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Lipase-Catalyzed Polycondensation in Water: A New Approach for Polyester Synthesis

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Abstract:

The lipase-catalyzed polycondensation of sebacic aid and 1,4-butanediol can be performed in a biphasic reaction system composed of an aqueous reaction phase and an organic extraction phase. The shift of the equilibrium to higher product yields can be realized by the integrated product removal (IPR). In this biphasic reaction system the product yield, molecular weight and polydispersity is depending on the partitioning of the substrates and the product between the phases. By the biphasic approach substrate inhibition as well as molecular weight and polydispersity can be controlled by medium engineering. The polyester concentration can be increased up to 98% by optimising the reaction conditions. The molecular weight is limited in the biphasic system due to its removal while the polydispersity is independent of the reaction conditions but connected to the biphasic approach. Additional to the conversion the partition coefficients have been investigated either experimentally and via calculation by the quantum chemical program COSMO-RS.

1. Introduction

Reaction engineering is a valuable tool to overcome bottlenecks in (bio) chemical reactions. Especially biphasic reaction systems promise to have a great potential to optimise reactions concerning space-time yield and selectivity. In biphasic media the second phase can either be used to increase the substrate concentration in the whole reaction system, or it allows an integrated product removal (IPR). Apart from this, the benefits of biphasic reaction media can help to suppress side and consecutive reactions.

Although biocatalysts are often sensitive against nonconventional reaction conditions such as, for example, organic solvents in their environment, biocatalytic reactions could be successfully carried out in biphasic media. Due to the low contact between the enzyme and the organic phase, the deactivating effect of the organic solvent is minimized. The applications of biphasic system in the field of biocatalysis vary from liquid−liquid over liquid−gas to gas−solid interfaces, which have been reviewed before.[1] Langermann et al. for example described the application of biphasic reaction media for the production of (*S*)-3-phenoxybenzaldehyde cyanohydrin from 3-phenoxybenzaldehyde catalysed by (*S*)-hydroxynitrile lyase from *Manihot esculenta*.[2] In general several advantages for the biphasic approach can be observed:[3−5]

(a) The solubility of hydrophobic compounds in the reaction system can be increased due to the organic phase working as substrate reservoir.
(b) In case of a higher partition coefficient for the products an integrated product removal (IPR) occurs, which potentially leads to higher grades of conversion.
(c) The easy removal of the organic phase enables an easy recycling of the enzyme within the aqueous phase.
(d) The low substrate and product concentrations in the aqueous phase avoid substrate and product inhibitions, which may cause higher residual activity.
(e) Nonenzymatic reactions, which are directly correlated with the substrate concentration within the aqueous phase and leading to nonfavored products, can be suppressed. Due to the partition coefficients the substrate concentration can be kept low.

Another example was reported by Zimmermann et al. for the production of *N*-acetylneuraminic acid via reactive extraction.[6] Furthermore, biphasic media consisting of two aqueous phases were successfully applied for selective protein purification by Dreyer et al.[7] In this work the phase-forming compounds of the ionic liquid-based aqueous two-phase systems (IL-based ATPS) induce a separation of the target protein and the cell fragments as well as a stabilisation of the enzyme.

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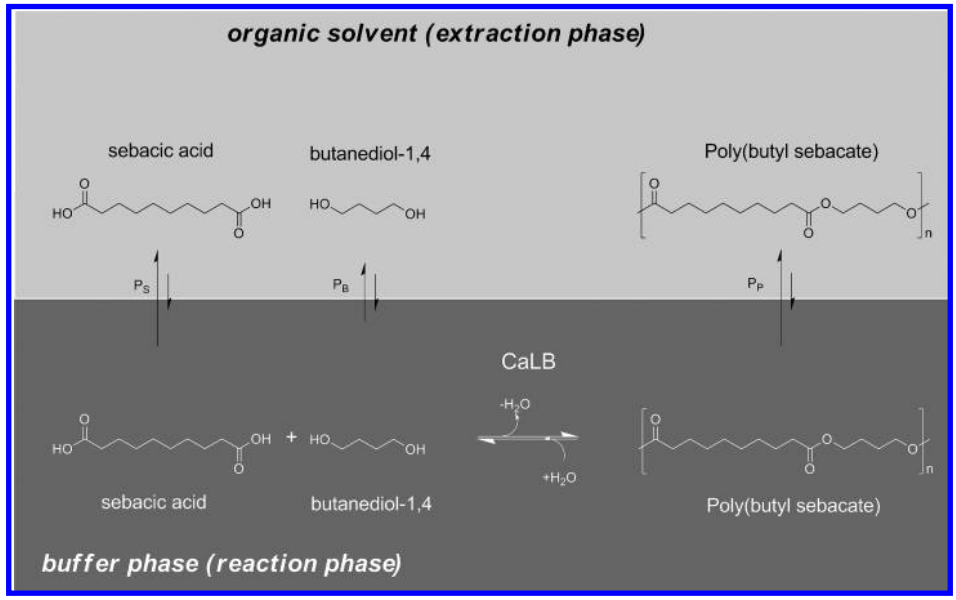
Scheme 1. **Schematic illustration of the polycondensation of sebacic acid and 1,4-butanediol in the biphasic reaction system**



The parameter which is mainly responsible for the efficiency of the biphasic approach is the partitioning coefficient. The distribution of all substances in the system affects the enzyme activity as well as the product concentration.

Due to previous work in the field of biphasic reaction media, we tried to apply a biphasic reaction system for lipase-catalysed polycondensation. Until today the biphasic reaction system has mostly been applied for lipase-catalysed ester hydrolysis. Liese et al. developed a diafiltration reactor for the enantioselective hydrolysis of 2-hydroxy-4-phenylbutyrate catalysed by the lipase from *Pseudomonas cepacia*.[8] Furthermore Salgin and co-workers investigated how the enantioselectivity and activity of *Candida rugosa* lipase (CrL) are influenced in biphasic media.[9] Recently, the kinetic resolution of racemic mixtures catalysed by porcine pancreatic lipase (PPL) was assayed in different neat organic solvents and biphasic systems.[10]

Apart from the hydrolysis, lipase-mediated ester synthesis has been carried out in biphasic media. Chen et al. used soluble lipase NS81020 for the production of fatty acid methyl ethers (FAME) in the biphasic water−oil system. The optimal water content was determined with 10 wt % water by oleic acid weight.[11] A centrifugal contact separator (CCS) has been applied by Kraai et al. for esterification of long chain fatty acid in a heptane−water system.[12] They further investigated the kinetics of the *Rhizomucor miehei*-catalysed esterification of oleic acid and 1-butanol in aqueous−organic biphasic systems. A ping-pong bi-bi mechanism with competitive inhibition of both substrates similar to monophasic systems was observed.[13] Due to the small lid of CAL-B it shows in contrast to the lipase from *R. miehei* no interface activation has to be taken into account.

