)	289

Table 2 Used additives, their abbreviation, and their functionality, f

	Isocyanates	Carbodiimides	Alcohols	Epoxy
Bifunctional	Hexamethylene diisocyanate HMDI	Stabaxol LF ST-LF	Diethylene glycol DEG	Diglycidylether bisphenol A DGE-BPA
Trifunctional	Poly(hexamethylene diisocyanate) PHMDI, $f = 3.6$	-	Glycerol GLYC	Trimethylol-propane triglycidyl ether TMP-TGE
Polyfunctional	-	Raschig 9000 R9, $f = 77.6$	poly(vinyl alcohol) PVAL, $f = 351.2$	poly(glycidyl methacrylate) PGMA, $f = 144.8$

All additives except for PGMA were purchased and used as received. PGMA was synthesized by atom transfer radical polymerization in emulsion according to Haloi et al.¹⁶ The conditions of the PGMA synthesis and the resulting properties of PGMA are listed in Table 3. The product was purified by

passing its chloroform solution through a 1.5 cm column of neutral alumina. The amount of copper in the purified polymer was < 0.002 mg/g (< 2 ppm) as analysed by atomic emission spectroscopy.

Table 3 The results of PGMA synthesis

GMA/CuBr/Pby/MBrP	H ₂ O (g)	Triton (g)	Triton (%/GMA)	M _n (kDa)	M _w (kDa)	PDI
150/1/1.5/1	40	4.0	14	20.9	23.1	1.11

3.2. Sample preparation methods

3.2.1. Reactions in the melt

A 30 ml internal chamber Brabender laboratory kneader operating at 185 °C and 45 RPM was used. The polymer was dried at 60 °C for 2 h before processing. The polymer was fed into the chamber within one minute and was homogenized continuously for 1 min. After that, the reactive agent was added, and the kneading continued for 3 minutes. Prepared polymer melt was pressed between two metal plates in a laboratory press to obtain a 1 mm thin specimen ideal for further handling.

The total sample weight was 25 g of PHB (m_{PHB}) plus the weight of the respective additive. The amount of added reagent was calculated from the number of PHB ends and the functionality of the reagents. PHB molecular weight is $M_n = 91\,490$ Da, and its substance amount, n_{PHB} , is:

$$n_{PHB} = \frac{m_{PHB}}{M_{n,PHB}} = \frac{25}{91490} = 0.273 \text{ mmol.} \quad \text{Eq. 8}$$

Considering that the polymer is degraded and there is only one reactive group per macromolecule, as one end is carboxyl and the other is crotonyl, the functionality of PHB is $f_{PHB} = 1$.

The functionality of the additive was taken into consideration when calculating its substance amount. When all PHB ends react with all functional groups of the additive, the molar ratio is:

$$n_{PHB} : n_{reagent} = 1 : \frac{1}{f_{reagent}}, \quad \text{Eq. 9}$$

where $n_{reagent}$ is the substance amount of the additive. In the case of an X -fold overdose of the reagent, the molar ratio is as follows:

$$n_{PHB} : n_{reagent} = 1 : \frac{X}{f_{reagent}}. \quad \text{Eq. 10}$$

The amounts of reagents for 2-fold, 10-fold, 20-fold, 40-fold and 100-fold overdose were calculated. However, especially at higher dosages, the release of the reagents after the processing was so intense that these samples were not taken into consideration, and higher amounts were not prepared. The samples, which were successfully kneaded, are listed in Table 4, Table 5 and Table 6.

Table 4 Samples with bifunctional additives prepared in the kneader with the amount of the additive

Overdose	HMDI	ST-LF	DEG	DGE-BPA
2-fold	M_HMDI_2	M_ST-LF_2	M_DEG_2	M_DGE-BPA_2
	44 µl ~ 0.2 wt%	99 mg ~ 0.4 wt%	26 µl ~ 0.1 wt%	93 mg ~ 0.4 wt%
10-fold	M_HMDI_10	M_ST-LF_10	M_DEG_10	M_DGE-BPA_10
	219 µl ~ 0.9 wt%	495 mg ~ 1.9 wt%	130 µl ~ 0.6 wt%	465 mg ~ 1.8 wt%

Table 5 Samples with trifunctional additives prepared in the kneader with the amount of the additive

Overdose	PHMDI	GLYC	TMP-TGE
2-fold	M_PHMDI_2	M_GLYC_2	M_TMP-TGE_2
	68 µl ~ 0.3 wt%	13 µl ~ 0.1 wt%	48 µl ~ 0.2 wt%
10-fold	M_PHMDI_10	M_GLYC_10	M_TMP-TGE_10
	339 µl ~ 1.5 wt%	67 µl ~ 0.3 wt%	238 µl ~ 1.1 wt%
20-fold	M_PHMDI_20	M_GLYC_20	M_TMP-TGE_20
	678 µl ~ 2.9 wt%	133 µl ~ 0.7 wt%	476 µl ~ 2.2 wt%

Table 6 Samples with polyfunctional additives prepared in the kneader with the amount of the additive

Overdose	R9	PVAI	PGMA
2-fold	M_R9_2	M_PVAI_2	M_PGMA_2
	67 mg ~ 0.3 wt%	24 mg ~ 0.1 wt%	78 mg ~ 0.3 wt%
10-fold	M_R9_10	M_PVAI_10	M_PGMA_10
	335 mg ~ 1.3 wt%	120 mg ~ 0.5 wt%	392 mg ~ 1.5 wt%
20-fold	M_R9_20	M_PVAI_20	M_PGMA_20
	671 mg ~ 2.6 wt%	241 mg ~ 1.0 wt%	783 mg ~ 3.0 wt%
40-fold	-	M_PVAI_40	M_PGMA_40
	-	482 mg ~ 1.9 wt%	1 566 mg ~ 5.9 wt%
100-fold	-	M_PVAI_100	M_PGMA_100
	-	1 205 mg ~ 4.6 wt%	3 916 mg ~ 13.5 wt%

3.2.2. Reactions in the solution

Firstly, PHB was pre-degraded in the hot press at 185 °C for 5 minutes. For the reaction, the pre-degraded polymer (11.76 g) was firstly dissolved in 150 ml of chloroform under reflux (around 63 °C) to form a 5 wt% solution. After complete dissolution, the reactive agent was added, and the mixture was kept under reflux for 6 h. Pre-degraded PHB without any reagent was treated the same way to get a reference sample (marked S_REF). The amount of the additive was calculated in the same way as for the kneaded samples according to equation Eq. 10. All prepared samples, their abbreviations and the amount of additives are listed in Table 7 for 2-fold and Table 8 for 100-fold molar overdose samples.

In addition, for low molecular weight additives comparison samples in 100-fold overdose amounts were prepared. PHB was dissolved in chloroform, and subsequently, the respective amount of the reagent was added, and the sample viscosity was characterised. Samples were marked with the word “mix” behind the sample name.

Table 7 Samples with 2-fold molar overdose of the additive towards the polymer prepared in the solution

	Isocyanates	Carbodiimides	Alcohols	Epoxy
Bifunctional	S_HMDI_2	S_ST-LF_2	S_DEG_2	S_DGE-BPA_2
	21 µl	47 mg	12 µl	44 mg
Trifunctional	S_PHMDI_2	-	S_GLYC_2	S_TMP-TGE_2
	32 µl	-	6 µl	22 µl
Polyfunctional	-	S_R9_2	S_PVAI_2	S_PGMA_2
	-	32 mg	11 mg	37 mg

Table 8 Samples with 100-fold molar overdose of the additive towards the polymer prepared in the solution

	Isocyanates	Carbodiimides	Alcohols	Epoxy
Bifunctional	S_HMDI_100	S_ST-LF_100	S_DEG_100	S_DGE-BPA_100
	1032 µl	2330 mg	610 µl	2188 mg
Trifunctional	S_PHMDI_100	-	S_GLYC_100	S_TMP-TGE_100
	1595 µl	-	313 µl	1120 µl
Polyfunctional	-	S_R9_100	S_PVAI_100	S_PGMA_100
	-	1578 mg	567 mg	1842 mg

3.3. *In situ* reactions in the rheometer

Rotational rheometer AR-G2 from TA instrument with the radiation oven was used for the measurements. An Environmental Test Chamber system and parallel plate geometry with a diameter of 25 mm were used for all tests.

Specimens for rheology were prepared on a laboratory hydraulic press in the 25 mm diameter mould at laboratory temperature. For the 100-fold overdose samples (Table 9), the amount of PHB was weighed so that the total amount of PHB plus the additive was 1.2 g. The additive was added to the powder, and the mixture was homogenized and pressed with 50 kN of force for 10 s. For the reference sample (R_REF), 1.2 g of polymer powder was pressed.

Table 9 Samples with 100-fold overdose of the additive for the frequency sweep test with the amount of the additive

	Isocyanates	Carbodiimides	Alcohols	Epoxy
Bifunctional	R_HMDI_100 88 μ l, 8.4 wt%	R_ST-LF_100 198.1 mg, 16.5 wt%	R_DEG_100 52 μ l, 5.5 wt%	R_DGE-BPA_100 186.0 mg, 15.7 wt%
Trifunctional	R_PHMDI_100 151.9 mg, 13.2 wt%		R_GLYC_100 27 μ l, 3.2 wt%	R_TMP-TGE_100 95.2 μ l, 9.9 wt%
Polyfunctional		R_R9_100 134.1 mg, 11.8 wt%	R_PVAI_100 48.2 mg, 4.6 wt%	R_PGMA_100 156.6 mg, 13.5 wt%

The scan consisting of four subsequent frequency sweeps (FS) was measured. The test was performed in the linear viscoelastic region with a 1% amplitude of deformation. The FS procedure used was:

1. preheating to 185 °C for 1 min with a setting of normal force for 1 N,
2. deactivation of normal force control,
3. FS at 185 °C, 1% amplitude of deformation and frequencies from 0.1 to 50 Hz,
4. conditioning for 5 s,
5. repeating from step 3 for 3 times.

Obtained data were evaluated according to Harrison and Melik and the values of time zero complex viscosity $\eta^*(\omega, t = 0)$, and viscosity loss factor R_{v1} were obtained for each frequency.¹¹

3.4. Samples characterisation methods

3.4.1. Gel permeation chromatography

Molecular weight was determined by gel permeation chromatography (GPC) on Agilent Technologies 1100 Series instrument with PLgel 5 μ m mixed C column thermostated to 30 °C with chloroform as the eluent at a flow rate of 1 ml/min. Linear polystyrene standards with narrow distribution were used for calibration (10 points). A refractive index detector was used for the eluent analysis. For each analysis, 5 mg of the sample was weighed and dissolved in 1 ml of solvent. The solution was always filtered using a 0.2 μ m PTFE syringe prior to the analysis. All samples to be compared were measured in one run.

3.4.2. Infrared spectroscopy

Fourier transform infrared spectrophotometry (FTIR) measurements were conducted on Bruker Tensor 27 machine in attenuated total reflection mode in spectral area 4000–600 cm^{-1} , with resolution

4 cm⁻¹ and number of scans 32. For the samples with hydroxyl and epoxy functional reagents, a diamond crystal was used due to its low refractive index and high hardness. Because diamond has absorbance in the region 2200–1800 cm⁻¹, for the samples with nitrogenous reagents, germanium crystal was used.

3.4.3. Thermal characterisation

Measurements were performed on DSC 2500 model from TA Instruments. All samples (10-15 mg) were hermetically sealed in aluminium pans. All measurements were carried under a nitrogen atmosphere. Two heating scans from –30 to 200 °C with a temperature ramp 10 °C/min were measured.

Crystallization temperature was evaluated from the first cooling cycle. The glass transition and melting temperatures and melting enthalpy were evaluated from the second heating cycle. Crystallinity X_c was calculated from measured data using the following equation:

$$X_c = \frac{\frac{\Delta H_m}{w_{PHB}}}{\Delta H_m^0} \cdot 100\%, \quad \text{Eq. 11}$$

where ΔH_m and ΔH_m^0 (J/g) are melting enthalpy of the second cycle and theoretical enthalpy of melting of 100% crystalline polymer (146 J/g for PHB) and w_{PHB} is mass fraction of PHB in the sample.

3.4.4. Viscosimetry

The viscosity of solutions was measured using a Brookfield RVDV-II + PX rotational viscometer with spindle number 3. The solutions were firstly tempered overnight at laboratory temperature and refilled with chloroform to the original weight (compensation for the chloroform evaporation). Approximately 150 ml of solution was used for the measurement. The solution temperature was 22 °C.

3.5. Biological properties

Laboratory biodegradability test *in vitro* was performed on the kneaded 10-fold overdose samples, together with the reference PHB sample. Tests were conducted on rounded testing specimens with 6 mm diameter, which were shape cut from the thin pressed discs prepared during kneading experiments. All measurements were performed in triplicates. The samples were firstly dried at 60 °C for 2 hours and put in the medium and kept at 37 °C in the incubator with their medium being regularly changed. Three sets of samples were prepared, which were incubated for 30, 60 and 90 days. After this time, the samples were dried and analysed. The test was performed in two environments:

- 1) in phosphate buffer saline (PBS) with pH 7.4 prepared according to Table 10,
- 2) in PBS with the addition of lipase in 10 µg/ml concentration.

