UV-induced crosslinking of the biopolyester poly(3-hydroxybutyrate)-*co*-(3-hydroxyvalerate)

Barbara Rupp,[a] Clemens Ebner,[a] Elisabeth Rossegger,[b] Christian Slugovc,[b] Franz Stelzer[b] and Frank Wiesbrock*[b]

Received 30th April 2010, Accepted 2nd July 2010
DOI: 10.1039/c0gc00066c

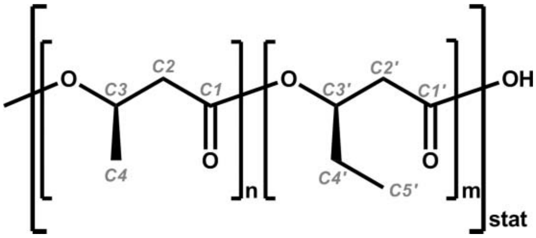
Films of the biopolyester poly(3-hydroxybutyrate)-*co*-(3-hydroxyvalerate) **PHB-HV**, a microbe-derived poly(hydroxyalkanoate), were crosslinked with 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone **BA** under UV irradiation. The degree of network formation reached 90% within irradiation times lower than 1 min. The 3 wt% concentration of **BA** in **PHB-HV** was found to be the optimum with respect to the rate of crosslinking relative to the rate of chain scission, which were derived from Charlesby-Pinner analyses. The **PHB-HV/BA** system could successfully be used for the generation of one- and two-step photolithographic imprints with reasonable resolution in the 50 μm range and, hence, proved to be an efficient system for finishing with biopolyesters.

1. Introduction

Biopolymers attract continuously growing interest, originating from the limited and ever decreasing availability of fossil resources and the inherent awareness of the carbon footprint as indicator for economic sustainability. In addition to common biopolymers like cellulose, starch, and proteins, microbe-derived macromolecules like poly(lactic acid) **PLA** and poly(hydroxyalkanoate)s **PHA**s have recently started to attract attention as biodegradable polymers from renewable resources as well.[1–5]

The fact that bacteria can produce polyesters like **PHA**s was unknown to polymer chemists until 1960 (and even to most biochemists and microbiologists until 1958),[6] although their presence in bacterial cells in isolable amounts was reported by Lemoigne in 1926.[7] Microbes produce **PHA**s as intracellular storage compounds for carbon and energy under nutrient-limited conditions in the presence of excess carbon sources.[8] Starting with the early discovery of poly(3-hydroxybutyrate) in 1926, as of today more than 150 different microbe-derived monomer units in the corresponding **PHA**s have been reported.[6,9–10] The composition and structure of the **PHA**s is determined by the producing type of microbe and its enzymatic specificity as well as by the carbon source provided.[11]

PHAs are categorized into two classes, depending on the size of the side chain on the C3-position (Scheme 1): short chain-length **PHA**s (scl-**PHA**s) are composed of repeating units of three to five carbon atoms (comprising units of, *e.g.*, 3-hydroxybutyrate 3-**HB** and 3-hydroxyvalerate 3-**HV**), while medium chain-length **PHA**s (mcl-**PHA**s) contain hydroxyalkanoate units with more than 6 (up to 14) carbon atoms per repeating unit.[12–13]



Scheme 1 Structure of the statistical copolyester poly(3-hydroxybutyrate)-*co*-(3-hydroxyvalerate) **PHB-HV**.

Owing to their natural origin, **PHA**s have exceptional stereochemical regularity: the chains are completely linear, and the chiral centres only possess *R* configuration, resulting in fully isotactic polyesters. Scl-**PHA**s are thermoplastics with a high degree of crystallinity, while mcl-**PHA**s are elastic or tacky materials with low degrees of crystallinity that exhibit, in particular in the case of unmodified mcl-**PHA**s, low melting points, which limits the number of potential applications for this class of (unmodified) **PHA**s.[8] UV-induced, γ-ray-induced and peroxide-mediated crosslinking have been reported in literature for mcl-**PHA**s with saturated[14–15] and unsaturated[16–20] side-chains; furthermore, chemical modifications that pave the way to chemical crosslinking have recently been shown for unsaturated mcl-**PHA**s as well.[21] Synthetic modifications involving the crosslinking of scl-**PHA**s, on the other hand, have been scarcely performed.