Apart from the biphasic systems consisting of an aqueous and an organic phase, different biphasic systems have been applied for esterifications and transesterifications. Lozano et al. prepared a kinetic resolution of *rac*-phenylethanol via esterification in ionic liquid/hexane and ionic liquid/supercritical CO_2 biphasic media.[14]

Many reports dealing with polyester production via lipase-catalysed polycondensation have been published in the last decades.[15,16] Lipase-catalysed polyester synthesis can be carried out via polycondensation of diacids or their esters with diols.[17,18] A shift of the thermodynamic equilibrium, necessary for high product concentrations, is generally created by continuous water removal. Various methods of integrated water removal can be found in literature, such as for example azeotrope distillation, pervaporation, or the use of molecular sieves.[19−22] In such monophasic systems partitioning is negligible, whereas in a biphasic system partitioning of all substances influences the yield and polyester chain lengths.[23] If a reaction system with a high partition coefficient for the polyester can be found, a

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product removal will occur which leads to high product concentration without water removal. The idea is to couple an integrated product removal of the polyester to a polycondensation in an aqueous−organic biphasic system.[24] The main advantages of the biphasic reaction systems described above can be transferred to the polycondensation in biphasic media as well.

In this work the benefits of biphasic reaction media for polycondensation of sebacic acid and 1,4-butanediol will be discussed (Scheme 1). Different reaction parameters such as organic solvent and reaction temperature have been varied to investigate their influence on the substrate and product partitioning. Due to the knowledge of the reaction mechanism, which is similar to the mechanism reported by Kraai et al. for the *R. miehei* lipase, the partition coefficients of the substrates should be high to minimise the substrate inhibition. In this report the challenges and applications of a biphasic reaction media for polycondensation reactions are in the centre of interest.[13]

Due to the phase transfer and the defined residence time of the growing chain in the reaction phase, molecular weight distribution is influenced. Low polydispersity indexes (PDIs) are needed for the production of these polyesters if they should be used as additives for high-molecular weight polymers to influence the thermal, chemical, and mechanical properties. The main requirement for these additives is a low PDI and therewith a reproducible product quality.

2. Experimental Section

Materials. Native lipase from *Candida antarctica* type B (CAL-B) (specific activity 6150 U/mL) was provided by Julich Chiral Solution, now part of Codexis, Inc. (Julich, Germany). Novozyme 435 (N435) (specific activity 3 U/mg) was obtained from Novozymes (Bagsvaerd, Denmark) and consists of *Candida antarctica* lipase type B (CAL-B) adsorbed within a macroporous polymethacrylate resin. Sebacic acid and 1,4-butanediol from Fluka (Seelze, Germany) and *tert*-butyl-methyl ether (MTBE), diisopropyl ether (DIPE), *tert*-butyl-ethyl ether (ETBE) from Merck (Darmstadt, Germany) and other organic solvents were of HPLC grade and used without further purification. The solvents for NMR and MS measurements d_1-chloroform from Merck (Darmstadt, Germany) and methanol with 0.1% formic acid from Riedel de Häen (Seelze, Germany) have also been used without further purification.

Analytical Methods. HPLC analyses were performed on HPLC equipment from Knauer (Berlin, Germany) with a K-501 pump, a Knauer Marathon Basic autosampler, and a Knauer K-2301 refractive index detector using an BIORAD Aminex HDR-87 H ion exclusion column with 0.006 mol L^{-1} H_2SO_4 as eluent. For data analysis Eurochrom 2000 software by Knauer was used. The number and weight average molecular weights (M_n and M_w, respectively) of the polyesters were measured by gel permeation chromatography (GPC) using a Knauer HPLC system with a model K-1001 pump and a light-scattering detector from Polymer Laboratories (PL-ELS 2100) (Darmstadt, Germany). The separation by molecular weight was performed with a ResiPore-column (300 mm × 7.5 mm) from Polymer Laboratories (Darmstadt, Germany) in a range of 400,000 g mol^{-1} down to 300 g mol^{-1}. The system was calibrated with polystyrol standards in a range of 377,400 g mol^{-1} to 580 g mol^{-1}. THF was used as mobile phase with a flow rate of 0.6 mL min^{-1}. Software Eurochrom 2000 was used, and for calculation of the molecular weight, special analysis software of Polymer Laboratories, the Cirrus-program, was used.

General Procedure for CAL-B-Catalysed Polycondensation of Sebacic Acid and 1,4-Butanediol. Sebacic acid (101.1 mg, 0.2 mol L^{-1}) and 1,4-butanediol (90.1 mg, 0.4 mol L^{-1}) were diluted in 2.5 mL of organic solvent. Afterwards 2.5 mL of the buffer solution (citric acid−Na_2HPO_4, 0.2 mol L^{-1}, pH 6.0) was added to the reaction mixture (the phase ratio α describes the volume of the organic solvent referring to the volume of the buffer phase; $\alpha = 1$). The reaction mixture was incubated at a temperature between 30 and 60 °C for 30 min. Ten percent (w/w) N435 (relatively to the weight of the monomers) or equal amount of the native CAL-B (with respect to the specific activity of the immobilized enzyme) was added to start the polycondensation. The reaction system was mixed by a vertical shaker, and periodically samples were taken from the organic phase of the reaction mixture, extracted with water, and analyzed by HPLC to determine the decreasing concentration of the monomers. After 48 h the reaction was stopped by removing the organic phase. The organic solvent was dried with Na_2SO_4 and evaporated in an argon flow at room temperature. Samples were rediluted in THF for GPC analysis. For the monophasic system 101.1 mg (0.2 mol L^{-1}) of sebacic acid and 90.1 mg (0.4 mol L^{-1}) of 1,4-butanediol were diluted in 5 mL of organic solvent. *In situ* water removal was ensured by the addition of molecular sieves.

General Procedure for Partition Coefficients of Substrates. The corresponding amount of diacid or diol was diluted with 2.5 mL of the organic solvent resulting in a concentration of 0.2 mol L^{-1} of the diacid or 0.4 mol L^{-1} of the diol. Afterwards 2.5 mL of the buffer solution (citric acid−Na_2HPO_4, 0.2 mol L^{-1}, pH 6.0) was added to the mixture (the phase ratio α describes the volume of the organic solvent referring to the volume of the buffer phase; $\alpha = 1$). Samples at $t_0 = 0$ have been taken to determine the concentration in the phases at this time. The reaction mixture was incubated and well mixed at a temperature between 30 and 60 °C for 30 min. By storing the mixtures at desired temperature in a water bath over 3 days, the phase equilibria were ensured to be reached. After this time samples of the mixtures have been taken and analysed via HPLC.