Table 10 PBS composition

Compound	Amount (g)	Molarity (mol/dm ³)
NaCl (MW 58.44 g/mol)	8.0	0.1370
KCl (MW 74.55 g/mol)	0.2	0.0027
Na ₂ HPO ₄ ·12H ₂ O (MW 358.14 g/mol)	3.6	0.0100
KH ₂ PO ₄ (MW 136.09 g/mol)	0.2	0.0018

4. RESULTS AND DISCUSSION

4.1. Thermal degradation of neat poly[(R)-3-hydroxybutyrate]

PHB degrades rapidly during the processing, which manifests in the change of colour, molecular weight decrease and also shift in the thermal properties. The effects of shear and temperature on PHB are clearly visible in Figure 3, showing the molecular weight (MW) of polymer processed in a hydraulic press and in a kneader at 185 °C for 5 minutes, also in comparison to the raw polymer. The high shear stress during kneading ensures conditions comparable to large-scale processing techniques, such as extrusion.

Both number and weight average MW (M_n and M_w) drop rapidly after the polymer is melted, M_w decreased from 530 kDa for raw powder PHB to 53% during pressing. This agrees well with the data in the literature, where around a 50% drop in MW is reported for the temperature of 190 °C.^{9,18} Further decrease is observed for the kneaded sample with final M_w of 163 kDa (59% of the pressed sample). The values corresponding to kneaded PHB were not achieved by pressing even at higher temperature.

The changes in thermal properties of processed PHB do not match the changes in MW. The glass transition temperature increased from -0.8 °C for raw PHB to 2.2 °C for kneaded sample; however it is not a profound change taking into consideration its evaluation difficulties. The average melting point of kneaded sample was around 1 °C higher than for neat PHB despite the drop in MW. Nevertheless, a significant drop of melting point is not observed before the MW decreases to around 50 kDa.¹⁸

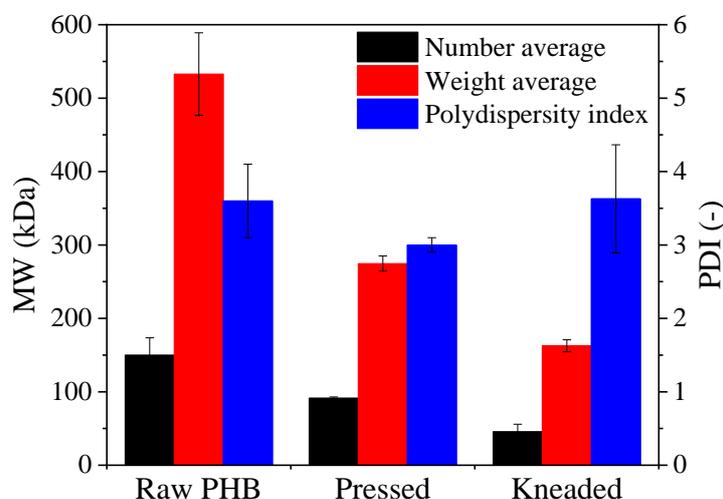


Figure 3 MW of raw PHB and processed at 185 °C for 5 minutes in the kneader and hydraulic press ($n = 3$)

4.2. Melt reactions in the kneader

4.2.1. Isocyanates addition

Two isocyanate additives were studied – bifunctional hexamethylene diisocyanate (HMDI) and its trimer poly(hexamethylene diisocyanate) (PHMDI). The relative torque during kneading for reference PHB sample, and both isocyanate samples is shown in Figure 4 and further evaluation is in Table 11.

As for HMDI, the sample with 2-fold molar overdose shows a more profound decrease of relative torque than the reference. These results are in agreement with the MW results, especially the M_w , which is

significantly lower than the reference (10% decrease). Moreover, the melting temperature of M_HMDI_2 is -1.3 °C lower than for the reference. On the other hand, the sample with a higher dosage of HMDI (10-fold overdose, 0.9 wt.%) exhibits 29% higher absolute torque at the end than neat PHB. At the same time, its M_n increased by 59% and M_w by 13% compared to the kneaded PHB. Also, the melting temperature of this sample is slightly higher compared to the reference. Moreover, the addition of HMDI led to a significant increase in T_g and crystallization temperature for both samples.

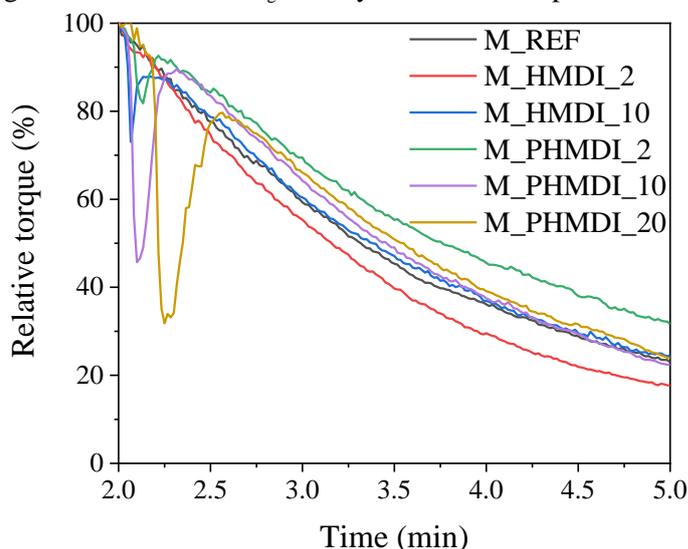


Figure 4 Relative torque during kneading of PHB after the addition of isocyanate additives

As for PHMDI, the sample with a 2-fold overdose of PHMDI has 61% higher torque at 5 min than PHB, and the slope of torque change is less steep than the reference during the whole kneading. In addition, M_PHMDI_2 has a 19% increase in M_w and a small increase in T_m as well, which makes it the most effective out of isocyanate samples. Higher amounts of the additive than 0.3 wt% did not show any further increase in studied characteristics compared to the sample M_PHMDI_2.

Table 11 The results of kneading experiment, GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurements and for kneaded samples with isocyanate additives expressed as the change of mean values (values differing for more than 2σ are underlined)

Sample name	M_{5m} (mN·m)	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
M_REF	1720 ± 160	46 ± 10	163 ± 8	2.2 ± 0.7	173.9 ± 0.3	59.8 ± 0.3	78.0 ± 1.6
	ΔM_{5m} (mN·m)	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
M_HMDI_2	-240	-5	-17	<u>+3.2</u>	<u>-1.3</u>	<u>-5.0</u>	<u>-3.8</u>
M_HMDI_10	<u>+624</u>	<u>+27</u>	<u>+21</u>	<u>+2.5</u>	<u>+0.7</u>	<u>-3.3</u>	<u>-4.4</u>
M_PHMDI_2	<u>+1208</u>	+3	<u>+31</u>	-1.0	<u>+0.9</u>	+0.3	<u>-12.4</u>
M_PHMDI_10	+315	<u>+21</u>	<u>+17</u>	+0.3	<u>+1.1</u>	<u>-6.4</u>	<u>-9.6</u>
M_PHMDI_20	<u>+644</u>	<u>+21</u>	+16	+1.2	<u>+1.4</u>	<u>-2.1</u>	<u>-9.1</u>

DSC curves of the reference and PHMDI samples are in Figure 5. It is visible that the crystallization ability of PHB is profoundly hindered by PHMDI addition. The crystallization peak is broader, and its maximum is shifted to lower temperatures by 9–12 °C. Moreover, all PHMDI samples have a small

peak of cold crystallization around 50 °C. This may be caused by the low nucleation activity of PHB ends with attached reagent. The crystallinity is decreased for the samples with 10- and 20- fold overdose.

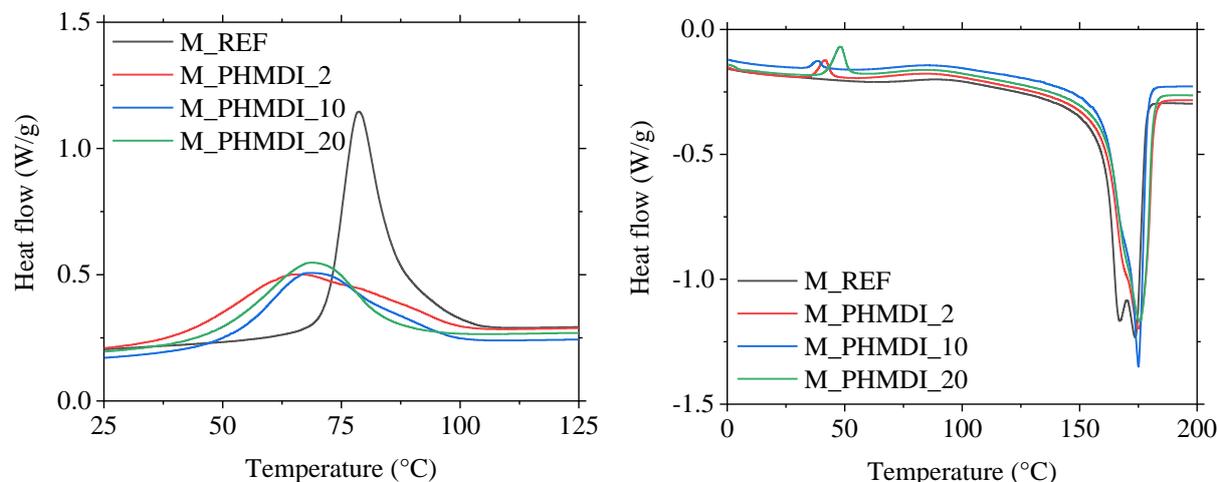


Figure 5 Crystallization (on the left) and melting (on the right) of PHMDI samples and the reference (exo up)

4.2.2. Carbodiimides addition

Additives with carbodiimide functional group with two functionalities were studied – bifunctional bis(2,6-diisopropylphenyl)carbodiimide, Stabaxol® 1 LF (ST-LF), and its polymeric form Raschig® 9000 (R9). The change of relative torque during kneading for PHB reference, and the samples kneaded with ST-LF and R9 addition is shown in Figure 6 and further evaluation in Table 12.

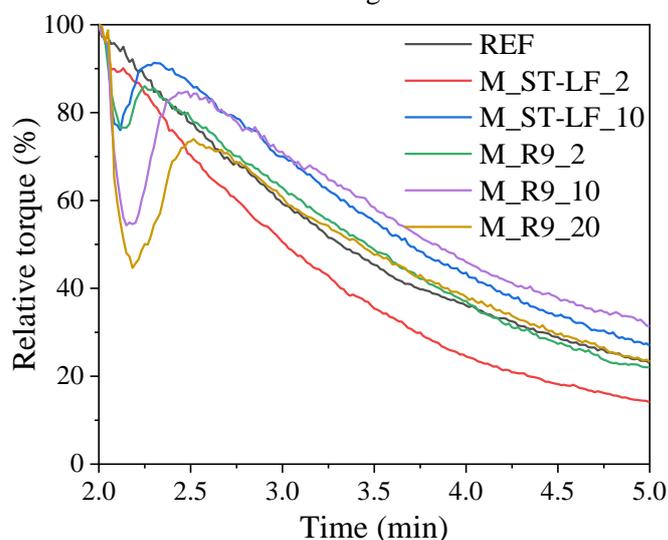


Figure 6 Relative torque during kneading of PHB after the addition of carbodiimide additives

The sample with a 2-fold overdose of ST-LF showed a significantly steeper decrease of torque during kneading and reached 31% lower final torque than kneaded PHB, which is the lowest value measured among all tested reagents and amounts. On the contrary, M_ST-LF_10 has 36% higher torque at the end of the test than the reference sample despite the 1.9 wt% addition of low MW reagent. The curve of relative torque for this sample lies above the reference during the whole experiment. The observed positive effect on the melt viscosity is in agreement with GPC results. Compared to the reference, the increase is 76% and 16% in M_n and M_w , respectively. The glass transition temperature is increased by 2 °C for this sample, while the melting temperature remains unchanged.

Table 12 The results of kneading experiment, GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurements and for kneaded samples with carbodiimide additives expressed as the change of mean values (values differing for more than 2σ are underlined)

Sample name	M_{5m} (mN·m)	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
M_REF	1720 \pm 160	46 \pm 10	163 \pm 8	2.2 \pm 0.7	173.9 \pm 0.3	59.8 \pm 0.3	78.0 \pm 1.6
	ΔM_{5m} (mN·m)	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
M_ST-LF_2	<u>-539</u>	-6	-5	+0.8	+0.0	-0.3	+0.9
M_ST-LF_10	<u>+766</u>	<u>+36</u>	<u>+25</u>	<u>+1.9</u>	+0.5	<u>-3.0</u>	<u>-3.7</u>
M_R9_2	+217	+18	-14	-1.1	+0.3	<u>-2.6</u>	-2.8
M_R9_10	<u>+1232</u>	<u>+28</u>	+13	+1.0	-0.2	<u>-4.7</u>	<u>-5.7</u>
M_R9_20	<u>+389</u>	<u>-22</u>	<u>+22</u>	<u>-1.9</u>	<u>-0.6</u>	<u>-3.4</u>	<u>-7.9</u>

The addition of polymeric carbodiimide in the 2-fold overdose amount had a negligible effect on studied characteristics, such as in the case of its bifunctional version. Raschig showed the highest impact in the 10-fold overdose (1.3 wt%), reaching 72% higher final torque. The effect on molecular weight is apparent only for M_n though. Further increase in the additive amount in the blend, to 2.6 wt%, had an ambiguous effect. The relative decrease of torque of M_R9_20 copied that of the reference PHB sample and ended with a 23% higher value at the end of processing. M_n decreased substantially by 48% while the M_w increased by 14%, which is the highest value out of carbodiimide-additivated samples. At the same time, the glass transition and the melting temperatures decreased.