The properties of **PHB** and related scl-**PHA**s are similar to those of polyethylene and polypropylene. The polyesters can be fabricated in production processes such as batch, semi-batch or continuous fermentation;[22] remarkably, for the production of **PHB** and related scl-**PHA**s, microbes are known with low or no specific requirements regarding the carbon nutrition sources.[23–26] With melting temperatures in the range of 170–180 °C, processing temperatures starting from 180 °C have to

---

[a] *Polymer Competence Center Leoben GmbH (PCCL), Roseggerstrasse 12, AT-8700, Leoben, Austria.*
[b] *Institute for Chemistry and Technology of Materials (ICTM), Graz University of Technology, Stremayrgasse 9/V, AT-8010, Graz, Austria. E-mail: f.wiesbrock@tugraz.at; Fax: +43 316 873 10 32283; Tel: +43 316 873 32283*

be applied, and thermal degradation involving chain scission occurs, adulterating the (mechanical) polymer properties.[27–28] At temperatures above 300 °C, polymer degradation yields low mass molecular compounds,[29] while chain scission noteworthy already starts at temperatures lower than the melting point.[30–32]

Hence, crosslinking of the scl-**PHA** polyesters has been proposed for further enhancement of the material properties, as the crosslinked material would also compensate for chain scission occurring at elevated temperatures. Thermally-induced crosslinking procedures involving benzoyl peroxide[33] and dicumyl peroxide[34] were reported in literature. Our key incentive for this study was the development of a method for the efficient crosslinking of **PHB-HV** films at low temperatures or, ideally, room temperature, in order to keep temperature-induced chain scission as well as energy costs at a minimum and to develop an ideal strategy for the crosslinking of scl-**PHA**s.

2. Results and discussion

2.1. Photochemical crosslinking

Radiation-induced processes are common methods for the crosslinking of polymers. Frequently, these processes exhibit low crosslinking yields even after prolonged irradiation times because of low radiation chemical yields. For crosslinking of **PHB-HV**, which is known for its low reactivity, bisazides were chosen as crosslinking agents in this study. Bisazides are known to form highly reactive nitrenes after electronic excitation, and (insoluble) networks are formed through the insertion of nitrenes into the carbon hydrogen bonds of the polyester chains (Scheme 2).[35]

For the quantification of the crosslinking ability of **BA** towards **PHB-HV**, sol–gel analyses were performed for mixtures with five different ratios of **BA** and **PHB-HV** (1, 2, 3, 4, and 5 wt%, corresponding to 0.24, 0.48, 0.72, 0.96, and 1.20 mol% with respect to the repetition units). The chloroform solutions were spin coated onto CaF_2 plates to form thin films of **PHB-HV/BA**. These plates were individually illuminated for the targeted times in the range from 0 to 180 s, and subsequently developed in dichloromethane to dissolve the non-crosslinked fractions of the polymer films.

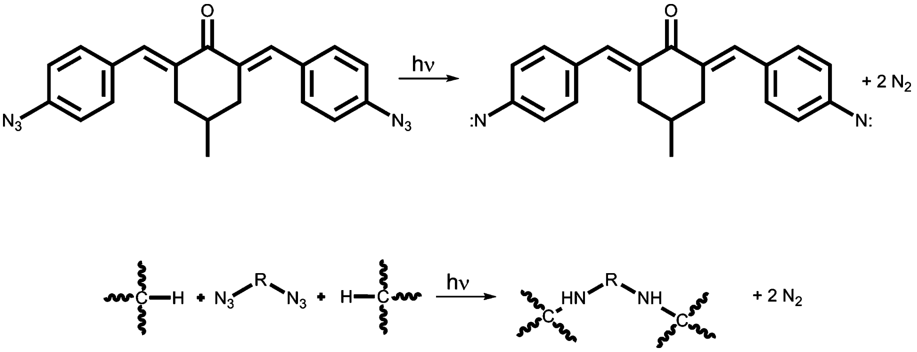
The decomposition of **BA** can be observed in FTIR-spectra measured before and after illumination, as the peak at 2118 cm^{-1}, representing the azide-groups in **BA**, disappears. Gel fractions of the individual samples were calculated from the ratio of the heights of the ester peaks at 1724 cm^{-1} after illumination (pht_0) and after development (pht_1), respectively (eqn (1)), to yield the (relative) percentage values:

$$\text{gel fraction}\,[\%] = \frac{pht_1}{pht_0} \times 100 \qquad (1)$$

For all **PHB-HV/BA** ratios investigated in this study, maximum crosslinking was achieved within the first minute of irradiation; the maximum value for the gel fraction increased from 70% (1 wt% of **BA**) to 80% (2 wt% of **BA**) and 90% (3, 4, and 5 wt% of **BA**) (Fig. 1).

The sol–gel analyses performed in this study relied on the assumption that the soluble fractions that dissolved during the development step were not composed of gels, whereas the insoluble (gel) fraction was composed of crosslinked gels. This assumption was supported by GPC analyses of the soluble fractions that clearly revealed the decrease of number average molecular weights from 191 down to 83 kDa with prolonged irradiation times, concomitant with an increasing broadness of the average molecular weight distribution, exhibited by the polydispersity indices PDI increasing from 2.4 to 3.8 (Fig. 2). It is assumed that the decreasing number average molecular weights originates from chain scission (see below: Charlesby-Pinner analyses); this idea is also supported by the simultaneously increasing PDI values. Furthermore, gravimetric control experiments of the gels crosslinked with 3 wt% of **BA** were in good agreement with the data obtained from IR measurements (3 s: 43%, 60 s: 95% of insoluble gel after development; see Fig. 1), verifying the inherent assumption that the ester percentage hydroxybutyrate *vs.* hydroxyvalerate in the film does not change during development.

BA can provide crosslinking of polymer chains according to two alternative mechanisms: it can abstract protons from the polyester chains to yield 2,6-bis(4-aminobenzylidene)-4-methylcyclohexanone (subsequent polyester chain recombination) or it can act as a crosslinking agent by insertion (Scheme 2).[35] In order to verify the insertion crosslinking mechanism proposed in literature, the sol fractions of the films containing 3 wt% of **BA** after irradiation times of 0, 3, and 20 s were investigated by ^{1}H-NMR spectroscopy (Fig. 3). The spectra revealed that already after irradiation times as low as 3 s, corresponding to crosslinking degrees of approx. 35% (Fig. 1), the soluble fraction did not contain any **BA** (or the



Scheme 2 Crosslinking of polymer chains with 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone **BA** under UV irradiation.