General Procedure for Partition Coefficients of Products. The corresponding amount of diacid or diol was diluted with 125 mL of MTBE, resulting in a concentration of 0.2 mol L^{-1} of the diacid or 0.4 mol L^{-1} of the diol. Afterwards 125 mL of the buffer solution (citric acid−Na_2HPO_4, 0.2 mol L^{-1}, pH 6.0) was added to the mixture ($\alpha = 1$). The reaction mixture was incubated at a temperature between 30 and 60 °C for 30 min. Ten percent (w/w) N435 was added to start the polycondensation. The reaction system was mixed by a vertical shaker, and periodically samples were taken from the organic phase of the reaction mixture, extracted with water, and analyzed by HPLC to determine the decreasing concentration of the mono-

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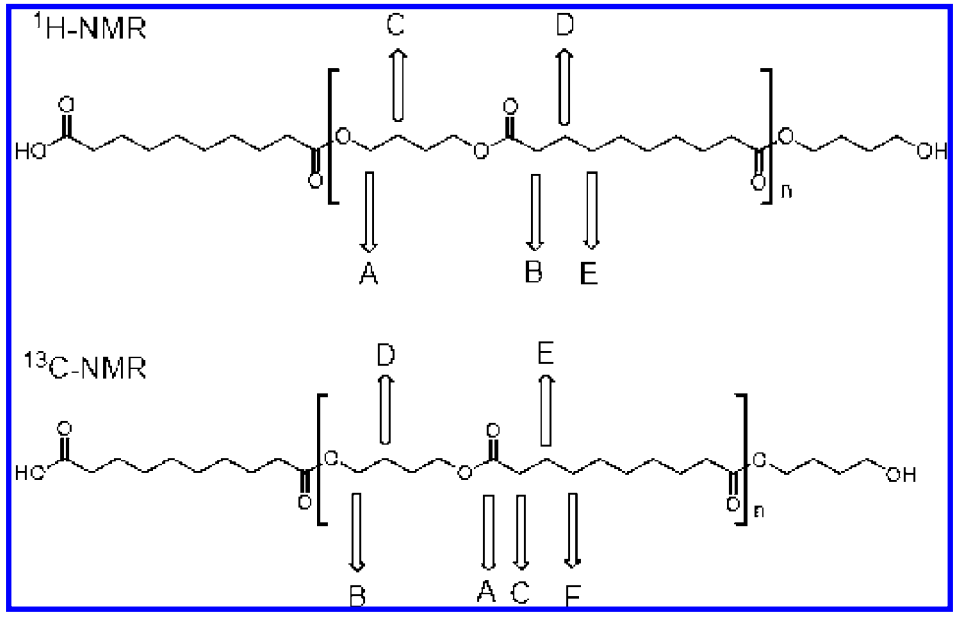
Scheme 2. **Structure determined via ^{1}H and ^{13}C NMR spectroscopy**



mers. After 48 h the reaction was stopped by removing the organic phase. The organic solvent was dried with Na_2SO_4 and evaporated in an argon flow at room temperature. Samples were rediluted in THF for GPC analysis. (Isolated yield: 97%, molecular weight: $M_n = 1960$ g mol^{-1}, $M_w = 2000$ g mol^{-1}, PDI = 1.02.) For other solvents the procedure was repeated accordingly.

COSMO Calculations. The theoretical calculations of liquid−liquid equilibrium and partition coefficients have been performed using SVP and TZVP data basis in COSMOtherm-C21-0107. The Cosmo-files have been prepared by COSMO*logic* GmbH & Co. KG (Leverkusen, Germany). $Log_{10}P$ calculations were performed at different temperatures (30−60 °C) and with two different solvents, *n*-heptane and MTBE.

General Procedure of NMR Measurements. Samples of poly(butyl sebacate) for NMR measurement have been diluted in 0.5 mL d_1-chloroform. The ^{1}H- and ^{13}C spectra were recorded using an instrument AC 250 F Aspect 3000 data System from Bruker (Karlsruhe, Germany) with 250 MHz. Furthermore an NMR-instrument from Bruker ARX 300 UNIX data system 300 MHz was used. The data were analysed via the software Mestrec.

Results for poly(butyl sebacate): ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.25−1.35 (m, 8H, E); 1.53−1.65 (m, 4H, D); 1.65−1.75 (m, 4H, C); 2.22−2.35 (m, 4H, B); 4.02−4.15 (m, 4H, A). ^{13}C (75 MHz, $CDCl_3$): δ [ppm] = 24.9 (8C, F); 29.0 (4C, E); 29.1 (4C, D); 34.2 (4C, C); 63.7 (4C, B); 173.8 (2C, A). See Scheme 2 for the structure as determined by NMR spectroscopy.

General Procedure of MS Measurements. Samples for mass spectrometry were diluted in 1.0 mL of methanol with 0.1% formic acid. The measurement was facilitated by adding the sample via direct injection with a syringe pump at a rate of 5 μL min^{-1} into the MS instruments, LCQ Advantage and LTQ, from Thermo Finnigan. The ionisation of the samples was performed by electrospray ionisation (ESI-mode 4.5 kV, positive full MS) in a mass range between 150 and 2000 amu (temperature of the transfer capillary: 270 °C, sheat gas (N_2): 10, sweep gas (He): 5). Additional measurements up to 4000 amu gave no further information. For data acquisition and analysis the software Xcalibur from Thermo Finnigan (Dreieich, Germany) has been used.

3. Results

In order to estimate whether a biphasic system is suitable for polycondensation reactions, we compared the polycondensation of sebacic acid and 1,4-butanediol under monophasic and biphasic reaction conditions.

In this case the monophasic system represents the common technique which uses the *in situ* removal of water for example by molecular sieves. The biphasic approach removes the polyester from the reaction mixture to achieve higher product concentrations. The organic phase extracts the polyester continuously from the aqueous reaction phase. As described above, the efficiency of esterifications, especially of polycondensations, is related to an efficient product removal to obtain high conversion. Indeed, thermodynamic data indicate that the polycondensation can effectively be performed due to the high equilibrium constant of the polycondensation estimated from thermodynamic calculations (see below).[25] Therefore, the driving force for the polyester to pass into the organic phase has to be taken into account if the biphasic system is under investigation. This means partition coefficients of the polyesters as well as of the monomers have a great impact on product concentration and molecular weight.