Thermal properties are depicted in Figure 7. Raschig 9000, with increasing addition, gradually slows the PHB crystallization process, broadening and shifting crystallization peak to lower temperatures. There is a mild cold crystallization for all PHB samples just before the melting. Moreover, the sample M_R9_20 exhibits a small cold crystallization peak around 54 °C and enthalpy of 1 J/g. Also, PHB double melting peak disappears with the increment of the additive to one single peak.

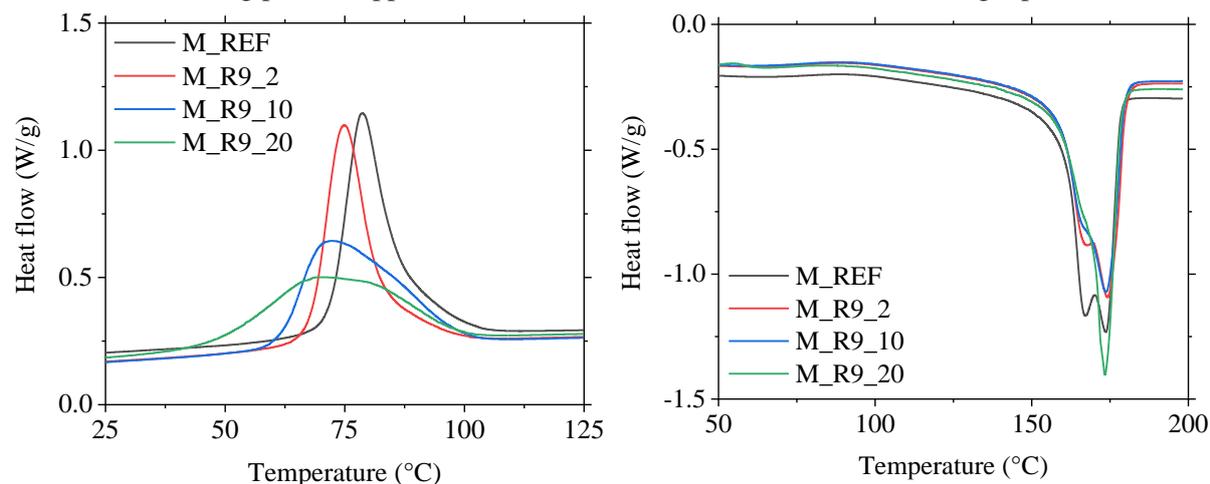


Figure 7 Crystallization (on the left) and corresponding melting (on the right) of R9 samples and the reference

4.2.3. Hydroxy compounds addition

Tested compounds with hydroxyl functional groups were bifunctional diethylene glycol (DEG), trifunctional glycerol (GLYC), and poly(vinyl alcohol) Mowiol® 4-88 with approximately 350

functional monomers (PVAI). The graphs of relative torque change during the kneading of neat PHB and PHB with hydroxyl reagents are in Figure 8. Because DEG addition did not lead to any significant changes in PHB properties, Table 13 shows the results for GLYC and PVAI only.

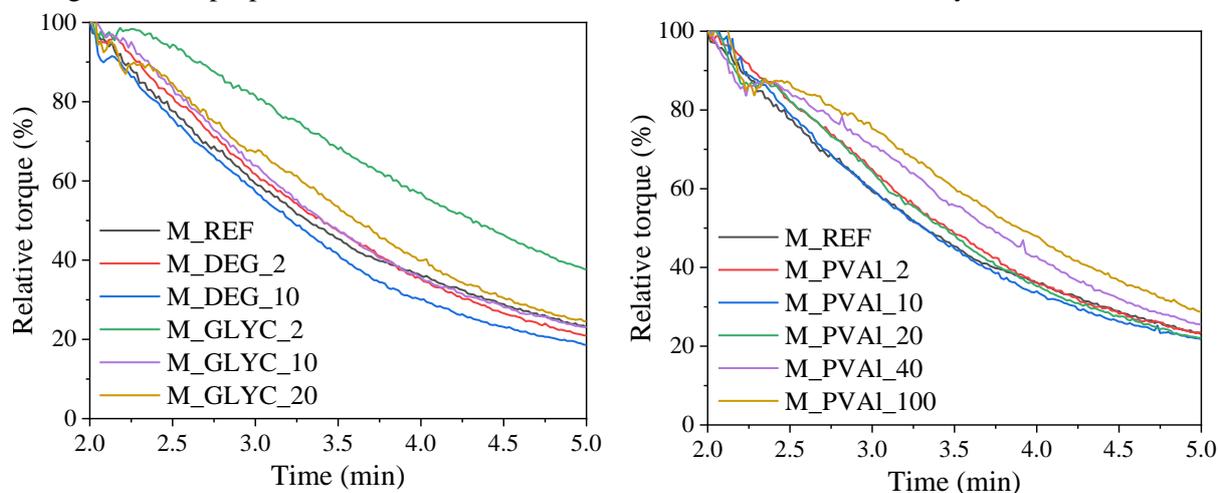


Figure 8 Relative torque during kneading of PHB with DEG and GLYC (on the left) and PVAI (on the right)

The sample with a 2-fold overdose of glycerol (only 0.1 wt%) had the highest torque at the end of the test of all kneaded samples, reaching more than two times higher value than the reference (139%). Correspondingly, it had the highest M_w obtained, 234 kDa, 44% higher than PHB processed at the same conditions. The melting temperature is among the highest measured for melt samples. However, a further increase in glycerol addition was counterproductive.

Table 13 The results of kneading experiment, GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurements and for kneaded samples with hydroxy additives expressed as the change of mean values (values differing for more than 2σ are underlined)

Sample name	M_{5m} (mN·m)	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
M_REF	1720 \pm 160	46 \pm 10	163 \pm 8	2.2 \pm 0.7	173.9 \pm 0.3	59.8 \pm 0.3	78.0 \pm 1.6
	ΔM_{5m} (mN·m)	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
M_GLYC_2	<u>+2385</u>	+7	<u>+71</u>	+0.3	<u>+1.2</u>	<u>-1.0</u>	-0.4
M_GLYC_10	<u>+389</u>	+19	+7	+0.1	<u>-3.8</u>	<u>-6.9</u>	<u>4.6</u>
M_GLYC_20	<u>+545</u>	<u>+30</u>	+10	-0.6	-0.5	<u>-5.2</u>	-2.8
M_PVAI_2	<u>+555</u>	+19	-9	<u>-1.7</u>	+0.5	<u>-3.4</u>	-0.9
M_PVAI_10	<u>+305</u>	<u>+23</u>	<u>+26</u>	<u>-2.4</u>	+0.2	<u>-2.6</u>	0.8
M_PVAI_20	<u>+374</u>	+9	<u>+31</u>	<u>-1.5</u>	+0.0	<u>-4.3</u>	<u>5.1</u>
M_PVAI_40	<u>+761</u>	<u>+26</u>	<u>+35</u>	<u>-2.5</u>	-0.1	<u>-3.8</u>	<u>-5.1</u>
M_PVAI_100	<u>+1120</u>	<u>+26</u>	<u>+24</u>	-1.3	<u>-0.9</u>	<u>-3.2</u>	<u>-15.4</u>

As can be seen from the relative torque evolution in Figure 8 on the right and also from the evaluation, there is a positive influence of poly(vinyl alcohol) addition into PHB on studied characteristics. PVAI led to the increase of 5 min torque for all tested dosages. Moreover, the values have a trend of -the higher the dosage, the higher the final torque- with the exception of the 2-fold overdose sample. The most increased final torque, 65% higher than neat PHB, was achieved for the sample with 100-fold overdose, which contained 4.6 wt% of PVAI. The relative torque evolution is also the most promising for this

sample. However, the molecular weight increase was more profound for the M_PVAI_10, M_PVAI_20 and M_PVAI_40 samples, reaching 16, 19 and 21% higher M_w than the reference, respectively.

For all PVAI samples, the glass transition temperature decreased slightly, and the crystallinity dropped by 3–4%. With further PVAI addition above 40-fold overdose, the crystallization of PHB is impaired, and the T_c drops reaching a minimal temperature observed among all samples, 62.5 °C for M_PVAI_100. The melting point of this sample is decreased consequently as well.

4.2.4. Epoxy compounds addition

Last but not least are the additives with epoxy groups: bifunctional diglycidyl ether of bisphenol A (DGE-BPA), trifunctional trimethylolpropane triglycidyl ether (TMP-TGE), and synthesised poly(glycidyl methacrylate) (PGMA). The change of relative torque during kneading for PHB reference and the samples with these additives is in Figure 9. The rest of the results is in Table 14.

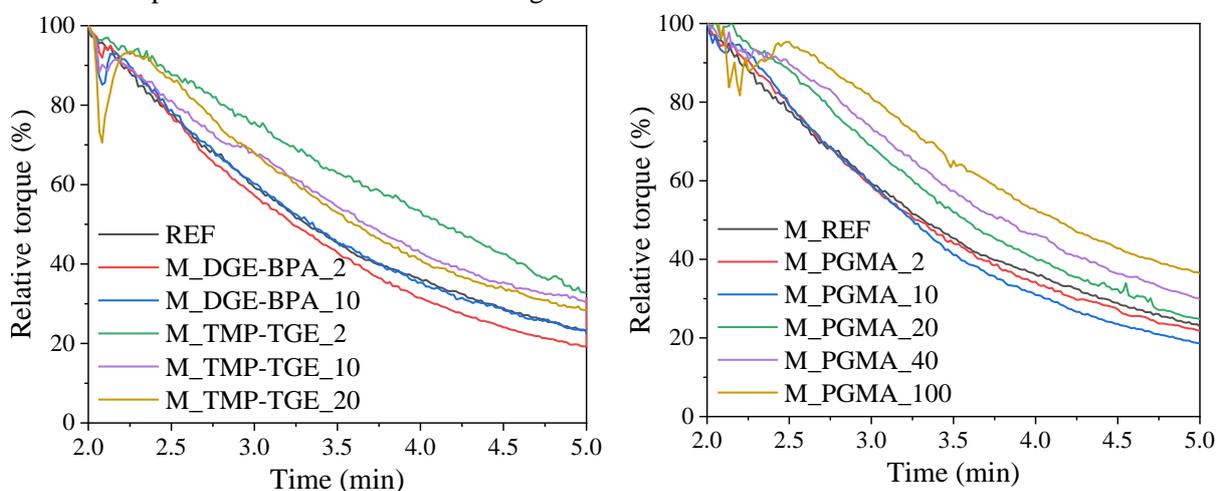


Figure 9 Relative torque during kneading of PHB with DGE-BPA and TMP-TGE (left) and PGMA (right)

Similarly to other bifunctional reagents, the addition of DGE-BPA in the 2-fold overdose amount (0.4 wt%) did not cause any convincing changes in observed characteristics, except for a minor drop in crystallinity. The sample M_DGE-BPA_10 has an identical slope of relative torque decline as the reference, notwithstanding it reached 22% higher torque at the end of the test. Together with that, the molecular weight of PHB increased by 65% for M_n and 16% for M_w .

TMP-TGE, on the contrary, showed the best performance in the lowest dosage (0.1 wt%). The sample M_TMP-TGE_2 reached 89% higher torque at the end of the kneading than PHB. The effect on M_w was also the most prominent, a 30% increase compared to PHB. With increasing the dosage of the reagent, the observed that end torque decreased, and so did the value of M_w , melting and glass transition temperatures and even the crystallinity and crystallization temperature.

PGMA addition showed positive effect from the amount of 3.0 wt% (20-fold overdose). The sample M_PGMA_20 had 41% higher final torque, and its curve of relative torque exceeded that of the reference PHB. It also led to a substantial increase in M_n . The sample M_PGMA_40 showed even better processing performance, 70% higher 5 min torque. For this sample, also the M_w increased by 15%. Maximum final torque among PGMA samples and one of the highest achieved was measured with the

100-fold overdose - 87% more than the neat PHB. Together with that, M_w rose by 25 %. For the sample M_PGMA_100, the crystallization temperature was 12.5 °C lower than for the reference, and even the melting temperature decreased considerably.

Table 14 The results of kneading experiment, GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurements and for kneaded samples with epoxy additives expressed as the change of mean values (values differing for more than 2σ are coloured)

Sample name	M_{5m} (mN·m)	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
M_REF	1720 \pm 160	46 \pm 10	163 \pm 8	2.2 \pm 0.7	173.9 \pm 0.3	59.8 \pm 0.3	78.0 \pm 1.6
	ΔM_{5m} (mN·m)	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
M_DGE-BPA_2	+25	+10	-6	+1.3	+0.2	<u>-1.9</u>	1.0
M_DGE-BPA_10	<u>+374</u>	<u>+30</u>	<u>+26</u>	+1.2	+0.5	<u>-2.5</u>	<u>-3.5</u>
M_TMP-TGE_2	<u>+1534</u>	+15	<u>+49</u>	+0.3	<u>+1.0</u>	<u>-1.8</u>	-1.3
M_TMP-TGE_10	<u>+1193</u>	<u>+21</u>	<u>+22</u>	-0.3	+0.3	<u>-1.9</u>	-2.2
M_TMP-TGE_20	<u>+948</u>	+18	-6	<u>-2.0</u>	-0.5	<u>-3.2</u>	<u>-4.0</u>
M_PGMA_2	+217	+14	<u>-24</u>	<u>-2.0</u>	-0.3	<u>-2.7</u>	-1.1
M_PGMA_10	-4	<u>+28</u>	+13	-1.1	-0.3	<u>-3.6</u>	<u>3.9</u>
M_PGMA_20	<u>+698</u>	+19	+7	<u>-2.1</u>	+0.1	<u>-1.0</u>	<u>5.8</u>
M_PGMA_40	<u>+1198</u>	<u>+30</u>	<u>+24</u>	<u>-1.7</u>	<u>-1.0</u>	<u>-2.8</u>	<u>5.0</u>
M_PGMA_100	<u>+1487</u>	<u>+30</u>	<u>+41</u>	<u>-2.1</u>	<u>-1.7</u>	<u>-1.8</u>	<u>-12.5</u>

4.2.5. Discussion

A laboratory kneader was used to study the processing changes in poly(3-hydroxybutyrate) with and without the addition of selected reagents. Prepared samples were characterised by means of processing performance, molecular weight and thermal properties determination.

