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Robot Assisted Polyurethane Chain Extension of Dihydroxy Telechelic Depsipeptides

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ABSTRACT

Depsipeptide-based multiblock copolymers synthesized from dihydroxy telechelic oligodepsipeptide precursors are promising candidate materials for biomedical and pharmaceutical applications. High molecular weight polymers in polyaddition reactions e.g. of diols with diisocyanates can only be reached when reactive groups are equivalent and a high conversion for this step growth polymerization is obtained. However, in depsipeptide-based multiblock urethanes reported so far, the stoichiometric ratio of the diisocyanate compound exceeded the theoretical value of 100% by far. In order to investigate the influence of the dosing system in this unusual behavior of the stoichiometric reaction two dosing devices, a solid dosing unit (SDU) and a gravimetric dosing unit (GDU) were used for a gravimetric transfer of an oligo(3-*sec*-butylmorpholine-2.5-dione) (OBMD) as model oligodepsipeptide. The OBMD precursor, which was transferred as a solid or as a highly viscous solution, was reacted with an isomeric mixture of 2,2,4- and 2,4,4-trimethylhexamethylene diisocyanate (TMDI) as chain extender. Two series of 49 reactions were performed and the chain extension efficacy of the building block was compared between the SDU and GDU as well as with respect to the Carothers equation. When the GDU was used the chain extension yielded higher molecular weights, proving the high accuracy of the dosing device, and the molar ratio of TMDI required for the high-throughput synthesis of the depsipeptide-based multiblock copolymers was similar to depsipeptide-based multiblock copolymers created in a classical synthesis approach.

INTRODUCTION

Robot assisted synthesis [1, 2] of multiblock copolymers, in which reaction parameters have been varied gradually has become a promising option to investigate structure-property relationships and is helpful to gain an insight into the mechanism of multiblock copolymer formation [3, 4]. Recently, high throughput (HT) synthesis of polyester-diols with diisocyanates by polyaddition reaction was reported where the volumetric transfer of viscous polymer solution was quantified and the influence of the diol to isocyanate molar ratio on the molecular weight was investigated [5]. However, an optimization of a volumetric transfer is required for viscous solutions to ensure accurate quantitative amount of components. Hence, a gravimetric transfer is needed.

Here, we investigated whether a polyaddition reaction of dihydroxy oligomers with diisocyanates can be performed by gravimetric controlled addition of the starting material with a robotic platform. Interesting for biomedical application are oligodepsipeptides - alternating copolymers of an α-amino acid and an α-hydroxy acid - as they provide thermal properties ranging over wide temperature intervals and a beneficial degradation behavior [6]. It has been

noticed, that the chain extension behavior of oligodepsipeptides deviates from the expected behavior as disproportional amounts of diisocyanates are necessary to obtain high molecular weight polyurethanes [6]. As the volumetric dosing of viscous polymer solution in HT synthesis has to be optimized to enable quantitative transfer, the solid dosing unit (SDU) and the gravimetric dosing unit for high viscous solutions (GDU) were chosen for the oligomer transfer. The oligodepsipeptide oligo(3-*sec*-butylmorpholine-2.5-dione) (OBMD) was selected as model component for HT synthesis as this oligodepsipeptide was used in several studies, which showed a significant deviation from the theoretical optimum molar ratio between the isocyanate and hydroxyl groups.

EXPERIMENTAL PART

Materials

Isomeric mixture of 2,2,4- and 2,4,4-trimethylhexamethylene diisocyanate (TMDI) was obtained from Sigma-Aldrich (Steinheim, Germany) and distilled prior use. *N*-Methyl-2-pyrrolidone (NMP) from Iris Biotech (Marktredwitz, Germany) was dried over 4 Å molecular sieves. All other solvents (Merck, Darmstadt, Germany) and chemicals (Sigma-Aldrich, Steinheim, Germany) were of commercial grade and were used as received unless noted otherwise.

Methods and Instruments

Molecular weight of the starting material was determined with the high throughput gel permeation chromatography (HT-GPC) system Tosoh EcoSEC HLC-8320 GPC including a refractive index detector (Tosoh Bioscience, Stuttgart, Germany) combined with a PSS Universal Data Center (PSS, Mainz, Germany), a viscometer ETA2010 (PSS), an EcoSEC UV detector 8320 (Tosoh Bioscience), and a light scattering detector SLD7100 (PSS). Two serially operated HT-GPC columns type PSS SDV analytical linear M 5 µm (PSS, Mainz, Germany), tetrahydrofuran (THF) as eluent (35 °C, flow rate 1.0 $mL \cdot min^{-1}$) with 0.05 wt% 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) as internal standard, and polystyrene standards (PSS, Mainz, Germany) were used for standard as well as universal calibration to determine the number-average of the molecular weight. Sample preparation of multiblock copolymers was done by mixing 20 µL of the reaction solutions in NMP with 1 mL THF/BHT leading to a polymer concentration of about 4 $mg \cdot mL^{-1}$.