First we describe the reaction of sebacic acid and 1,4-butanediol in mono- and biphasic systems to evaluate the feasibility of the biphasic system (Scheme 1). Second, benefits and limitations of the biphasic approach for the condensation of acids and diols will be discussed. Finally the influence of partition coefficients is discussed in more detail. For this quantum chemical calculations via COSMO-RS were used. The experimental results will be compared to the calculated ones to classify the usability of COSMO-RS for such applications.

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Table 1. **Comparison of the monophasic (with water removal) and biphasic reaction media used for polycondensation of sebacic acid and 1,4-butanediol[a]**

system	product concentration/%	M_n/g mol^{-1}	M_w/g mol^{-1}	PDI
monophasic				
"bulk" (diol in excess)	84 ± 3	1420 ± 30	2230 ± 40	1.6
diisopropyl ether (DIPE)	54 ± 2	1330 ± 20	1830 ± 20	1.4
biphasic				
diisopropyl ether (DIPE)/Puffer	96 ± 4	1840 ± 40	1890 ± 30	1.03
tert-butyl methyl ether (MTBE)/Puffer	86 ± 3	2470 ± 50	2520 ± 60	1.02

[a] Reagents and conditions: 0.2 mol L^{-1} sebacic acid, 0.4 mol L^{-1} 1,4-butanediol, total volume 5 mL, $\alpha = 1$, organic solvent: 1,4-butanediol, DIPE or MTBE, buffer: citric acid−Na_2HPO_4 (0.2 mol/L, pH 6.0), 10% (w/w) N435, 50 °C, 990 rpm, 48 h, standard deviation (product concentration) < 5%, standard deviation (M_n; M_w) < 60 g/mol, standard deviation (PDI) < 0.01).

Comparison of the Monophasic and Biphasic Reaction System. Sebacic acid and 1,4-butanediol can be polymerised via polycondensation catalysed by the lipase B from *Candida antarctica*. The yield of this polycondensation is depending on the shift of the equilibrium position. An effective product removal is necessary to obtain high product concentration. In a previous work Gibbs energy and equilibrium constant have been investigated.[25] The values estimated from this theoretical study are $\Delta G_{pol} = -91.3$ kJ mol^{-1} and $K_{pol} = 9.9 \cdot 10^{15}$. As these values are quite high and would as a result predict, that complete conversion without any attempts to shift the equilibrium to the product site, further experimental verification is under way.

Monophasic reaction systems, consisting of pure organic solvent, have to be coupled to an effective water removal. This provides a high molecular weight of the product because chain growth is not limited in these systems. As shown by Mahapatro et al. molecular weights of more than 40,000 g mol^{-1} can be obtained after 3 days of reaction time at 70 °C.[26] Contrary to this, in the biphasic systems the yield as well as the molecular weight is connected to the residence time of the growing chain in the surrounding of the lipase. In experiments high product concentrations could be obtained for the polyester via the biphasic approach (Table 1).

Compared to the monophasic system using the same organic solvent diisopropyl ether, the polyester concentration is more than 40% higher in the biphasic system. In the monophasic "bulk" system, meaning 1,4-butanediol is used as solvent, product yields of more than 80% based on the acid are accessible. Although high product concentration can be achieved, bulk system is not favored because high diol concentrations lead to lipase inhibition. Romero and co-workers found out that the alcoholic components as well as short-chain acids inhibit CAL-B depending on the concentration.[27] Similar results were published by Kraai et al. for another lipase following the reaction mechanism the same as that of CAL-B.[13]

In monophasic systems we obtained molecular weights between 1800 and 2200 g mol^{-1}. In contrast to the results of Mahapatro et al., who were able to create molecular weights of more than 40,000 g mol^{-1}, we obtained lower molecular weights. This discrepancy between our results and the data from the literature can be ascribed to the reaction conditions. First of all the reaction temperature in our experiments is lower than in experiments of Mahapatro and co-workers. Apart from this diisopropyl ether seems not to be the solvent of choice for the monophasic approach. Due to the fact that the monophasic system is not operated under optimum conditions in this case, the yield and the molecular weight are lower. Nevertheless, it can be derived from the data presented in Table 1 that the biphasic system could successfully be applied for the polycondensation of sebacic acid and 1,4-butanediol.

An important benefit of the biphasic reaction system is the very low polydispersity of the product. In the monophasic systems a polydispersity up to 2, depending on the solvent and reaction time, has to be accepted. In contrast to this the biphasic system creates polyesters with a polydispersity index (PDI) of only 1.2. We suppose this is due to the defined residence time in the aqueous phase surrounding the biocatalyst.

By estimating the polyester structure via NMR and MS, we found a linear structure without ring formation (Schemes S1 and S2 of Supporting Information). Our measurements indicate a highly symmetric polymer which correspond to data published earlier. Witt et al. published similar results for the polyester formed from 1,3-propanediol and sebacic acid.[28]

A further advantage of the biphasic reaction media is the possibility that the native CAL-B can be applied in these systems. By using the native instead of the immobilised lipase the reaction rate is increased. Compared to the immobilized lipase which shows a reaction rate of 0.082 mol h^{-1} the native enzyme converts sebacic acid and 1,4-butanediol with a rate of 0.094 mol h^{-1}. Hence, an increase of reaction rate and higher space-time yields and process efficency could be realized. This also confirms that the reaction indeed is catalysed by the lipase and not by the support of the Novozyme 435. As reported by Hollmann et al. monomers, such as tartaric acid and glycerol, are also converted in experiments with the enzyme-free carrier material of Novozyme 435.[29]

Influence of Extraction Phase on Polycondensation. The partitioning of all compounds between the extraction and reaction phase is of major importance for the polycondensation. On the one hand the monomer concentration in the water phase has to be high enough to eliminate mass transfer limitations. On the other hand high concentrations of diol and diacid in the surroundings of the lipase lead to inhibition. Romero et al. found out that especially diols in high concentrations inhibit the CAL-

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Table 2. **Polyester solubility in different organic solvents at 30 °C[a]**

extraction phase	product concentration/%	M_w/g mol^{-1}	polyester solubility/g L^{-1}
chloroform	87	1150	140
MTBE	98	1890	110
DIPE	87	2520	105
p-xylene	74	3930	100
ETBE	68	4340	100
n-hexane	66	2260	45

[a] Reagents and conditions: 0.2 mol sebacic acid, 0.4 mol 1,4-butanediol, total volume 5 mL, α = 1 or 2, organic solvent: MTBE, buffer: citric acid−Na_2HPO_4 (0.2 mol L^{-1}, pH 6.0), 10% (w/w) N435, 50 °C, 990 rpm, 48 h, standard deviation (product concentration) < 2%, (M_w) < 40 g mol^{-1}, polyester solubility: poly(butyl sebacate) was synthesized via the biphasic approach in MTBE/ buffer at 60°C, product concentration: 97%, molecular weight: M_n = 1960 g mol^{-1}, M_w = 2000 g mol^{-1}, PDI = 1.02.