Generally, all bifunctional additives showed better performance for the higher tested dosage, as expected due to the higher probability of the additive finding two polymer ends. The only exception is diethylene glycol, which showed no effect on PHB. Hexamethylene diisocyanate, bis(2,6-diisopropylphenyl)carbodiimide and diglycidyl ether of bisphenol A addition in 10-fold molar overdose towards PHB chain ends led to a rise in torque values at the end of the processing. The highest value was measured for carbodiimide reagent Stabaxol® 1 LF, 45% higher than neat PHB. In addition, the sample M_ST-LF_10 was the only one among the bifunctional additives with relative torque during kneading, surpassing that of the reference. It is important to note that all three named additives led to 13–16% higher weight average molecular weight compared to the reference, which indicates the stabilization effect of these three reagents or chain extension reaction between them and PHB chain ends.

As opposed to bifunctional ones, all trifunctional additives were effective in increasing the absolute values of processing torque and compensating for the loss of molecular weight in all tested dosages. Quite surprisingly, they were the most effective in the smallest amounts tested, as manifested by a profound change in the slope of the relative processing torque. On the contrary, the curves of 10- and 20-fold overdose samples lie close to each other and to the reference curve. However, we have to take

into consideration the possible plasticizing effect of the reagents, which might play a role, especially for 20-fold samples. Nevertheless, glycerol in 2-fold dosage exhibited the greatest suppression of the relative torque decrease among all samples and reached more than two times higher torque after 5 minutes of kneading at 185 °C than the reference. M_GLYC_2 had the highest MW measured as well – 44% more than PHB. Trimethylolpropane triglycidyl ether and poly(hexamethylene diisocyanate) additivated samples in the 2-fold overdose follow, both with better results than all bifunctional reagents.

As far as polyfunctional additives are concerned, their effect on final processing torque and molecular weight increases with higher dosages with few discrepancies. The highest numbers were achieved for the sample M_PGMA_100, which has an 87% higher value of torque after 5 min of processing and 25% higher weight average molecular weight.

When discussing the molecular weight change with the addition of reagents with the functionality of more than 2, we also have to take into consideration the limits of gel permeation chromatography, which gives the most reliable numbers for linear polymers. As the analysis takes place in a diluted solution where the polymer is in the form of a random coil, its retention time is given by its intrinsic viscosity, which is lower for a branched polymer compared to a linear one with the same molecular weight.

The thermal properties of prepared samples were characterised by differential scanning calorimetry. All samples except two exhibited a decrease in PHB crystallinity, which is very positive given the poor mechanical properties of PHB caused, among other things, by its high crystallinity. The highest decrease was observed for the samples with glycerol. For PHMDI, Raschig, PGMA and high dosage PVAI samples, the crystallization peaks were broadened profoundly and most of them also shifted to lower temperatures, showing worsened ability of PHB to crystallize. All of them had also an increase in molecular weight, suggesting that the reagent incorporated into the polymer structure, changing its thermal behaviour.

4.3. The reactions in the solution

Due to the high temperature of processing, for some additives, only a small dosage samples could be prepared in the melt. Therefore, the reactions in the solution were carried out. For that, powder PHB was pre-degraded for 5 min at 185 °C in the press in order to get closer to the melt processing conditions (MW dropped to 270 kDa). In the same way as in the melt, studied reagents can act as chain extenders, single or multi-way branching points for PHB. This change of structure should reflect in infrared spectra, MW and the solution viscosity, which was measured on 5 wt% solutions using an oscillation viscometer.

4.3.1. Isocyanates reagents

Prepared solutions of PHB treated with studied isocyanate compounds, hexamethylene diisocyanate (HMDI) and poly(hexamethylene diisocyanate) (PHMDI), and the reference PHB were firstly subjected to the viscosity measurement, with results in Figure 10 and further characterisation in Table 15.

The solution of neat PHB pre-degraded in a press and kept in chloroform under reflux for 6 hours has a viscosity of 116.7 Pa·s. The simple addition of 8.4 wt% HMDI (S_HMDI_100 mix) prior to the

measurement has a negligible effect on the viscosity. On the contrary, the solutions to which HMDI was added before the reflux time show an increase in viscosity by 4% and 8% for 2-fold and 100-fold samples, respectively. However, only the sample S_HMDI_100 had higher M_w than neat PHB (19%).

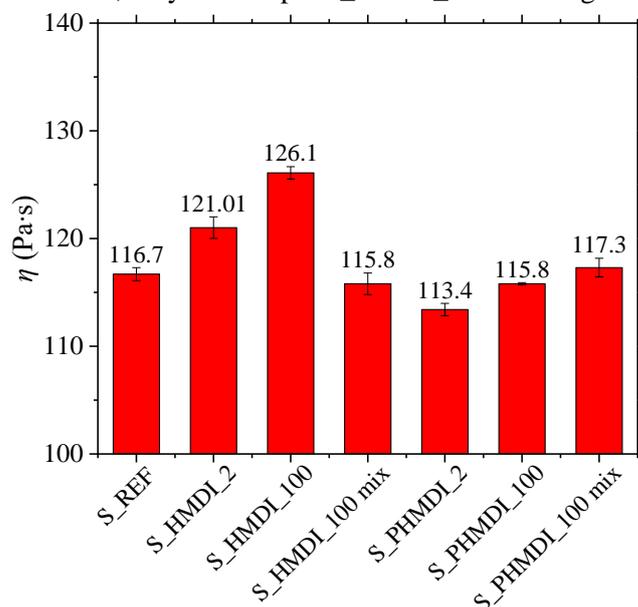


Figure 10 The comparison of viscosity of 5 wt% solutions of PHB (REF) and PHB with isocyanate reagents

Table 15 The results of GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurement and for samples prepared by solution method with isocyanate additives expressed as the change of mean values (values differing for more than 2σ are underlined)

Sample name	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
S_REF	47 ± 6	268 ± 16	-4.4 ± 0.7	172.2 ± 0.3	60.0 ± 0.6	73.8 ± 1.2
	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
S_HMDI_2	<u>-17</u>	+7	<u>2.2</u>	-0.4	<u>-2.4</u>	0.7
S_HMDI_100	<u>-16</u>	<u>+51</u>	<u>3.7</u>	0.0	<u>6.4</u>	2.3
S_PHMDI_2	-8	-10	<u>3.6</u>	<u>3.1</u>	<u>-9.7</u>	<u>-4.1</u>
S_PHMDI_100	<u>+39</u>	-8	<u>2.1</u>	-0.1	<u>8.0</u>	<u>-2.9</u>

The FTIR spectra of the samples with bifunctional HMDI (S_HMDI_100 and only mixture of PHB and the reagent, S_HMDI_100 mix) and the reagent itself were measured and are shown in Figure 11. Pure HMDI has absorption peak corresponding to its functional isocyanate group NCO at 2251 cm^{-1} .¹⁹ This peak is also visible in the spectrum of the sample S_HMDI_100 mix, although it is very weak. On the other hand, in the spectrum of the reactive sample S_HMDI_100, the NCO peak is absent while a mild peak of -NH bonds between $3200\text{--}3500 \text{ cm}^{-1}$ and amide peaks around 1600 cm^{-1} appears. This proves the reaction between isocyanate and carboxyl groups forming amides.

In the case of PHMDI, the viscosities in Figure 10 do not look very promising at first sight. A simple addition of this reagent in 13.2 hm% did not affect the viscosity, and the reactive sample S_PHMDI_100 did neither. Moreover, the sample with a 2-fold overdose amount of the reagent showed 3% lower viscosity than the reference. The M_w was not changed, only for the higher dosage sample the M_n is 81% higher than for PHB. It is important to note that this does not disprove the reaction between PHMDI and

PHB. PHMDI has a functionality slightly higher than 3 and therefore, star-like branched macromolecules may form. These structures have a lower viscosity than their linear analogues.²⁰

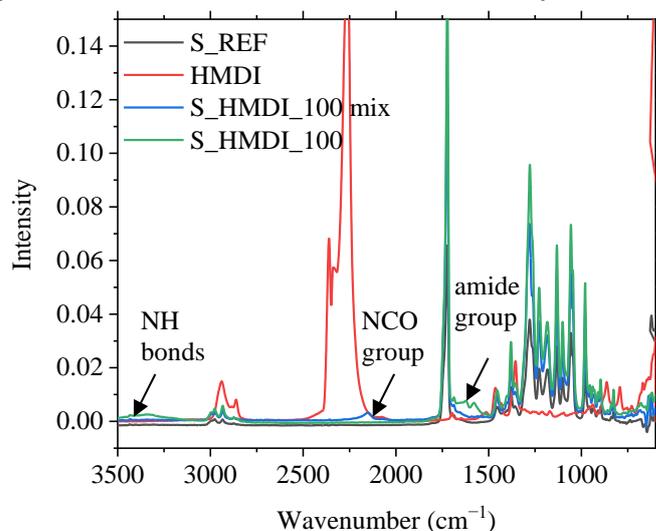


Figure 11 FTIR spectra of PHB reference, pure HMDI, the sample *S_HMDI_100* mix (as prepared), and *S_HMDI_100* (after the reaction)

The infrared spectra of the reference and PHB with 13.2% PHMDI added pre, and after 6 hours under reflux in chloroform are in Figure 12. PHMDI has clear peaks of NCO group at 2361 and 2271 cm⁻¹. The sample, where PHMDI was only mixed with PHB without any reaction time (as prepared) contains peaks corresponding to the reagent's functional group. These are absent in the sample *S_PHMDI_100*, which indicates that the reaction took place.

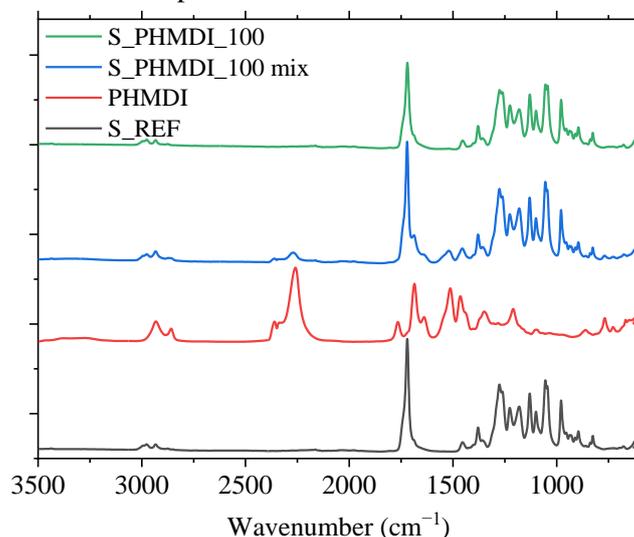


Figure 12 FTIR spectra of PHB reference, PHMDI, the sample *S_PHMDI_100* mix (as prepared), and *S_PHMDI_100* (after the reaction)

4.3.2. Carbodiimide reagents

The viscosity values of prepared samples with carbodiimide additives Stabaxol and Raschig are in Figure 13. In Table 16, there are the results of MW and thermal characterization.

The addition of a 2-fold molar overdose of Stabaxol did not have any positive effect. On the other hand, when a 100-fold overdose was used, the solution viscosity is 4% higher than the reference, even though

the simple addition of ST-LF decreased the viscosity by 3%. The increase in molecular weight for S_ST-LF_100 is profound – 82% increase in M_n and 19% in M_w . The glass transition temperature of S_ST-LF_100 increased by 4 °C, and its melting and crystallization temperatures dropped majorly.