Water contents were determined by Karl-Fischer (KF) titration using the automated KF titrator AQUA 40.00 (ECH, Halle, Germany).

Hydroxyl end group determination (OH number) was performed by the acetyl anhydride method using a 716 DMS Titrino (Metrohm, Filderstadt, Germany) for the potentiometric back-titration with a TitriPUR tetrabutylammonium hydroxide standard solution (Merck, Darmstadt, Germany).

^{1}H-NMR spectra were recorded at 25 °C in DMSO-d6 with a Bruker Avance 500 spectrometer (500 MHz, Bruker, Karlsruhe, Germany) with a relaxation time of 2 seconds.

Polyaddition reactions were carried out in vial reactors employing the automated parallel synthesizer platform Accelerator SLTII/106 (Chemspeed Technologies, Augst, Switzerland).

Automated volumetric transfers of NMP, TMDI, and the catalyst were performed with a 4-needle head (septum piercing needles), connected to syringe pumps (2x1 mL, 1x10 mL, 1x 25 mL). The SDU (for transfer of solids) and the GDU (for transfer of highly viscous solutions) were used for an automated gravimetric transfer of the OBMD as powder or as viscous solution, respectively.

Synthesis of OBMD

The oligodepsipeptide OBMD (Figure 1) was synthesized according to the procedure described elsewhere [6]. Ethylene glycol was used as initiator in an initiator : monomer ratio of 1 : 28.

^{1}H-NMR (500 MHz, DMSO): δ [ppm] = 0.75-0.94 (m, 6H, CH_3-5 and 7); 1.14-1.51 (m, 2H, CH_2-6); 1.77-1.93 (m, 1H, CH-4); 3.83-3.91 (t, ^{3}J(H,H) = 5 Hz, 4H, CH_2-8); 4.33-4.38 (t, ^{3}J(H,H) = 5 Hz, 1H, CH-3); 4.51-4.67 (AB-system, ^{AB}J(H,H) = 15 Hz, 2H, CH_2-1); 8.32-8.37 (d, ^{3}J(H,H) = 5 Hz, 1H, NH-2).

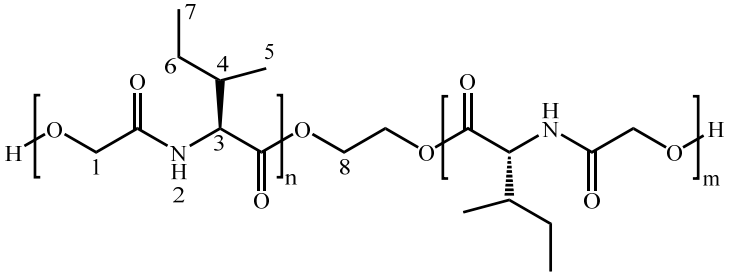

Figure 1. The oligodepsipeptide OBMD.

Pre-Preparatory Procedures

The tubings of the robotic volumetric transfer system were rinsed with dry NMP to ensure the lowest water content possible within the system. Therefore, each of the four syringe pumps was programmed to aspirate 50 mL of dry NMP from the reservoir of the robotic synthesizer, which was afterwards discarded. OBMD was dried in vacuum at elevated temperatures and dissolved in dry NMP by weighing it with mg-accuracy in a cartridge for the GDU resulting in a concentration of c_{OBMD} = 3.38 g·mL^{-1}. The water content of the NMP was determined by KF (< 50 ppm). Freshly distilled TMDI was weighed into a volumetric flask with mg-accuracy and filled up with NMP resulting in a concentration of c_{TMDI} = 39.7 mg·mL^{-1}. Apparatures and TMDI solution were equilibrated to the temperature within the robotic platform. The catalyst solution was prepared by dissolving 130 mg of dibutyltin dilaurate (DBTDL) in 20 mL NMP in a container.

Robotic Applications

Prior performing the robot assisted polyaddition reactions the vial reactors were dried at 180 °C for 24 h. Depending on the dosing unit used in this study the oligomer was transferred as solid or as highly viscous solution to each reactor. Afterwards TMDI and the catalyst solution

(0.1 wt% with respect to oligomer) were transferred to the reactors and the reaction mixture was mixed by vortexing at 80 °C for 24 h under argon atmosphere. The molar ratio of isocyanate to hydroxyl groups (x_{NCO}) was calculated on the basis of c_{TMDI}, the dispensed volume of TMDI solution (V_{TMDI}), the mass of the oligomer (m_{OBMD}), and the molecular weights of oligomer and TMDI ($\overline{M}_{n,OBMD}$, M_{TMDI}) according to Equation 1.

$$x_{NCO} = n_{TMDI} \cdot n_{OBMD}^{-1} = c_{TMDI} \cdot V_{TMDI} \cdot \overline{M}_{n,OBMD} \cdot (m_{OBMD} \cdot M_{TMDI})^{-1} \quad (1)$$

Error Analysis

Data are provided as "values ± error". Errors are estimated as the error of GPC measurements (±10%).