B.[27] Kraai et al. determined a decrease in initial reaction rate if the 1-butanol concentrations is increased above 0.1 mol L^{-1} in *n*-hexane.[13] Therewith, the monomer concentration in the aqueous reaction phase has to be limited to exclude substrate inhibition.[30] Furthermore, the phase transfer of the polyester has to be as effective as possible to obtain high product yield, whereas this rapid transfer might lead to low molecular weight due to the low residence time in the lipase environment.

To evaluate how the substrate and product partitionings influence the molecular weight and the yield of the polyester, different organic solvents have been applied for the biphasic approach. Table 2 indicates the great impact of the solvent choice on the molecular weight and the polyester concentration. The product concentration can be correlated to the polyester solubility in the organic phase. For example, chloroform shows a high solubility for the poly(butyl sebacate). This results in a high yield for the condensation, because of a high driving force for the polyester to pass into the organic phase. Apart from the product concentration, the molecular weight of the product is also important. Therefore, a high driving force such as that in chloroform is not favorable because it creates short chain lengths. In biphasic systems with an ether extraction phase the driving force of the phase transfer is lower due to the lower polyester solubility (Table 2). Hence, the molecular weight is higher, whereas the product concentration is still high (MTBE: 98% product concentration and 2000 g mol^{-1}). A negative effect of the lower driving force is a lower reaction rate. Because of this a compromise has to be found among product concentration, molecular weight, and reaction rate.

Nevertheless, molecular weight is limited in biphasic systems depending on the extractive efficiency of the organic solvent. The main advantage of the biphasic reaction media is not the production of high molecular weight polyesters but the synthesis of polyesters with defined molecular weight. Due to the phase transfer and the defined residence time of the growing chain in the reaction phase, molecular weight distribution is narrow. PDIs between 1.02 and 1.05 ± 0.007 could be detected. With this narrow molecular weight distribution the production of polyesters with defined molecular weight can be achieved. Such short-chain polyesters can be used as additives for high molecular weight polymers to influence the thermal, chemical, and mechanical properties. The main requirement for these additives is a low PDI and therewith a reproducible product quality. Polyesters from the classical monophasic system mostly range between 1.5 and 1.8. Mahapatro et al. reported a polydispersity of PDI between 1.5 and 2.0 for the polycondensation of adipic acid with different aliphatic diols in diphenylether at 70 °C.[26,31] In contrast Sahoo et al. determined polydispersities between 1.01 and 1.17 for the polycondensation of sebacic acid with poly(ethylene glycol).[32] In addition, the biphasic system simplifies product isolation.

Influence of Temperature on Polycondensation. Partition coefficients (*P*) are influenced by the reaction temperature. With increasing temperature, the monomer concentrations in the reaction phase change. Partition coefficients were measured according to eq 1: $P_{\text{sebacicacid}}$ of sebacic acid increases from 71 to 103, and $P_{\text{butanediol-1,4}}$ increases from 0.8 to 1.1.

$$P_{\text{substance}} = \frac{C^{\text{org}}_{\text{substance}}}{C^{\text{aq}}_{\text{substance}}} \quad (1)$$

As a result the lipase activity is affected due to the inhibiting effect of 1,4-butanediol. Anderson et al. mentioned that the CAL-B shows no inhibiting effect of long-chain acids with corresponds to our data.[33] Small alcohols and diols, such as 1,4-butanediol, are known to cause a loss of lipase activity. As reported by Swarts et al. the esterification of propionic acid and 1-butanol follows the ping-pong bi-bi mechanism with alcohol inhibition.[34] Partition coefficients of 1,4-butanediol indicate a lower concentration of butanediol in the aqueous phase at 60 °C than at 30 °C which should result in a lower inhibition.

Apart from the diol partitioning the temperature itself affects the lipase. An increase of reaction temperature increases the reaction rate, whereas over a certain enzyme-dependent temperature unfolding and denaturation take place.

The effect of temperature shown in Figure 1 is a complex phenomenon which is composed of all effects previously discussed. At low lipase concentrations of 5%(w/w) Novozyme 435 the deactivating effect of protein leaching from the solid support (data not shown) and thermal unfolding is detectable. By adding the Novozyme 435 in portions over the reaction time (dark bar), thermal deactivation can be minimized and therewith higher product concentrations are accessible. In the case of higher lipase concentrations (≥10%(w/w) Novozyme 435) the thermal deactivation is not obvious because active CAL-B is present until the equilibrium position is reached. Suggesting that in the systems with more than 10%(w/w) Novozyme 435 have reached the equilibrium position, portion wise addition of the catalyst should not have any effect on the polyester product concentration. Further experiments did not show any increase in product concentration when adding the 10%(w/w) Novozyme 435 in portions to the reaction mixture. These results

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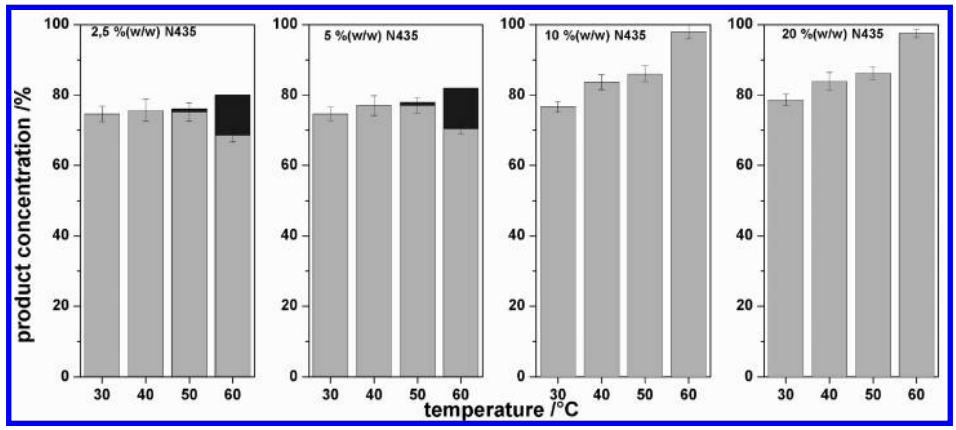