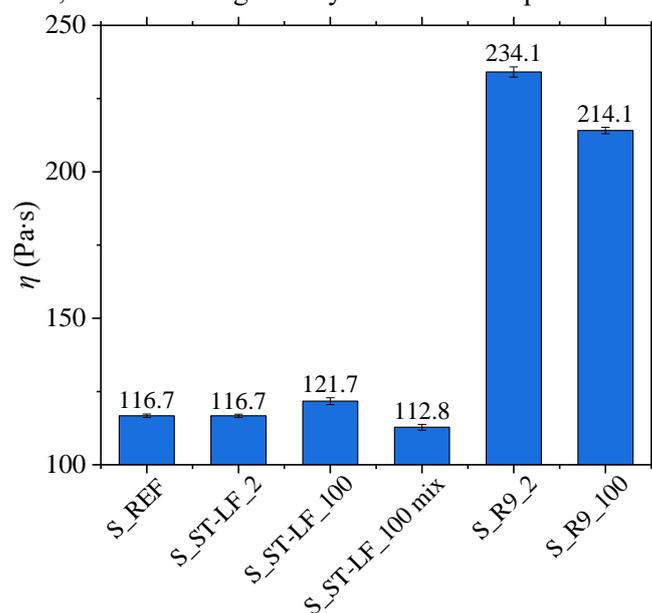


Figure 13 The comparison of viscosity of 5 wt% solutions of PHB (REF), and PHB with carbodiimide reagents. The FTIR spectra of bifunctional carbodiimide Stabaxol LF, reference PHB and the samples with a 100-fold overdose of ST-LF can be found in Figure 14. The most intensive peak of Stabaxol is 2156 cm^{-1} , corresponding to its functional group $\text{N}=\text{C}=\text{N}$.²¹

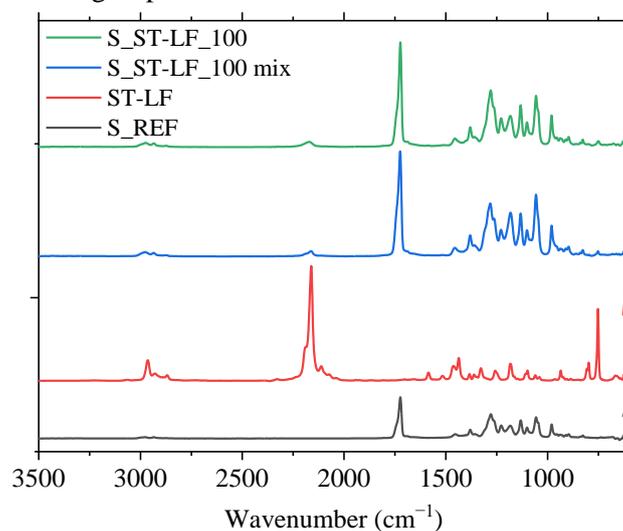


Figure 14 FTIR spectra of PHB reference, pure ST-LF, the sample S_ST-LF_100 mix (as prepared), and S_ST-LF_100 (after the reaction)

The NCN group of Stabaxol LF should be consumed during the reaction with carboxyl ends forming urea derivative with new characteristic FTIR peaks. However, there are no amide characteristics peaks, as described for HMDI. Although there is an indisputable effect on PHB's MW and solution viscosity, the reaction was not proven by FTIR. This is probably caused by the high overdose, as there are so many bonds that are supposed to be consumed that we can't see the decrease.

Table 16 The results of GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurement, and for samples prepared by solution method with carbodiimide additives expressed as the change of mean values (values differing for more than 2σ are underlined)

Sample name	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
S_REF	47 \pm 6	268 \pm 16	-4.4 \pm 0.7	172.2 \pm 0.3	60.0 \pm 0.6	73.8 \pm 1.2
	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
S_ST-LF_2	<u>-22</u>	-7	-0.1	-0.1	+0.8	<u>+3.1</u>
S_ST-LF_100	<u>+39</u>	<u>+50</u>	<u>+4.3</u>	<u>-5.5</u>	<u>+3.0</u>	<u>-8.7</u>
S_R9_2	<u>+86</u>	<u>+130</u>	<u>+3.1</u>	<u>+0.8</u>	<u>-4.8</u>	<u>-4.0</u>
S_R9_100	<u>-28</u>	-28	-1.3	<u>-1.2</u>	<u>-4.7</u>	<u>-5.3</u>

Raschig 9000, reached 101% higher viscosity compared to the reference on 2-fold molar overdose (only 0.3 wt%), see Figure 13. The increase in solution viscosity can be attributed to the changes in MW. The sample S_R9_2 has 181% higher M_n and 49% higher M_w than the reference, which are the highest values achieved for solution samples. Its thermal properties were affected as well, T_g and T_m rose while crystallinity and crystallization temperature dropped. The sample S_R9_100 showed an increase in viscosity as well, 83% more than neat PHB. However, there was no positive influence of MW observed. The reason is a great amount of unreacted Raschig in the S_R9_100 sample lowering the M_w . The overdose was confirmed also by FTIR spectra, such as for Stabaxol.

4.3.3. Alcohol reagents

Due to polyvinyl alcohol's insolubility in chloroform, it could not be used in this test. Therefore, only samples with diethylene glycol and glycerol were prepared. The results of viscosity measurement for all samples are in Figure 15, and further characterization is in Table 17.

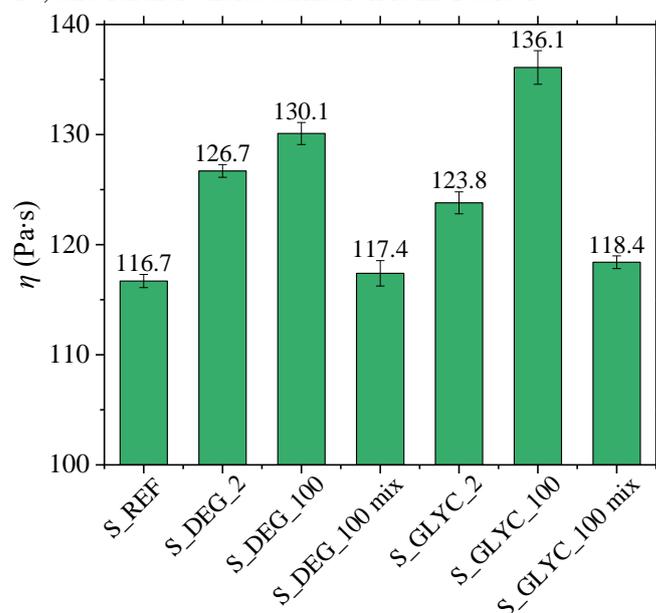


Figure 15 The comparison of viscosity of 5 wt% solutions of PHB (REF), and PHB with hydroxyl reagents

Comparing the reference sample and the sample S_DEG_100 mix, in which the PHB was treated the same way and 5.5 wt% of DEG was added into it, we can see no difference in the solution viscosity. On the other hand, the samples after the reaction time show a 10 and 13% increase in viscosity for 2-

fold and 100-fold samples, respectively. Moreover, the sample S_DEG_2 shows a distinctly increased M_n (by 36%). The glass transition temperature is slightly lower, and the crystallization temperature higher than the reference. The sample with a 100-fold overdose of DEG has lower M_n than neat PHB, and its M_w is unchanged. The melting point of this sample is decreased slightly. The FTIR spectra of diethylene glycol, reference PHB and the sample S_DEG_100 are in Figure 16.

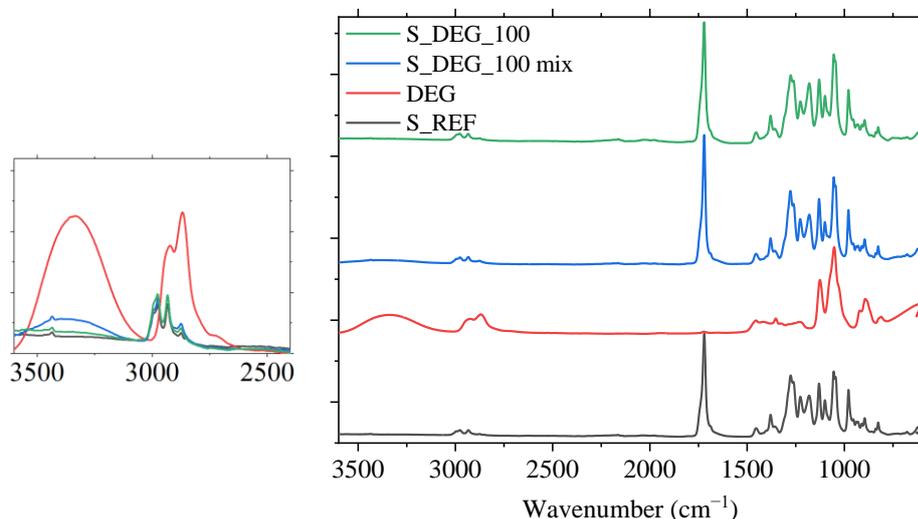


Figure 16 FTIR spectra of the reference PHB, pure diethylene glycol, the sample S_DEG_100 mix (as prepared), and S_DEG_100 (after the reaction)

DEG has prominent wide peak corresponding to its end hydroxyl groups at 3336 cm^{-1} . This peak is visible also in the spectrum of the non-reactive sample S_DEG_100 mix. However, it is not present in the S_DEG_100 sample, which suggests the functional groups have been consumed.

Table 17 The results of GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurements, and for samples prepared by solution method with hydroxy additives expressed as the change of mean values (values differing for more than 2σ are underlined)

Sample name	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
S-REF	47 ± 6	268 ± 16	-4.4 ± 0.7	172.2 ± 0.3	60.0 ± 0.6	73.8 ± 1.2
	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
S_DEG_2	<u>+36</u>	+27	<u>-1.5</u>	+0.0	+0.4	<u>+3.4</u>
S_DEG_100	<u>-22</u>	+7	-0.4	<u>-2.3</u>	<u>+3.3</u>	+0.1
S_GLYC_2	<u>+41</u>	+13	+0.1	+0.2	<u>-1.5</u>	<u>-2.4</u>
S_GLYC_100	<u>+33</u>	+3	+0.3	<u>-3.5</u>	+0.8	+2.3

In the case of glycerol, the pure addition of 3.2 wt% into the PHB solution caused a slight 1% increase in the solution viscosity, while reactive sample S_GLYC_2 has 6% higher and S_GLYC_100 17% higher viscosity than the reference PHB. Both samples showed a profound increase in M_n . The 2-fold overdose sample showed an 87% increase, and the 100-fold sample showed a 70% increase compared to the neat PHB. Such as for DEG, the 100-fold sample had a lower melting point than non-additivated PHB, other thermal characteristics did not change.

The infrared spectra of both prepared samples in the amount of 100-fold overdose (3.2 wt%), reactive sample S_GLYC_100 and non-reactive sample S_GLYC_100 mix were measured, and are shown in

Figure 17, together with glycerol spectrum. In the sample S_GLYC_100 mix as well as in glycerol, we can see a dominant peak of the hydroxyl functional group at 3284 cm^{-1} (analogous to the case of DEG). This peak is not present in the S_GLYC_100 sample after the reaction. This means that the functional groups of the additive have been consumed during the reaction time in the solution.

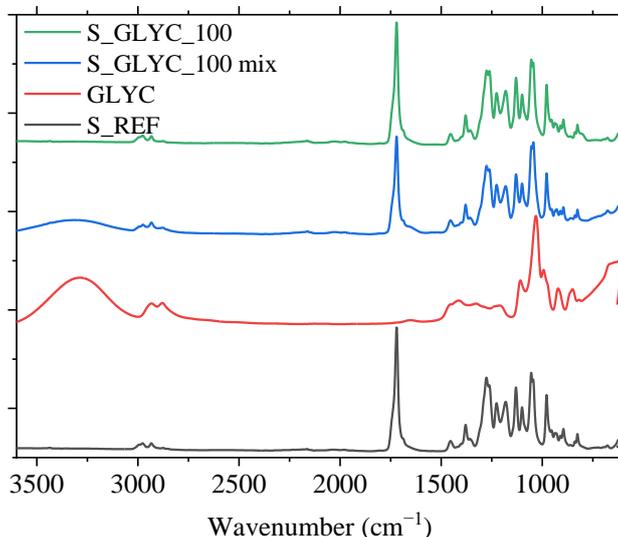


Figure 17 FTIR spectra of the reference PHB, pure glycerol, the sample S_GLYC_100 mix (as prepared), and S_GLYC_100 (after the reaction))

4.3.4. Epoxy reagents

The last subchapter will present the results for tested additives with the epoxy group: diglycidyl ether of bisphenol A, trimethylolpropane triglycidyl ether and poly(glycidyl methacrylate). Obtained values of solution viscosity are presented in Figure 18, and further characterization is given in Table 18.

Starting with the bifunctional DGE-BPA, a viscosity increase was observed for both tested dosages, 3% for a 2-fold overdose and 4% for a 100-fold overdose sample. This increase is especially noticeable when we take into consideration the fact that the addition of 15.7% DGE-BPA (S_DGE-BPA_100 mix) causes a 3% decrease in viscosity. Both samples also showed an increase in M_n , which is 72% higher than the reference for S_DGE-BPA_2, and 109% higher for the sample S_DGE-BPA_100. The sample S_DGE-BPA_100 also shows interesting thermal behaviour, $20\text{ }^\circ\text{C}$ lower crystallization temperature than PHB (the lowest value among all prepared samples both in the melt and in the solution). Moreover, this sample exhibited a small peak of cold crystallization during the following heating cycle, just under $50\text{ }^\circ\text{C}$. Its melting was affected as well, dropping by almost $7\text{ }^\circ\text{C}$.

TMP-TGE was more effective in increasing the solution viscosity in 2-fold molar overdose amount. The sample with 0.2 wt% addition had 5% higher viscosity than the reference and 83% higher M_n . In line with this, also its characteristic temperatures, T_g and T_m , rose. In the case of a 100-fold sample, the viscosity dropped by 4% in comparison with the reference. This is partially the effect of the simple addition of the additive, as it causes a 2% decrease in viscosity (Figure 18). However, the sample has a 118% increase in M_n , and its thermal properties were affected as well, with higher T_g and $4\text{ }^\circ\text{C}$ lower T_m .