RESULTS AND DISCUSSION

Synthesis and Characterization of OBMD

The dihydroxy oligodepsipeptide was synthesized by coordination-insertion ring-opening polymerization of BMD, which was initiated with ethylene glycol and catalyzed by tin(II) 2-ethylhexanoate. An initiator to monomer ratio of 1 : 28 was used and the molecular weight of the resulting oligomer was analyzed by GPC, NMR, and end group titration of the telechelic hydroxyl groups (Table 1). The number average molecular weight of 5800 g·mol^{-1} has been taken as basis for all following calculations as the results of the hydroxyl titration, NMR, as well as GPC (universally calibrated) were almost identical.

Table1. Determination of the molecular weight of OBMD by different characterization methods.

	$\overline{M}_n$ [g·mol^{-1}]			
	OH number	^{1}H-NMR	GPC[a)]	GPC[b)]
OBMD	5800 ± 400	5700 ± 200	5800 ± 600	6800 ± 700

[a)] determined by universally calibrated GPC (incl. viscosimetry), THF as eluent
[b)] determined by standard calibrated GPC, THF as eluent

Synthesis of Polyurethanes with Gravimetric Dispensing

The polyaddition reaction of OBMD with TMDI was moved to HT synthesis by using a robotic synthesizer equipped with a gravimetric transfer system. The oligomer was transferred to the reactors by means of gravimetric dosing. An optimization of the gravimetric dosing was not required as the exact amount of the OBMD transported was monitored by the HT synthesis software, which adapted all subsequent additions of compounds according to the molar ratio. The diisocyanate was added, whereby x_{NCO} was varied between 95 mol% and 215 mol% as the optimum of this polyaddition reaction was expected to exceed 100 mol% according to the results from other oligodepsipeptides [6]. The polyurethane synthesis was performed at 80 °C and was catalyzed by DBTDL. The resulting polyurethanes were analyzed by HT-GPC measurements in order to determine the molecular weight, whereby the polymers were taken directly from the

reaction solutions without further purification. For this reason, the exact polymer concentration of samples remained unknown excluding the analysis via universal calibration. However, standard calibration was used to determine $\overline{M}_n$, which can be used to compare the efficiency of the polyaddition reaction. Furthermore, a Carothers fit of the polyaddition reaction was performed according to equation 2, 3, and 4 [5] including the apparent number-average molecular weight $\overline{M}_{n,app}$, the apparent average block length $\overline{M}_{blocks}$ (arithmetic mean of molecular weight of OBMD and TMDI), the ratio of reactive groups r, the conversion of reactive groups p, and the deviation x_{shift} of the optimal molar ratio (100 mol%).

$$\overline{M}_n = \overline{M}_{blocks} \cdot (1 + r) \cdot (1 + r - 2 \cdot p \cdot r)^{-1} \tag{2}$$

$$r = x_{NCO} - x_{shift} \qquad \text{for } x_{NCO} \leq 100\% \tag{3}$$

$$r = (x_{NCO} - x_{shift})^{-1} \qquad \text{for } x_{NCO} > 100\% \tag{4}$$

In case of gravimetric dosing by means of SDU the resulting values of $\overline{M}_n$ are presented in Figure 2a exhibiting the successful polyaddition reaction by the increase in molecular weight to about 14000 g·mol^{-1}. The highest molecular weight was achieved at x_{NCO} = 197 mol%. This would correspond to x_{shift} = 97 mol% resulting in p = 39% and an average block length of $\overline{M}_{blocks}$ = 8200 g·mol^{-1} according to the Carothers fit.