Figure 1. **Influence of temperature on the product concentration (grey bars: enzyme amount added at once; dark grey bars: enzyme amount added in 3 to 4 portions over the reaction time; conditions: 0.2 mol L^{-1} sebacic acid, 0.4 mol L^{-1} 1,4-butanediol, total volume 5 mL, $\alpha = 1$, organic solvent: MTBE, buffer: citric acid−Na_2HPO_4(0.2 mol L^{-1}, pH 6.0), 10% (w/w) N435, 50 °C, 990 rpm, 48 h, standard deviation (product concentration) ≤ 2%).**

Table 3. **Influence of the reaction temperature and the lipase content on the molecular weight of the product[a]**

temperature	M_w (5% (w/w))/g mol^{-1}	M_w (10% (w/w))/g mol^{-1}
30	960	1140
40	1380	2320
50	1790	2700
60	1860	1890

[a] Reagents and conditions: 0.2 mol L^{-1} sebacic acid, 0.4 mol L^{-1} 1,4-butanediol, total volume 5 mL, $\alpha = 1$, organic solvent: MTBE, buffer: citric acid−Na_2HPO_4 (0.2 mol L^{-1}, pH 6.0), 10% (w/w) N435, 50 °C, 990 rpm, 48 h, standard deviation (M_w) < 40 g mol^{-1}.

have proven our suggestion that polycondensation yield is not influenced by the general enzyme concentration but by the active lipase content. Therewith a thermal deactivation of the immobilized lipase B from *C. antarctica* has been detected in biphasic media. This deactivation process consists of two parts. First the lipase is detached from the macroporous resin, and second a deactivation by both the monomers and the organic solvent takes place.

Hollmann et al. reported that polymerisation can be catalysed by the lipases support.[29] In our experiments concerning the polycondensation of sebacic acid and 1,4-butanediol we excluded a polymerisation catalysed by the support material. We could exclude this in our experiments as we observed polyester concentrations 5−10% higher in the case of the native enzyme when compared to the supported enzyme.

According to the molecular weight of the produced polyester a temperature effect is detected (Table 3). With increasing reaction temperature from 30 to 60 °C the molecular weight increases up to 2800 g mol^{-1} at 50 °C. Due to the increased lipase activity the chain growth at the same time is higher than that at lower temperatures, meaning a higher molecular weight of the polyester can be obtained in a shorter time. At temperatures above 50 °C the driving force for the polyester to pass into the extraction phase is increased which causes a lower residence time in the surrounding of the CAL-B. The reason for this is a displacement of the liquid−liquid equilibrium (Schemes S3 and S4 of Supporting Information).

Influence of pH on the Polycondensation. The polycondensation of sebacic acid and 1,4-butanediol in biphasic systems depends on a range of parameters. Due to the effect of pH on both the lipase activity and the phase partitioning of all compounds, we investigated in what way the product concentration and the molecular weight depend on the pH of the reaction phase.

Although the activity measurement indicated a high dependence on pH, no effect on product concentration and molecular weight could be detected. The product concentration in experiments with pHs that varied between 3 and 9 ranged between 65.8 ± 3.4 g L^{-1} (pH = 2) and 82.1 ± 4.5 g L^{-1} (pH = 6). A limitation due to a saturation of the organic phase with the polyester is responsible for a lower product concentration at neutral pH. The concentration of the polyester in the organic phase approaches 105 g L^{-1} in MTBE and 95 g L^{-1} in DIPE the saturation concentration (Table 2). Hence, it should be possible to obtain higher product concentrations by increasing the phase ratio α ($\alpha = c_{org\ phase}/c_{aq.\ phase}$). Therewith a higher capacity of the organic phase leads to a higher driving force of polyester phase transfer due to a larger difference of its chemical potential in the two phases. The data summarised in Table 4 indicate the phase saturation as the limiting factor. By increasing

Table 4. **Influence of the pH and phase ratio on the product concentration and molecular weight of the product[a]**

pH (aqueous phase)	product concentration/% ($\alpha = 1$)	M_w/% ($\alpha = 1$)	PDI ($\alpha = 1$)	product concentration/% ($\alpha = 2$)	M_w/% ($\alpha = 2$)	PDI ($\alpha = 2$)
5	75	3900	1.02	78	3900	1.03
6	82	4030	1.03	92	4040	1.02
7	74	4090	1.03	89	4070	1.03
8	78	3470	1.04	87	3500	1.04
9	77	3,900	1.06	80	3700	1.05

[a] Reagents and conditions: 0.2 mol L^{-1} sebacic acid, 0.4 mol L^{-1} 1,4-butanediol, total volume 5 mL, $\alpha = 1$ or 2, organic solvent: MTBE, 10% (w/w) N435, 50 °C, 990 rpm, 48 h, standard deviation (product concentration) < 3%, (M_w) < 40 g mol^{-1}, (PDI) < 0.006.

the capacity of the organic phase ($\alpha = 2$) we were able to raise the product concentration at neutral pH from 74% up to 89% (pH = 7), meaning an increase of 15%. In contrast to the product concentration, both molecular weight and PDI are not affected by the increase of the phase ratio. Due to the setup of the reaction system the residence time of the growing chain is limited and not connected to the phase ratio. Therefore, the molecular weight and the PDI, depending on residence time of the chain in the aqueous phase, are only influenced by the choice of organic solvent.

Influence of Monomer Structure on Polycondensation. To prove the usefulness of the biphasic reaction system a set of aliphatic monomers has been tested. We found that with increasing molecular weight of the monomers in the range of C_2 to C_{10} the product concentration and the molecular weight are influenced. Long-chain monomers have a higher partition coefficient and therewith a higher driving force to pass into the organic phase. Due to this the transport of the polyester out of the reaction phase is fast, and an efficient shift to high product concentrations occurs. In addition, kinetic parameters might also influence product concentration and molecular weight. Since lipases are reported to favorably accept long-chain substrates similar to their natural substrates, this also contributes to the higher yield.[33,35,36] In addition to this the inhibiting effect of the alcohols as well as of the acid has to be taken into account. With increasing chain length of the monomers, the solubility in the reaction phase ($P_{momomer}$ increases) decreases and causes therewith a lower inhibiting effect. This results in a higher reaction velocity and higher product concentrations (Figure 2A).[18,23,30,37] Yadav et al. studied the dependence of CAL-B inhibition on alcohol chain length. They found a proportional correlation of increasing chain length and cumulative inhibition.[38]

Contrary to the effect on the polyester yield the molecular weight is not influenced by the monomer chain length. Figure 2B shows a slight increase of the M_w with the monomer chain length which can be transferred to the increasing molecular weight of the repeating unit ($M_{RUofpoly(propyloxalate)} = 130$ g mol^{-1}, $M_{RUofpoly(decanylsebacate)} = 340$ g mol^{-1}).