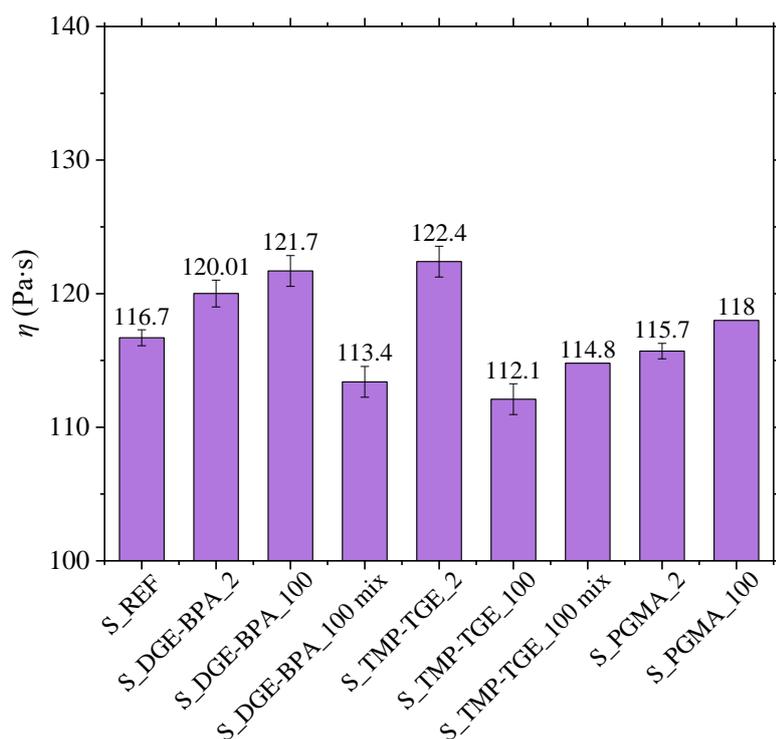


Figure 18 The comparison of viscosity of 5 wt% solutions of PHB (REF), and PHB with epoxy reagents

As far as PGMA is concerned, its effect on the solution viscosity is minor, with only a 1% increase for the sample with 100-fold overdose, as can be seen in Figure 18. The sample S_PGMA_2 showed no significant change in the MW and had the lowest PHB T_g among all prepared samples in the melt and in the solution, -8.5 °C. S_PGMA_100 had a 101% increase in M_n . The crystallization of PHB with the was hindered, with a 16 °C drop of T_c and 3% of the crystallinity for this sample

Table 18 The results of GPC and DSC analysis for neat PHB expressed as mean value \pm standard deviation from 5 measurement, and for samples prepared by solution method with hydroxy additives expressed as the change of mean values (values differing for more than 2σ are underlined)

Sample name	M_n (kDa)	M_w (kDa)	T_g (°C)	T_m (°C)	X_c (%)	T_c (°C)
REF	47 ± 6	268 ± 16	-4.4 ± 0.7	172.2 ± 0.3	60.0 ± 0.6	73.8 ± 1.2
	ΔM_n (kDa)	ΔM_w (kDa)	ΔT_g (°C)	ΔT_m (°C)	ΔX_c (%)	ΔT_c (°C)
S_DGE-BPA_2	<u>+34</u>	+0	<u>+1.6</u>	-0.2	+0.2	-0.5
S_DGE-BPA_100	<u>+52</u>	+30	-1.2	<u>-6.6</u>	<u>+2.1</u>	<u>-20.0</u>
S_TMP-TGE_2	<u>+39</u>	-8	<u>+3.5</u>	<u>+1.1</u>	<u>-2.0</u>	+0.9
S_TMP-TGE_100	<u>+56</u>	+20	<u>+4.4</u>	<u>-3.8</u>	+0.8	<u>-4.8</u>
M_PGMA_2	-4	-9	<u>-4.0</u>	-0.1	<u>-4.6</u>	+1.3
M_PGMA_100	<u>+48</u>	-16	<u>+1.6</u>	<u>+1.8</u>	<u>-3.1</u>	<u>-15.6</u>

4.3.5. Discussion

Solution reaction offered a convenient way of the preparation of high-dosage samples with a 100-fold molar overdose to polymer reactive chain ends. For each additive, also low-dosage (2-fold overdose) samples were prepared. The viscosity of prepared solutions was measured, and dried samples were characterised by means of molecular weight, thermal properties and their infrared spectra.

For a better understanding of the changes in viscosity, comparative samples where the additives were simply mixed with PHB solution prior to the measurement were prepared for all bifunctional and trifunctional additives. In most cases, the pure addition had zero or slightly negative effect on the solution viscosity, only in the case of glycerol, it caused a 2% increase in viscosity. Therefore, observed increases in viscosity can be attributed to the changes in PHB molecular structure. The greatest change in viscosity was achieved for the samples with Raschig 9000, where the increase is one order higher than for the rest of the samples – 101% higher than for the reference. The sample with a 100-fold amount of glycerol follows with 17% higher viscosity than neat PHB. The only two reagents causing a decrease in the solution viscosity compared to the reference were trifunctional poly(hexamethylene diisocyanate) and trimethylolpropane triglycidyl ether. However, due to the limited informative value of viscosity for non-linear macromolecules, it is necessary to look also at other measured characteristics.

For the majority of samples, the changes were reflected in the number average MW probably due to the high mobility of low molecular weight fraction of the polymer in the solution and, therefore, good accessibility towards chain extension and branching reaction. Moreover, the weight average of the reference PHB prepared by the solution method was higher than kneaded PHB, and its determination is burdened by high measurement error. Therefore, although there are some more promising samples, only in three cases a significant increase was achieved. That was for 100-fold samples with hexamethylene diisocyanate and Stabaxol and a 2-fold sample with Raschig 9000.

As for the melting point, for 2-fold samples, it either did not change or increased and if so, the crystallinity of the sample dropped at the same time. On the other hand, 100-fold samples often exhibit lower melting point and higher crystallinity compared to the reference. This may be attributed to the plasticizing effect of the additives in such high dosages. The exceptions are Raschig and PGMA, which decreased the crystallinity, and the latter also increased the melting point. Higher dosage samples with carbodiimide and epoxy additives caused a shift of the crystallization peak to lower temperatures.

Further insight into the interaction between PHB and the additives was gained from infrared spectra measurement. For that, non-reactive samples with 100-fold dosage were prepared by simple mixing of PHB with the additive and were compared with the samples prepared by the reaction in the solution. For isocyanate reagents, the consumption of the reactive NCO group was confirmed by the disappearance of the characteristic peak at 2250 cm^{-1} in the spectra of the reactive samples. In the case of HMDI, the formation of an amide group was observed as well. In the case of carbodiimide samples, on the other hand, no reduction of the peak intensity of the reagent's reactive groups was observed. For Raschig, the addition of 100-fold molar overdose (11.8 wt%) is counterproductive, as also shown by viscosity and molecular weight results. For example, for the blend of PHB with polylactide, more than 0.5 wt% of Raschig was proven ineffective.²³ Diethylene glycol and glycerol both have a wide peak of hydroxyl functional group between 3500 and 3200 cm^{-1} visible in the spectra of non-reactive samples. The peak disappears for the samples after the reaction time, meaning the reactive groups were consumed. In the case of epoxy additivated samples, the changes were not detected, as the characteristic peak of the oxirane group had too low intensity.

4.4. Rheological studies

Kneading gave us information about the processing changes of poly(3-hydroxybutyrate) with selected reagents, and solution reaction enabled us to study the high dosages of the additives. Using rheology, we can study the possible reaction of PHB with selected additives *in situ* and with great precision.

4.4.1. Degradation kinetics

A measurement containing four subsequent frequency steps (FS) was carried out. Diethylene glycol and glycerol addition was not studied in this test due to their extreme evaporation in 100-fold overdose dosage. The representative of the measurement record for the reference is in Figure 19. As the material acts more solid-like with increasing frequency, the viscosity decreases, which can be observed for all four FS. A gradual shift to lower viscosities due to the thermal degradation is also clear. You can easily notice that the first point measured is quite remote. This is a consequence of insufficient melting at the start of the measurement. Therefore, this frequency was excluded from further calculations.

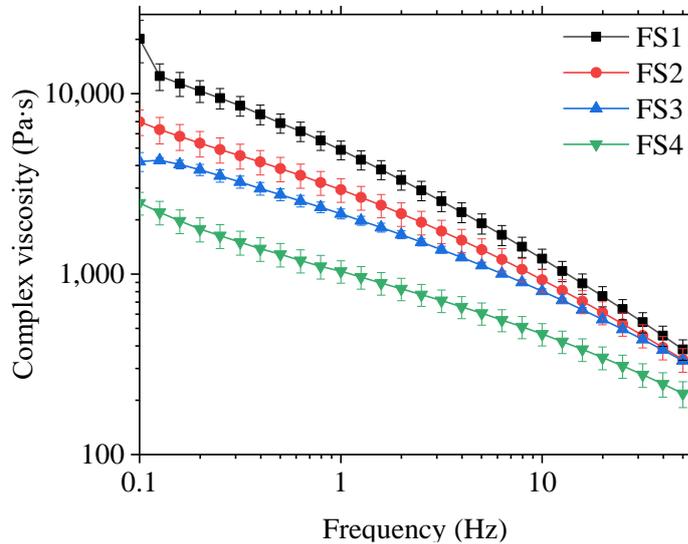


Figure 19 Frequency dependency of complex viscosity for four subsequent runs

Logarithms of obtained viscosity values were plotted as a function of the measurement time for each frequency separately. Afterwards, the data for each frequency were fitted with linear regression. For example, for 0.13 Hz:

$$y = 4.18 - 0.059x, R^2 = 0.9901. \quad \text{Eq. 12}$$

Looking at Eq. 6, from the first coefficient of the obtained linear equation, we can calculate the complex viscosity at time zero for this frequency $\eta^*(\omega, t = 0)$, and the second coefficient corresponds to the first-order viscosity loss rate R_{v1} for given frequency as follows:

$$\begin{aligned} \eta_0^*(0.13 \text{ Hz}, t = 0) &= 10^{4.18} = 15262 \text{ Pa} \cdot \text{s}, \\ R_{v1} &= 0.059 \text{ min}^{-1}. \end{aligned} \quad \text{Eq. 13}$$

This procedure was repeated for each frequency, obtained data are in Figure 20. The time zero viscosity rapidly decreases with frequency, as observed for measured data. The viscosity loss rate starts at 0.06 min^{-1} , and is constant up to 0.5 Hz, slowly decreasing until 1 Hz from when it falls more rapidly.

At 50 Hz, it reaches 0.02 min^{-1} . At higher frequencies, the molecular movement is hindered and the degradation is suppressed. Harrison and Melik described the same shape for PHB copolymers.¹¹

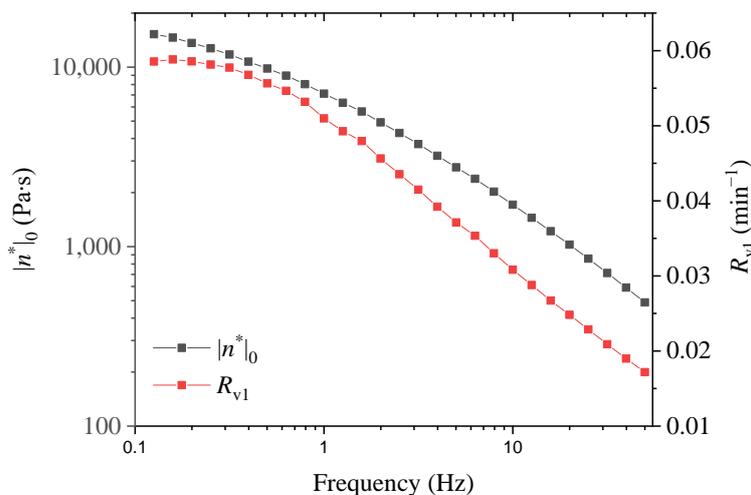


Figure 20 Frequency dependency of complex viscosity at time zero and viscosity loss rate for the reference PHB

The data measured for each sample were processed in the same ways as those of the reference using first-order fits for the complex viscosity-measurement time data sets. Obtained time zero viscosity-frequency plots compared with the reference are in Figure 21.

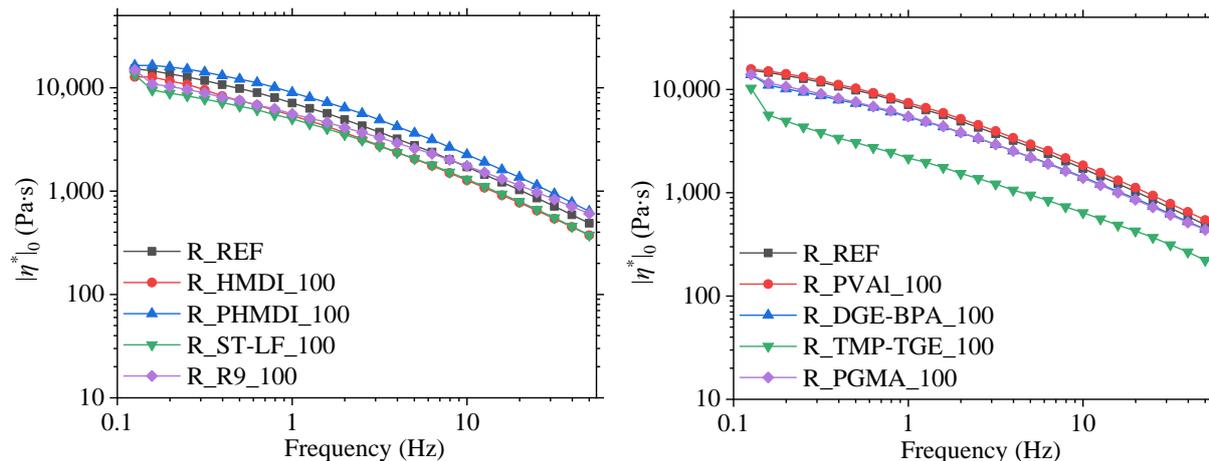


Figure 21 Calculated values of the viscosity at time zero – prior to any degradation - for the reference, and the samples with nitrogenous additives (on the left), and oxygenous derivates (on the right)

In the left graph, we can see that R_PHMDI_100 lies above the reference curve for all tested frequencies. PHB mixed HMDI and Stabaxol exhibit lower time zero viscosity than neat PHB. The curve of the R_R9_100 sample lies below the reference up to 10 Hz frequency, from where it slowly rises. As for the oxygenated additives (right graph), none of the samples reached higher time zero viscosity than the reference. Among them, the PVAI-PHB blend reached the highest values copying the curve of the neat PHB. The epoxy additivated samples R_DGE-BPA_100 and R_PGMA_100 exhibited almost identical values of calculated zero time viscosity a little lower than the reference PHB. The sample with trifunctional TMP-TGE, as expected from the FS curves, had the lowest values among tested mixtures. In fact, its time zero viscosity at a given frequency is less than half of the value of other epoxy reagents.