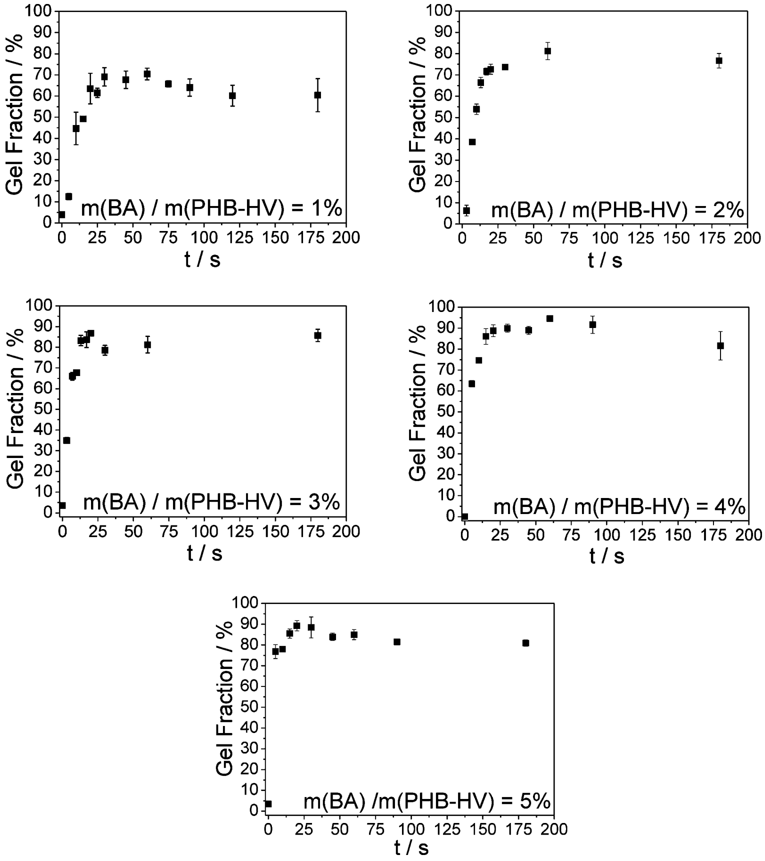


Fig. 1 Correlation of gel fractions with irradiation times for thin **PHB-HV** polymer films containing 1, 2, 3, 4, and 5 wt% of **BA** (with respect to **PHB-HV**). All samples were irradiated individually; experiments were repeated in triplicate.

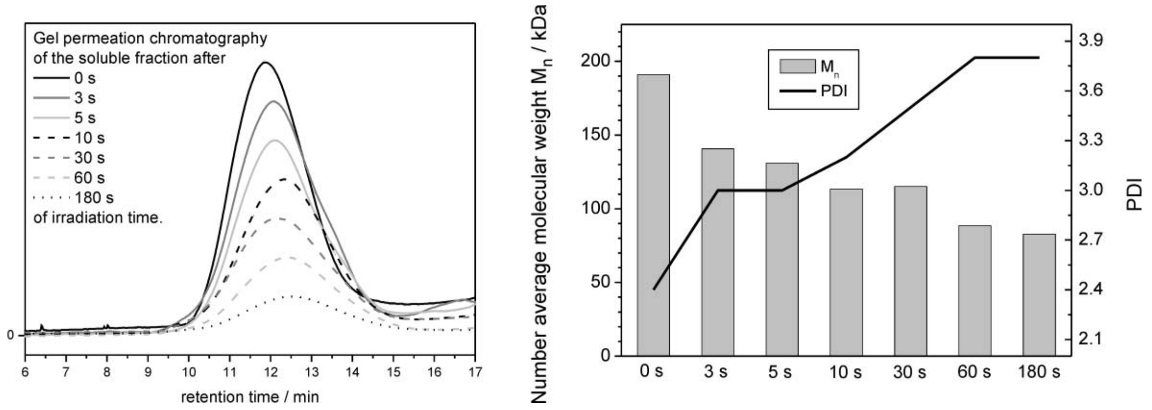


Fig. 2 GPC traces (left) and the calculated number average molecular weights and polydispersity indices (right) of the soluble polymer fraction obtained after the indicated irradiation times. The crosslinking experiments were performed with 3 wt% of **BA**.

2,6-bis(4-aminobenzylidene)-4-methylcyclohexanone derivative). It is assumed that this observation refers to two phenomena: (i) the validity of the insertion crosslinking mechanism (Scheme 2) and (ii) the fast initiation of the **BA** photoinitiator and its immediate reaction with the **PHB-HV** chains, also observed in the IR spectra that exhibited complete disappearance of the peak representing the azide group within the first 10 s of irradiation time. Final proof to support the insertion crosslinking mechanism was obtained from a crosslinking study with benzophenone as crosslinking agent under otherwise identical conditions: benzophenone, which can provide crosslinking by proton abstraction exclusively, failed to form any gel fraction in **PHB-HV** films.

Maximum amounts of gel fraction were obtained starting from **BA** concentrations of 3 wt% (Fig. 1). Increasing the amount of **BA** to higher concentrations of 4 and 5 wt% resulted in slightly longer irradiation times required to reach the maximum amount of gel fraction and, more remarkably, the diminishing of the amount of gel fraction during prolonged irradiation times (Fig. 1). A higher probability of chain scission (predominantly Norrish II type reactions), caused by the higher concentration of **BA** and the resulting higher concentration of radicals in