Influence of Partition Coefficients on Polycondensation. As pointed out in the previous sections, the partitioning of all substances between the reaction and extraction phases is of fundamental importance to define the optimum reaction conditions. Due to the high experimental effort, a reduction of necessary experiments is desirable. For this a quantum chemical calculation program can be used for calculating the partition coefficients. This can help to estimate whether a biphasic system is a feasible reaction system for a given reaction.

We compared our experimental data to the partition coefficients calculated via COSMO-RS, the conductor-like screening model for real solvents. COSMO is based on the classical dielectric theory and approximates solvents to the most important feature, i.e. their electrostatic interaction with the solute.[39] In contrast to the group contribution methods, the molecules are not divided into fragments, losing any information about intramolecular interactions. COSMO-RS is a theory which describes the interactions in a fluid as well as local contact interactions of molecular surfaces, and the interaction energies are quantified by the values of the two screening charge densities σ and σ' which form a molecular contact. The most important contributions to the interaction energy functional are the electrostatic misfit energy and hydrogen bonding.[40] To calculate partition coefficients of benzothiophene (BT) and benzothiophene 1,1-dioxide (BTDO) in the octane/acetonitrile system Shimoyama et al. used the COSMO (conductor-like screening model)-based activity coefficient models COSMO-SAC (segment activity coefficient) and COSMO-UNIQUAC.[41] It can be used for the prediction of blood−brain partitioning (log BB) and human serum albumin binding (log *K*(HSA)) of neutral

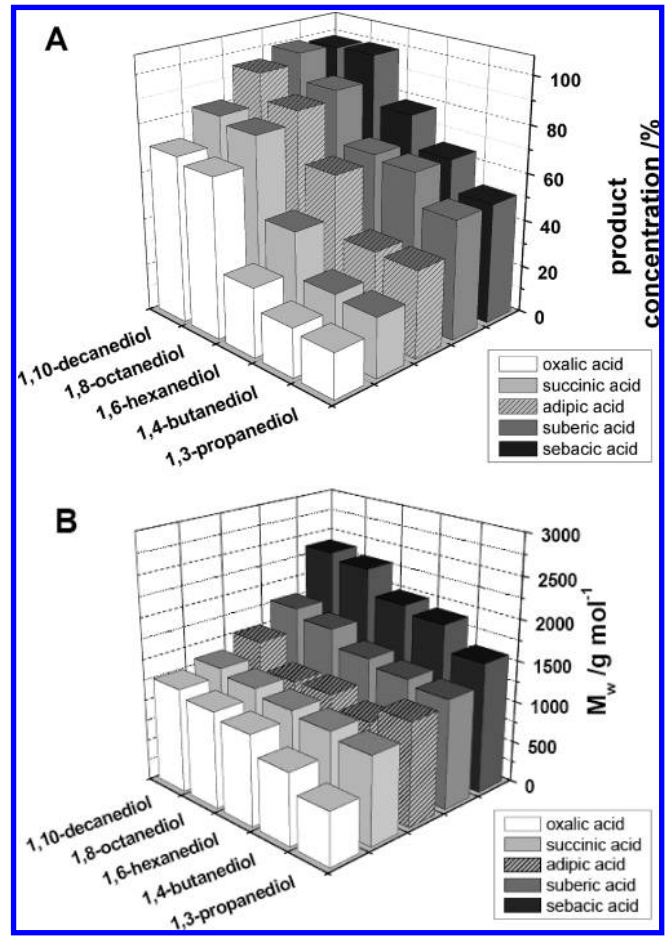

Figure 2. **Influence of monomer chain length on the product concentration (A) and molecular weight (B) (conditions: 0.2 mol L^{-1} diacid, 0.4 mol L^{-1} diol, total volume 5 mL, $\alpha = 1$, organic solvent: MTBE, buffer: citric acid−Na_2HPO_4 (0.2 mol L^{-1}, pH 6.0), 10%(w/w) N435, 50 °C, 990 rpm, 48 h, standard deviation (product concentration) ≤ 1.8%, standard deviation (M_w) < 40 g mol^{-1}).**

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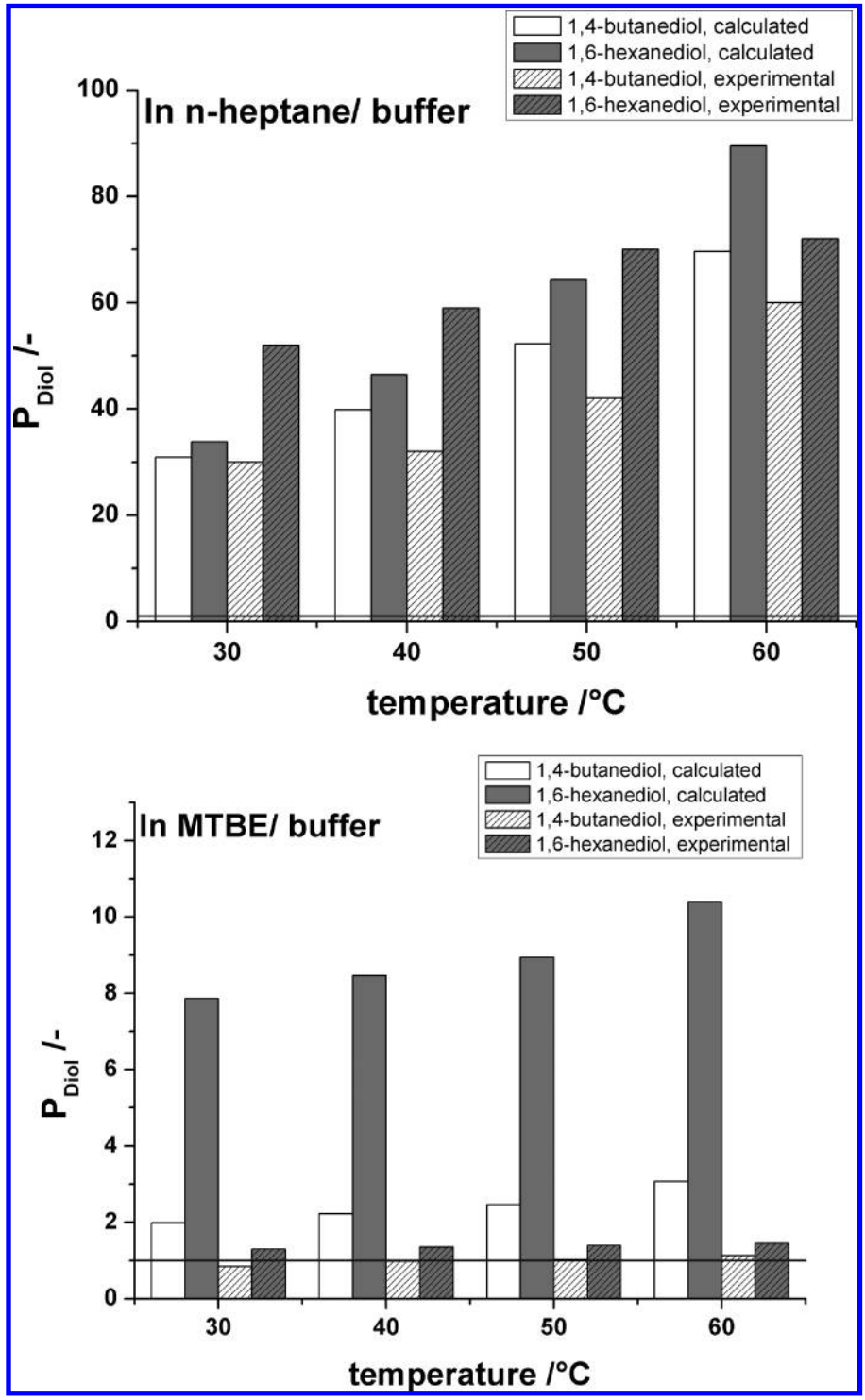