Also, viscosity loss rates as a function of frequency were calculated for all samples and compared with the values calculated for the reference PHB. The resulting curves for all samples are in Figure 22. In contrast with the zero time viscosity, with viscosity loss factor, we hope to obtain lower values than neat PHB. This was achieved for three samples in the limited frequency region. R_PHMDI_100, although being the only sample which reached higher time zero viscosity, had an R_{v1} factor lower than the reference only for the frequencies under 1.3 Hz. Moreover, R_R9_100 showed the lowest viscosity loss rate than the reference in the frequency region ranging from 0.13 to 16 Hz. Except for the two highest frequencies measured, the sample with HMDI had lower values of the viscosity loss coefficient than the reference in the whole frequency region.

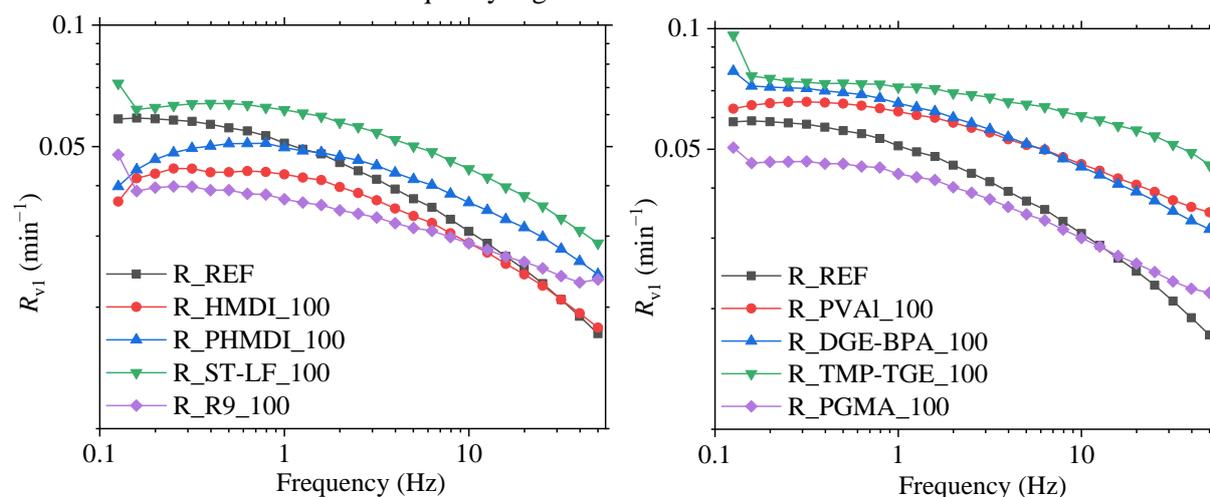


Figure 22 Calculated viscosity loss rate for each frequency for the reference, and the samples with nitrogenous additives (on the left), and oxygenous derivatives (on the right)

The highest values of the viscosity loss factor among all tested samples were obtained for the PHB additivated with TMP-TGE. The curve corresponding to R_DGE-BPA_100 reaches comparable values of R_{v1} as the R_TMP-TGE_100 for the frequencies up to 1 Hz but afterwards drops. Similarly, PVAI addition did not have any positive effect on the viscosity loss rate despite the promising values of the time zero viscosity. Last but not least, the sample with PGMA showed distinctly lower R_{v1} than neat PHB for the frequencies 0.13–16 Hz, with values comparable to those of R_HMDI_100.

4.4.2. Discussion

In this chapter, the kinetics of poly(3-hydroxybutyrate) degradation was studied using rheology test composed of four identical subsequent frequency sweeps at 185 °C. The parameters of the test were set so that it is as short as possible and, at the same time, covers a wide range of frequencies. The final frequency range is 0.1–50 Hz, and the length of the test is 18 minutes therefore, the random chain scission kinetics is applicable during the whole test.²⁵ The test results were processed using a kinetic model from the literature¹¹, and for each measured frequency, two parameters were calculated:

- time zero viscosity, a theoretical value of complex viscosity, in case of no degradation of the material. It tells us information about the influence of the reagent on the absolute viscosity,
- viscosity loss factor, a kinetic parameter characterizing the viscosity decrease in time..

With these two parameters, we can separate the effect of simple the addition of the reagent on PHB melt viscosity and the reagent's effect on the degradation rate. In previous tests, we had both of these effects working simultaneously. However, as most of the reagents are low-viscosity liquids, their addition causes a decrease in the viscosity of PHB, which does not necessarily mean they act as pro-degradants. Likewise, polymeric additives can increase the melt viscosity without having stabilizing effect.

This was observed for HMDI samples, which had lower time zero viscosity than neat PHB due to the addition of 8.4 wt% of low-molecular weight fraction. Nevertheless, the viscosity loss factor was lower than for PHB for almost all measured frequencies except the last two. PHMDI additivated sample alone reached higher time zero viscosities than the reference, but its viscosity loss rate was lower only in the low frequency range. The lowest overall viscosity loss factor was obtained for the sample with Raschig addition. On the contrary, Stabaxol, did not prove itself an effective stabilizer for PHB. Poly(vinyl alcohol) was the only representative of hydroxyl-functional compounds. Although the sample with PVAI had promising values of viscosity matching those of the reference, it was only due to the simple mixing rule, as its loss viscosity rate is higher than for neat PHB. Moreover, both epoxy reagents with low functionality, diglycidyl ether of bisphenol A and trimethylolpropane triglycidyl ether failed to suppress PHB's natural degradation and decrease its viscosity loss rate. The sample with poly(glycidyl methacrylate), on the other hand, showed comparable zero time viscosity with the reference and, at the same time, lower viscosity loss rate for a high range of frequencies, making it one of the most effective reagents in PHB thermal stabilization.

4.5. Biodegradability *in vitro*

Enzymatic biodegradability *in vitro* test with simultaneous measurement in purely abiotic conditions was performed in order to investigate if the addition of the reagent changes the biodegradability behaviour of this polymer. During the test, the samples did not show significant decrease in weight.

More prominent changes were observed in the results of molar mass measurement. The changes in the weight average molecular weight are presented in Figure 23 for the nonenzymatic test and in Figure 24 for samples treated with lipase solution. Obtained data were analysed using one-way ANOVA test in order to find out if there is a statistically significant difference between the individual samples.

In abiotic conditions the sample M_R9_10 showed the highest final MW loss, -31%, which is statistically higher than all the remaining samples except those with PGMA and PVAI. M_PGMA_10 and M_PVAI_10 also showed a profound decrease in M_w at the end of the test, -22% and -19%, respectively. The reference PHB showed 15% molar mass loss after 90 days of this test. The lowest change in M_w after 90 days was observed for the samples M_PHMDI_10 and M_ST-LF_10.

In the presence of lipase enzyme, most of the samples had a greater MW loss than in purely abiotic conditions. After 90 days in the medium, most of the samples exhibited the highest decrease in M_w measured in the time frame of the measurement. The neat PHB showed 20% molar mass loss at the end of the test. A significantly higher decrease than for the reference was measured only for M_R9_10,

–35%, such as in the non-enzymatic test. The samples with PGMA and GLYC also showed a profound decrease in M_w . The smallest drop was observed for M_ST-LF_10 (–6%) and M_HMDI_10 (–9%).

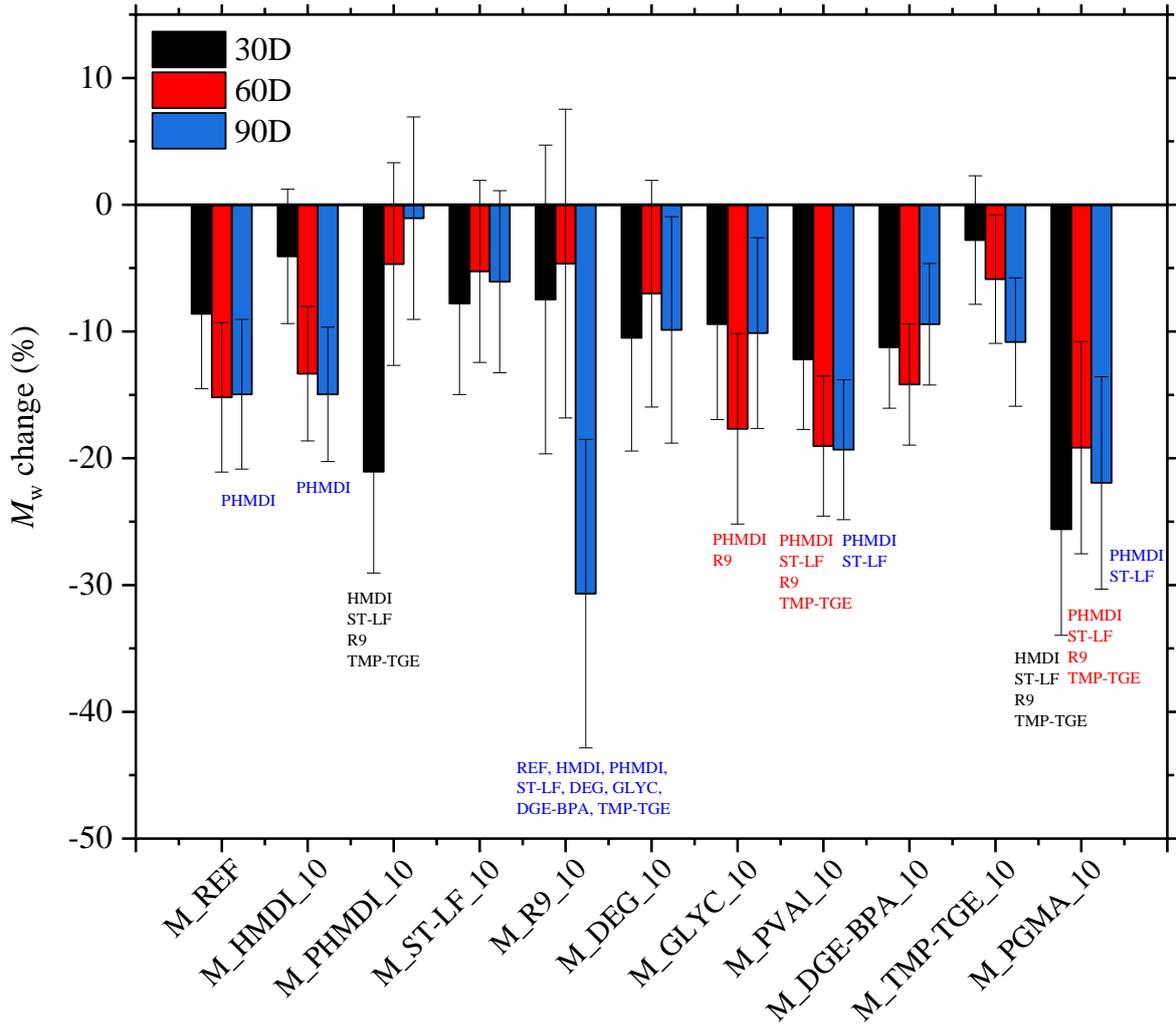


Figure 23 Weight average molecular weight change during the biodegradation test in abiotic conditions, marks above columns indicates the samples with statistically significant differences ($p < 0.05$, Fisher)

The data gathered from the measurement in non-enzymatic conditions were treated with a first-order kinetic model for the change of number average molecular weight in time in the case of random cleavage given in Eq. 1. The expression was expanded to obtain a linear expression:

$$\ln M_n^0 = \ln M_n^t - kt, \quad \text{Eq. 14}$$

where M_n^0 is the number average molecular weight at time 0, M_n^t is the number average MW at time t , and k is the rate constant. Obtained linear regression for the reference in abiotic conditions is:

$$y = 10.74 - 8.11 \cdot 10^{-3}x, R^2 = 0.5783. \quad \text{Eq. 15}$$

As you can see from the R^2 value, the data measured for the reference sample do not fit the model well. Whereas with Stabaxol, glycerol, PVA1, TMP-TGE and PGMA, the first-order kinetics work very well ($R^2 > 0.8$) and the results of the kinetic model fit for these samples are given in Table 19. Obtained rate constants range from $-2.5 \cdot 10^{-3}$ in the case of M_TMP-TGE_10 to $-8.24 \cdot 10^{-3} \text{ days}^{-1}$ for M_PGMA_10.