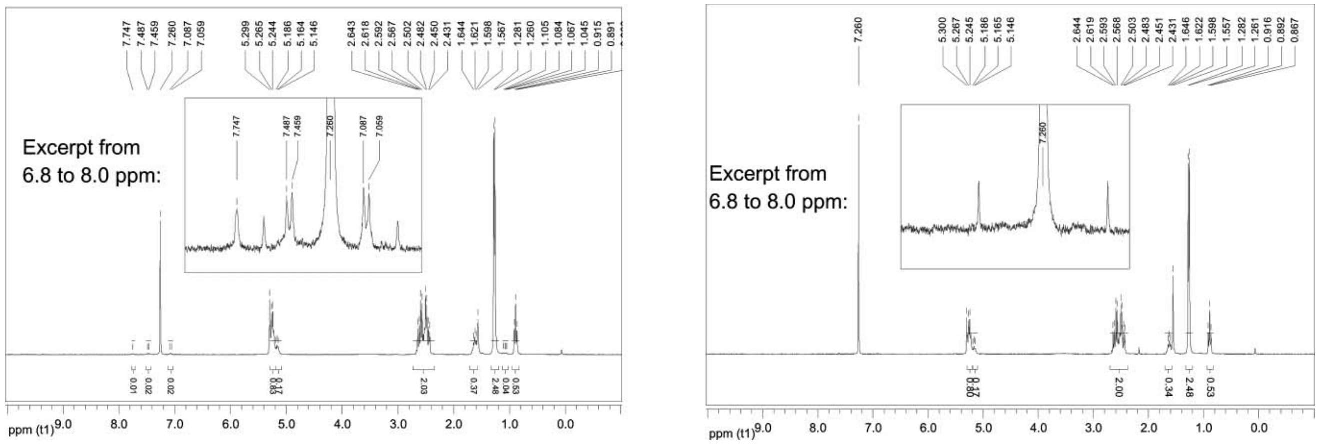


Fig. 3 ^{1}H-NMR spectra of the sol fractions of **PHB-HV/BA** films initially containing 3 wt% of **BA** after irradiation times of 0 s (left) and 3 s (right).

Table 1 Rates of crosslinking (v_q) and chain scission (v_p), and the respective ratios v_q/v_p for the **PHB-HV/BA** mixtures with **BA** contents from 1 to 5 wt%.

BA/PHB-HV [wt%]	10^3 v_q/min^{-1}	10^3 v_p/min^{-1}	v_q/v_p
1	9.32	7.05	1.32
2	12.3	7.79	1.58
3	16.9	8.27	2.04
4	29.9	31.3	0.96
5	68.1	73.9	0.92

the polyester chains, was assumed to cause these phenomena, and the crosslinking of **PHB-HV** with **BA** was subjected to Charlesby-Pinner analyses.

2.2. Charlesby-Pinner analyses

Crosslinking reactions, in which the probabilities of crosslinking and chain scission are independent of each other and constant for all units, can be described by eqn (2), which correlates the sol fraction (represented by the term $s + s^{0.5}$) with the crosslinking/chain scission density (q/p) and the number average polymerization degree (P_n^0).[36–37] From gel permeation chromatography, P_n^0 was calculated to be 1200 for the **PHB-HV** samples investigated.

$$s + \sqrt{s} = \frac{p}{q} + \frac{1}{P_n^0 \times q} \tag{2}$$

Assuming that crosslinking and chain scission have a constant rate, *e.g.* $p = v_p \times t$ and $q = v_q \times t$, eqn (2) can be transformed accordingly:

$$s + \sqrt{s} = \frac{v_p}{v_q} + \frac{1}{v_p \times P_n^0} \times \frac{1}{t} \tag{3}$$

Hence, the term $s + s^{0.5}$ is expected to correlate linearly with the reciprocal time for irradiation times shorter than the time required reaching the maximum value for the gel fraction. This expectation could be verified for all **PHB-HV/BA** mixtures investigated (Fig. 4), and the rates of crosslinking and chain scission (eqn (3)) were obtained from linear regression (Table 1).

For **BA** contents from 1 to 3 wt%, the rate of crosslinking v_q is higher than that of chain scission v_p and increases significantly from 9.32×10^{-3} min^{-1} to 16.9×10^{-3} min^{-1}, while the rate of chain scission v_p only slightly increases from 7.05×10^{-3} min^{-1} to 8.27×10^{-3} min^{-1}. At 4 wt% of **BA** content, on the other hand, the rate of crosslinking of 29.9×10^{-3} min^{-1} is found to be lower than the rate of chain scission of 31.3×10^{-3} min^{-1}; this trend is reproduced for 5 wt% of **BA** content as well. Consequently, for the investigated **PHB-HV/BA** mixtures, the ratio of the rates of crosslinking and chain scission exhibits a maximum for 3 wt% of **BA** content (Fig. 5), verifying the assumption that **BA** concentrations higher than 3 wt% unfavourably support the rate of chain scission to the disadvantage of the rate of crosslinking.