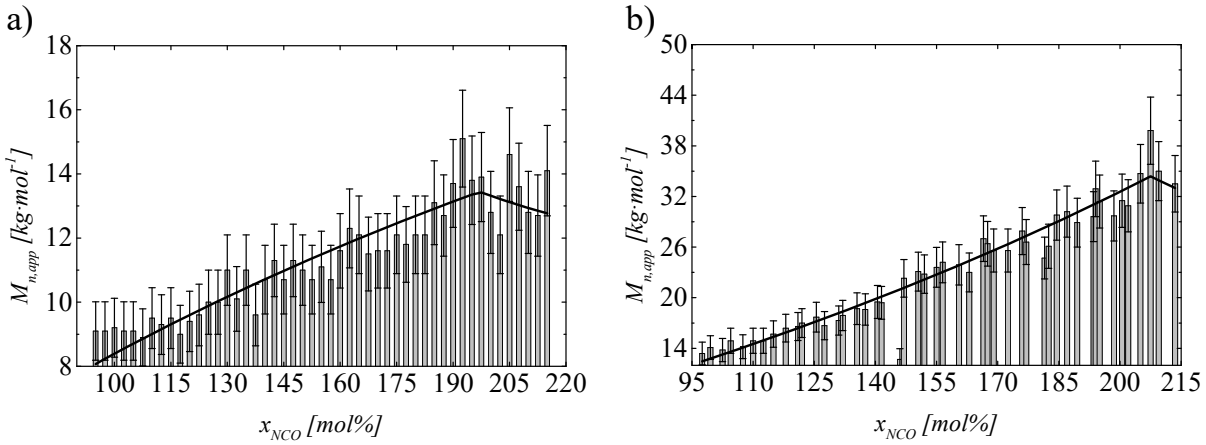

Figure 2. Number average molecular weight (—) as function of the molar ratio of isocyanate groups x_{NCO} for OBMD by means of a) SDU or b) GDU for gravimetric dispensing. Carothers fit (-) of polyaddition. Error bars indicate the error of GPC measurements (±10%).

Figure 2b shows the obtained molecular weights as well as the Carothers fit, which were obtained when the GDU was used for gravimetric dispensing. The successful addition reaction was reflected by the increase in molecular weight to 39000 g·mol^{-1} with an optimum located at x_{NCO} = 207.5 mol% (x_{shift} = 107.5 mol%). According to the calculation based on Carothers equation a conversion of 59% was achieved resulting in $\overline{M}_{blocks}$ = 14100 g·mol^{-1}.

In both cases (SDU and GDU) the detected shift (x_{shift} of around 100 mol%) confirmed the required amount of diisocyanates for the polyaddition of depsipeptides [6]. This deviation

from the theoretical optimum of polyaddition reactions (as described by the Carothers equation) is assumed to originate either from systematic errors for the determination of the molecular weight of the oligodepsipeptides or from a reaction mechanism, which is significantly different from the chain extension of polyesters with diisocyanates [5]. As the molecular weight of OBMD was determined by three different, independent methods (NMR, end group titration, and universally calibrated GPC) with the same result (within margin of error of these methods) it is assumed that such a high deviation from the theoretical ratio of functional groups originating from these measurements is unlikely. For this reason we assume that OBMD building blocks do not only react with their end groups but also with groups along the polymeric chain. However, this reaction only seems to lead to a deactivation of isocyanate groups as crosslinking, which would result in insoluble networks, was not observed. The exact mechanism of isocyanate deactivation, however, remains unknown.

In comparison to the solid dosing unit SDU, a pronounced increase in conversion and molecular weight was obtained for the liquid dosing unit GDU. This effect cannot be simply explained by the different modes of addition – after all about the same optimal molar ratio (around 200 mol%) was found for both dispensing methods. Taking a closer look on the whole processes of dispensing we assume that the water content of the reactions with the SDU was higher compared to the reactions performed with the GDU. This assumption is based on longer dispensing times of the SDU compared to the GDU and the higher surface area of solid oligodepsipeptide with the connected higher water absorption tendency.

CONCLUSIONS

Two different devices for gravimetric dosing were explored to perform a polyaddition reaction on a robotic synthesizer platform. The dihydroxy telechelic oligodepsipeptide OBMD and the diisocyanate TMDI were used as model components. The OBMD was transferred either by a gravimetric dosing unit SDU (for solids) or by GDU (for high viscous solutions). Two series of 49 reactions were performed and the molar ratio between isocyanate and hydroxyl groups was varied between 95 mol% and 215 mol%. Optimum ratios of x_{NCO} = 197 mol% for the SDU and x_{NCO} = 207.5 mol% for the GDU were detected, whereby the polyaddition reaction by a gravimetric transfer with the GDU resulted in higher molecular weights of about 39000 g·mol^{-1} and a high conversion of 59%. Therefore, application of GDU in the robotic synthesizer platform is generally preferable, however, both modes facilitated the determination of the optimal ratio of reactants and confirmed the significant deviation of the chain extension behavior of oligodepsipeptides blocks in the multiblock copolymer synthesis as reported in literature [6]. This deviation is assumed to be caused by an isocyanate deactivation by the oligodepsipeptides. However, the fully automated chain extension procedure was found to be a useful tool to identify reactant ratios in complex polyaddition reactions.

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