Table 19 A linear kinetic model fit for the degradability test in abiotic conditions for the samples with $R^2 > 0.8$: rate constant, calculated and measured values of time zero number average molecular weight

Sample name	k (day ⁻¹)	$\ln M_{n0}$	R_2	Calculated M_{n0} (Da)	Measured M_{n0} (Da)
M_ST-LF_10	$-3.78 \cdot 10^{-3}$	11.23	0.9171	75328	75680
M_GLYC_10	$-4.93 \cdot 10^{-3}$	11.04	0.8774	62268	65048
M_PVAL_10	$-6.93 \cdot 10^{-3}$	11.09	0.8352	65377	62178
M_TMP-TGE_10	$-2.49 \cdot 10^{-3}$	11.10	0.9434	66120	66983
M_PGMA_10	$-8.24 \cdot 10^{-3}$	10.82	0.8518	50054	51503

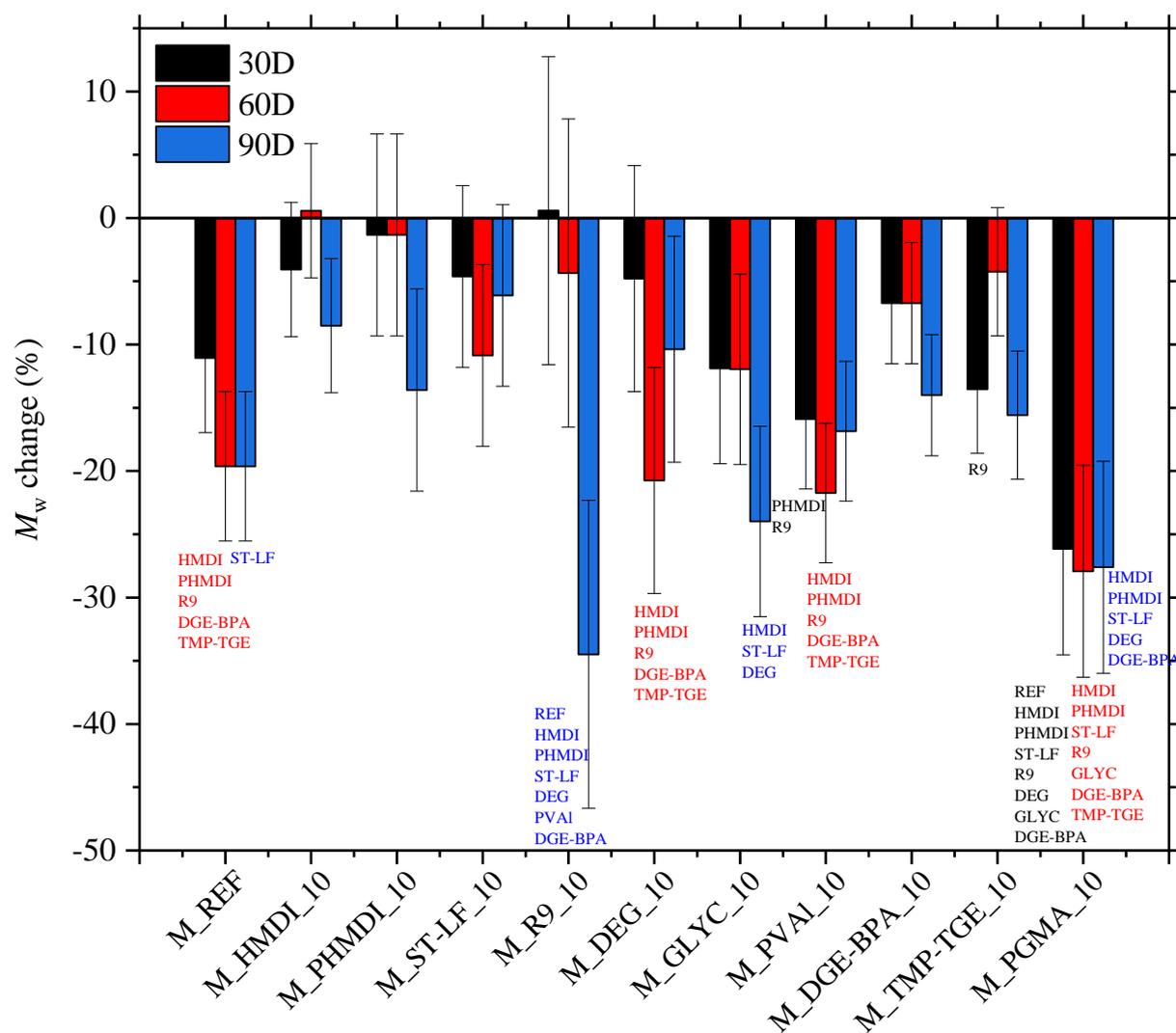


Figure 24 Weight average molecular weight change during the biodegradation test in enzymatic solution, marks above columns indicates the samples with statistically significant differences ($p < 0.05$, Fisher)

4.5.1. Discussion

In this last part of the work, the samples prepared by kneading poly(3-hydroxybutyrate) with studied additives were subjected to a laboratory enzymatic biodegradation test with lipase with parallel measurement in purely abiotic conditions phosphate buffer saline. For each sampling time (30, 60 and 90 days), individual sets of the testing specimen were prepared, which may be a source of discrepancies. Overall, a bulk hydrolysis behaviour was observed, as MW of PHB decreased profoundly while the weight remained unchanged. Weight loss is expected in the more advanced stages of degradation.⁴

In nonenzymatic conditions, the reference PHB reached a 15% drop in weight average molecular weight at the end of the test. For thin films, up to a 75% decrease can be expected.⁶ In the presence of lipase, the degradation of most of the samples was enhanced, only with HMDI the biodegradation was hindered. The reference achieved a 19% loss in M_w after 90 days in medium containing lipase.

The changes in the weight average molecular weight were analysed using the ANOVA test. The samples with polyfunctional additives, Raschig 9000, poly(vinyl alcohol) and poly(glycidyl methacrylate), showed the most significant M_w drop in both environments. For the sample with Raschig, the drop was especially profound at the end of the test and was statistically higher than for the reference PHB, -31% in non-enzymatic and -35% in enzymatic conditions. On the contrary, samples additivated with carbodiimide Stabaxol, hexamethylene diisocyanate, and poly(hexamethylene diisocyanate) showed the lowest decrease in M_w during both tests. Furthermore, the change in the number average molecular weight was analyzed and fitted to the first-order kinetic model for random cleavage, which should match the conditions of the test. For the samples with Stabaxol, glycerol, PVAI, TMP-TGE and PGMA, reliable values of rate constants for degradation by hydrolysis were obtained. The sample with PGMA has the greatest value, $-8.24 \cdot 10^{-3} \text{ days}^{-1}$.

5. CONCLUSION

This thesis's two questions of interest were: (i) Is it possible to suppress the thermal degradation of poly(3-hydroxybutyrate) during processing? and (ii) If so, will this affect its biodegradability?

In order to answer the first question, ten compounds of different functionality (2-, 3- or polyfunctional) and with four different functional groups were studied (functionality in brackets):

- 1) Isocyanates –hexamethylene diisocyanate (2), and poly(hexamethylene diisocyanate) (3),
- 2) Carbodiimides – Stabaxol® 1 LF (2), and Raschig® 9000 (77.6)
- 3) Alcohols – diethylene glycol (2), glycerol (3), and poly(vinyl alcohol) (351.2)
- 4) Epoxides – diglycidyl ether of bisphenol A (2), trimethylolpropane triglycidyl ether (3), and polyfunctional poly(glycidyl methacrylate) (144.8) synthesised in this work by atom transfer radical polymerisation in the emulsion, having the final molecular weight around 20 kDa.

Mentioned additives were mixed with poly(3-hydroxybutyrate) in the melt, which is the target application, and also in the solution. Moreover, the effect of the additive amount expressed as the molar overdose toward the reactive carboxyl chain ends of the polymer was studied.

Poly(3-hydroxybutyrate) undergoes cis-elimination during the processing using traditional machines for plastic production, it and its molecular weight, and overall performance deteriorates. During 5 minutes at 185 °C, its weight average molecular weight drops to 53% in the press and to 30% in the kneader, where the decrease is also accompanied by a decrease of the torque to 20% of initial values. The complex viscosity measured at 1 Hz of frequency and 1 % of the relative deformations decreases to 47% of the initial value after 5 minutes at the selected temperature.

The kinetics of the polymer's degradation was successfully estimated by measuring four subsequent frequency sweeps and fitting the measured data into the model for the first-order reaction of random cleavage of the macromolecules. Two values were calculated: (i) time zero viscosity, a theoretical value of complex viscosity in the case of no degradation; and (ii) the viscosity loss factor, a kinetic parameter which characterizes the viscosity decrease in time. This allowed to distinguish between the change in viscosity caused by a simple addition of the reagents and the supportive/suppressive effect on the degradation. Polymeric carbodiimide Raschig 9000, hexamethylene diisocyanate, poly(glycidyl methacrylate), and poly(hexamethylene diisocyanate), in this order, had substantially lower viscosity loss factor than pure poly(3-hydroxybutyrate) in the lower frequency range. The sample with Raschig had a 20–30% lower value of the viscosity loss factor than the reference in the region 0.1–5 Hz.

All four reagents also showed a positive effect during the kneading and solution test. The positive effect of hexamethylene diisocyanate and poly(glycidyl methacrylate) on molecular weight, processing torque during kneading, and the solution viscosity increases with increasing dosage. Hexamethylene diisocyanate led to a 13% increase in the weight average molecular weight of the polymer in a 10-fold overdose in the kneader and a 19% increase on a 100-fold overdose in the solution. Moreover, its reaction with the polymer was proved by infrared spectroscopy, as the original functional isocyanate groups were consumed, and the peaks of newly formed amide bonds arose. Poly(glycidyl methacrylate) is the most effective in the melt, where its addition in a 100-fold amount led to an 87% increase in the final torque after five minutes of kneading and a 25% higher final weight average molecular weight. Raschig's addition above 10-fold overdose (approximately 1.3 wt%) is counterproductive. In the kneader, the sample with a 10-fold dosage showed the greatest effect on the torque change, while in the solution, the 2-fold overdose addition caused a staggering 101% increase of the solution viscosity and 49% increase in weight average molecular weight of poly(3-hydroxybutyrate). Poly(hexamethylene diisocyanate) in the kneader successfully increased both the final melt torque (by 61%) and the resulting molecular weight (by 19%) in a 2-fold overdose. Remaining additives showed some positive effect in the performed tests, however, their effectivity was not supported by the kinetic measurement.

As for the second question asked in this thesis, the test of the enzymatic biodegradability with lipase was conducted for all melt-prepared samples in a 10-fold dosage. Also, abiotic degradation was monitored parallelly. After three months, the weight of the samples remained unchanged. On the other hand, the effect of molecular weight was significant, which corresponds to the bulk behaviour. For all samples except that with hexamethylene diisocyanate, the extent of the biodegradation was greater with the lipase than in the buffer. For some samples, the first-order kinetics was applied to the measured data of the number average molecular weight, and the degradation rate constant was calculated. The highest rate was obtained for the sample with poly(glycidyl methacrylate), $-8.24 \cdot 10^{-3} \text{ days}^{-1}$.

To conclude, the goals of the doctoral thesis were successfully achieved. Four reagents capable of suppressing the degradation rate of poly(3-hydroxybutyrate) were identified, and their effect was quantified. In addition, the melt-prepared samples with those additives showed a loss of molecular weight during three months in abiotic conditions and in the presence of lipase as well.

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Knowledge

Materials Biopolymers, composites
Methods Thermal and thermomechanical analysis – TGA, DSC, DMA, HDT; mechanical test – tensile, flexural, and compression tests, fracture toughness; rheology; FTIR; surface analysis using OCA and confocal microscopy; 3D printing using FDM

8. ABSTRACT

This thesis deals with melt reactions of bacterial biopolymer poly(3-hydroxybutyrate) with selected reactive agents. Compounds of different functional groups; isocyanates, carbodiimides, alcohols and epoxides; and functionalities; from two to polyfunctional; were exploited. The aim is to characterize the kinetics of the polymer's thermal degradation during processing with and without the addition of aforementioned reagents in different dosages. For that, rheological measurement consisting of four subsequent frequency sweeps covering a range of 0.1–50 Hz was carried out and obtained data were evaluated to gain a viscosity loss rate. This kinetic parameter enabled the comparison of the effects of individual reagents. Moreover, reactive samples were prepared by kneading, where melt torque was recorded and discussed, and also by solution method, where solution viscosity was measured. The thermal properties, molecular weight and infrared spectra of such prepared samples were studied. Among the tested additives, polymeric carbodiimide Raschig 9000, hexamethylene diisocyanate, poly(glycidyl methacrylate) synthesized for the purpose of this work, and poly(hexamethylene diisocyanate) showed the best overall results in performed experiments. Notably, the sample with a 100-fold molar overdose of Raschig had a 20–30% lower value of the viscosity loss factor than the reference in the frequency region 0.1–5 Hz. In addition, an enzymatic biodegradability test with lipase and in abiotic conditions was carried out. Poly(3-hydroxybutyrate) additivated with poly(glycidyl methacrylate) showed the highest rate of molecular weight decrease.