2.3. Photolithography

The optimum **PHB-HV/BA** system (3 wt% of **BA**) was used as negative photoresist for photolithographic imprints from a dedicated photo mask, both in one-step photolithographic processes on Si wafers as well as in two-step processes on glass/ITO (indium tin oxide)/chrome substrates (Fig. 6). In both cases, the **PHB-HV/BA** system was spin coated on the substrates to form the top layer, which was UV-irradiated through a quartz mask for 30 s and subsequently developed in dichloromethane in order to dissolve the non-illuminated segments of the polymer film. The illuminated parts were crosslinked and became insoluble, reproducing the geometric pattern of the photo mask.

The good adhesion of the crosslinked **PHB-HV** to the chrome surface as well as its low reactivity (towards oxidation) was illustrated by a second step of the photolithographic process on the glass/ITO/chrome substrates, when non-protected parts of the chrome layer were etched with an aqueous ammonium cerium(IV) nitrate solution to reveal the ITO layer underneath. The ammonium cerium(IV) nitrate solution could not access the surface of the chrome layer where it was covered with crosslinked **PHB-HV**, and, hence, these areas of the chrome layer were stable towards oxidation and the geometric pattern of the quartz mask was reproduced within the chrome layer. After two steps, the image pattern of the photo mask (original pattern size: 10 mm × 4 mm) was reproduced with a reasonable resolution of 50 µm (Fig. 6).

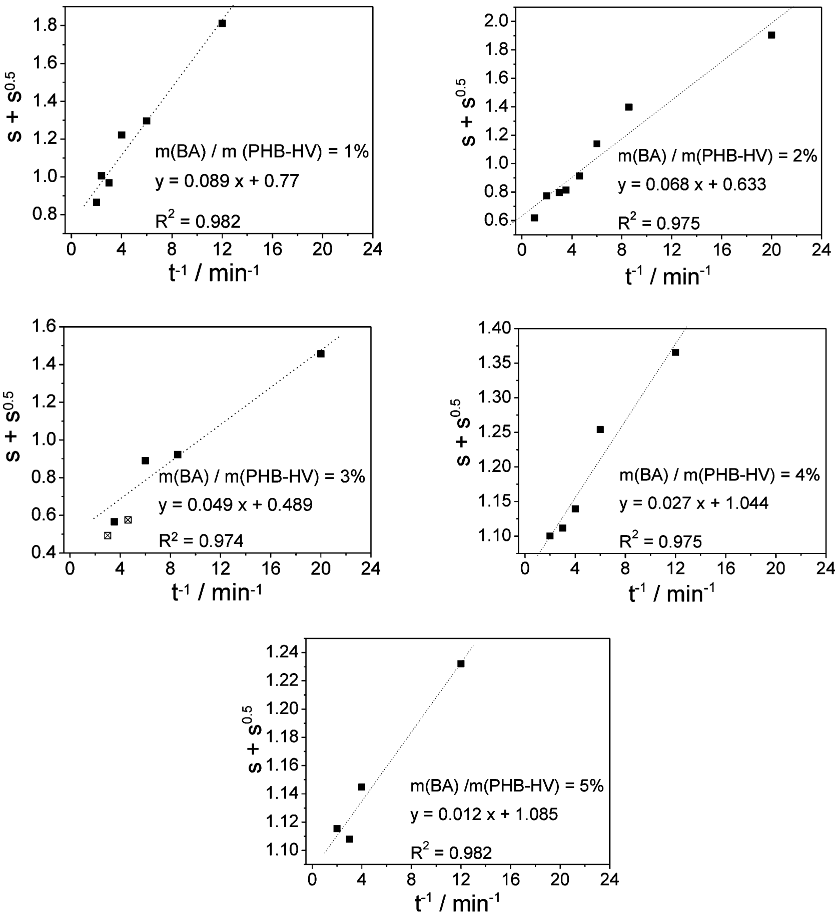


Fig. 4 Correlation of the sol fractions, represented by the term s + s$^{0.5}$, with the reciprocal times t^{-1}, according to Charlesby-Pinner analyses (eqn (3)). The sets of data can be described by a linear trend for all investigated **PHB-HV/BA** mixtures, ranging from 1 to 5 wt% of **BA**.