Figure 3. **Calculated and experimentally determined partition coefficients of 1,4-butanediol and 1,6-hexanediol in *n*-heptane (a) and MTBE (b) at 50 °C.**

molecules.[42] Apart from COSMO-RS other quantum chemical programms can be applied to calculate partition coefficients, such as the classical group contribution method, UNIQUAC. The determination of which method is suitable for a special problem cannot be discussed in general but has to be investigated for the regarded system. In the case of partition coefficients and their influence on polycondensation we used the COSMO-RS method for calculations because of the importance of the interaction between substrate, product, and solvent. In order to take into account these interactions a conductor-like screening model is most suitable for the determination of partition coefficients.

The calculated partition coefficients of 1,4-butanediol in *n*-heptane and MTBE show similar tendencies and agree within acceptable ranges (Figure 3). The differences between calculation and experimental data in MTBE are higher than in

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Table 5. **Calculated and experimentally determined partition coefficients of diacids and diesters in MTBE at 50°C**

substance	P_X (experimental)	P_X (calculated)
adipic acid	2.6	6.34
sebacic acid	99	243
adipic acid dihexyl ester	>10,000	290,000
sebacic acid dibutyl ester	>10,000	275,000

n-heptane which is caused by either the experimental setup or some simplifications during the calculation process.[43] Nevertheless, the correlation between the partition coefficient of the diol and reaction parameters such as organic solvent and temperature can be identified by this approach. To estimate if the correlation between the calculated and experimentally determined partition coefficients is adequate, a discussion on the application of the data has to be performed. Due to the use of the calculated data to reduce the number of experiments, a lower correlation is acceptable which is caused by some of the simplifications during the calculation process. For the given problem the accuracy of the calculation is high enough due to the fact that the deviations are not caused only by the calculation method. An important simplification is the endless dilution, applied for the calculation, which cannot be realized in the experimental setup. To overcome this a process simulator based on the activity coefficients could be applied such as aspenPlus or PRO/II. Nevertheless, the results of the COSMO-RS calculations allow us to estimate the affinity of the substrates to the organic or aqueous phase, respectively. As a result, mass transfer limitations and the degree of inhibition induced by high diol concentrations in the reaction phase can be estimated by COSMO-RS.

Similar results were established for diacids and their diesters. The experimental results confirm the tendencies which are determined via COSMO-RS calculations. Adipic acid has a partition coefficient into the organic phase lower than that of sebacic acid, and therefore, the inhibition caused by adipic acid is higher than it is with sebacic acid (Table 5). This corresponds well with our observations, that CAL-B is inhibited when in contact with short-chain diacids. Although from the high $P_{\text{sebacicacid}}$ of 99 a mass transfer limitation can be supposed, no evidence of this was found in our investigations. Due to the low reaction rate of the polycondensation and the fast phase transfer because of the high surface area between the phases, mass transfer is not limiting the reaction. Furthermore, partition coefficients of substances can be calculated that are not accessible by experiment. In the case of adipic acid dihexyl ester or sebacic acid dibutyl ester the partition coefficients are extremely high. Due to the experimental setup the concentration of the esters in the aqueous phase are below the detection limit of the HPLC, and therefore the experimental partition coefficient can only be stated as greater than 10,000. By using COSMO-RS the partitioning can be specified. Out of the COSMO-RS data the adipic acid dihexyl ester shows a higher driving force to pass into the organic phase than that of the sebacic acid dibutyl ester.

In general it can be concluded that COSMO-RS is a suitable tool to estimate the partitioning of a certain substance or monomer in a biphasic reaction media. This method can be applied to pre-estimate the substrate and product inhibitions and mass transfer limitations. It can be used to reduce the expenditure of experimental time and therewith process development costs.

Conclusions

The polycondensation of sebacic acid and 1,4-butandiol could be successfully performed in biphasic reaction media consisting of an aqueous reaction phase and an organic extraction phase. By the variation of different reaction parameters we were able to increase the polyester yield up to 98% and the molecular weight up to 4,000 g mol^{-1}.

The polyester is obtained with a very narrow molecular weight distribution (PDI between 1.02−1.05). Such products are of interest as additives. In total 10 g of the poly(butyl sebacate) was synthesized to prove the efficiency of the process. The influence of substrate and product partitioning on the polyester yield and the molecular weight was investigated and calculated. COSMO-RS was applied as a quantum chemical program to calculate the partition coefficients of the monomers; the tendencies of the calculated partition coefficients veer towards the experimental data and can indicate the efficiency of phase transfer.

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Supporting Information Available

Data of the NMR and MS (Figures S1 and S2) and results from LLE calculations for *n*-heptane/water and MTBE/water (Tables S3 and S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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