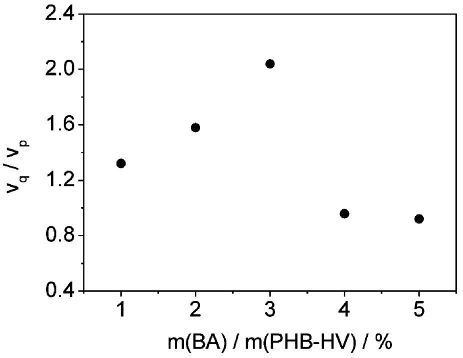


Fig. 5 Correlation of the ratio of the rates of crosslinking and chain scission (v_q/v_p) with the concentration of bisazide **BA** in the **PHB-HV/BA** mixtures.

3. Experimental

3.1. Materials

Commercial samples of poly(3-hydroxybutyrate)-*co*-(3-hydroxyvalerate) **PHB-HV** with 18.25 mol% of **PHV** were provided by M. Koller and G. Braunegg (Institute of Biotechnology and Biochemical Engineering, Graz University of Technology). They were purified by soxhlet extraction in ethanol (24 h), dried under vacuum, and subsequently subjected to two precipitation cycles, in which the copolymer was dissolved in dichloromethane (within 4–5 days under vigorous stirring), filtered, and precipitated in ethanol. The overall recovery yield after soxhlet extraction and two precipitation cycles was 21%.

^{1}H-NMR of **PHB-HV**: δ (ppm) = 0.88 (0.55; t, 3×0.1825 H = 0.55 H, $^3J_{H,H}$ = 6.9 Hz, H5′); 1.26 (2.48; d, 3×0.8175 H = 2.45 H, $^3J_{H,H}$ = 6.5 Hz, H4); 1.57–1.64 (0.38; m, 2×0.1825 H = 0.37 H, H4′); 2.44–2.64 (2.05; m, 2×0.8175 H + 2×0.1825 H = 2 H, H2 und H2′); 5.12–5.18 (0.18; m (dd), 0.1825 H, H3′); 5.22–5.28 (0.83; m (dd), 0.8125 H, H3).

GPC-Analysis of **PHB-HV**: Sample 1: M_n = 106.7 kDa, PDI = 2.8 (used for sol–gel-analyses, ^{1}H-NMR and photolithography); sample 2: M_n = 191.1 kDa, PDI = 2.4 (used after consumption of sample 1 for the mass balance experiments and gel permeation chromatography experiments of the soluble fractions).

The bisazide crosslinker 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone (97%) **BA**, dichloromethane (≥ 99.8%), chloroform (≥ 99%), and ethanol (99.8%) were bought from Aldrich and used without further purification.

3.2. Instrumentation

FT-IR spectra were recorded with a Perkin Elmer Spectrum One instrument and a DTGS-detector (spectral range between 4000 cm^{-1} and 800 cm^{-1}). All FT-IR spectra were taken in

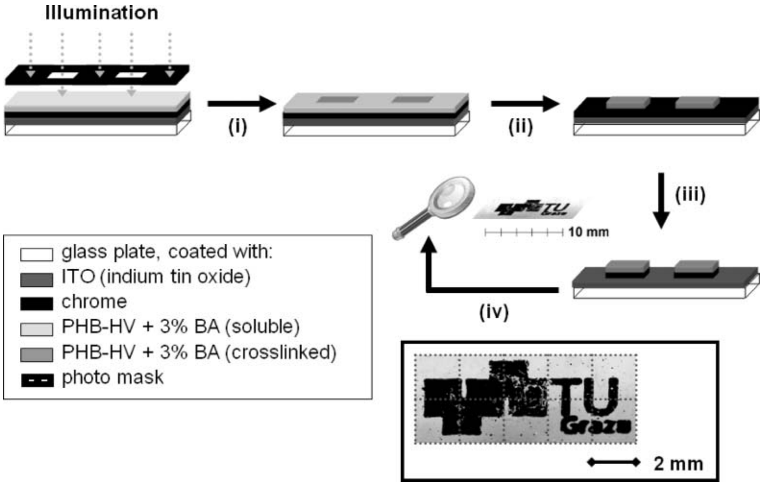


Fig. 6 Scheme of the photolithographic process on glass/ITO/chrome substrates that were covered with a spin coated **PHB-HV/BA** film. (i) Removal of the mask after UV irradiation. (ii) Development of the polymer layer in dichloromethane. (iii) Removal of the non-protected parts of the chrome layer by etching with ammonium cerium(IV) nitrate. The photography of the pattern formed by the chrome/**PHB-HV** pattern is shown in (iv).

transmission mode; the IR beam was directed through the spin coated films of the samples on CaF_2 windows and measured after it had passed the film. A blank sample (CaF_2 window) was subtracted as background. ^{1}H-NMR spectra were recorded on Bruker 300-MHz spectrometer, with relaxation delays of 5 s. The solvent residual peak of $CDCl_3$ was used for referencing the spectra to 7.26 ppm. Peak shapes are indicated as follows: s (singlet), d (doublet), dd (doublet of doublet), m (multiplet). Average molecular weights and polydispersity indices were determined by size exclusion chromatography GPC using $CHCl_3/Et_3N/^{iso}PrOH$ (94/4/2) as eluent. The measurements were performed with a Merck Hitachi L-6000A pump, separation columns from Polymer Standards Service, 8 × 300 mm STV linear XL 5 µm-grade size, and a differential refractometer Waters 410 detector. Poly(styrene) standards from Polymer Standard Service were used for calibration. Thin film samples were prepared on the Karl Suss CT-62 Spin Coater. For UV illumination of spin coated samples on CaF_2 plates, the mercury lamp EFOS Novacure from EXFO was used. Prior to polychromatic irradiation with 3000 mW cm^{-2}, the samples were placed in a dedicated chamber that was flushed with nitrogen (distance chamber - light source = 7 cm).

3.3. Preparation and characterization of the samples

Solutions of **PHB-HV** (c = 20 g L^{-1}) containing **BA** (1, 2, 3, 4, and 5 wt% of the polymer, respectively) with a total volume of 20 mL were freshly prepared in chloroform and stored shielded from light throughout the experiment. From these solutions, thin films were spin coated onto CaF_2 plates (20 s, 1000 rpm) to yield films with thicknesses in the range of few micrometres. After recording the FT-IR spectra of the **PHB-HV** layers, the CaF_2 plates were UV-illuminated in nitrogen atmosphere individually for the targeted times. The samples were then developed in dichloromethane for 20 min. After drying, another set of FT-IR spectra was recorded. All experiments were repeated in triplicate.

From the difference in absorbance of the ester band at 1724 cm^{-1} in the corresponding IR spectra, the residual film thickness (*i.e.* the insoluble fraction, also referred to as gel fraction W) was calculated; the sol fraction was calculated from the difference 1 – W.

For the gel permeation chromatography of the soluble fractions, solutions of **PHB-HV** (c = 50 g L^{-1}) were used in otherwise identical routines.

The gravimetric control experiments were performed with flat films of approx. 80 mg that were irradiated for the targeted times and developed in dichloromethane; the dichloromethane was removed by filtration and subsequent recovery of the developed film.

3.4. Photolithographic process

A freshly prepared chloroform solution of **PHB-HV** (c = 50 g L^{-1}) containing 3 wt% of **BA** was spin coated on Si wafers and glass/indium tin oxide ITO/chrome substrates at 1000 rpm for 20 s. The films were covered with a quartz mask and irradiated for 30 s. Afterwards, the substrates were developed in dichloromethane for 20 min and (in the case of glass/ITO/chrome substrates) subsequently treated with a freshly prepared aqueous solution of ammonium cerium(IV) nitrate (100 g L^{-1}).

4. Conclusions and outlook

Films of **PHB-HV**, a biopolyester of the class of scl-**PHA**s, were crosslinked with bisazide **BA** under UV irradiation at room temperature; energy-consuming heating processes were not required to realize crosslinking. **BA** reacted as crosslinking agent through the insertion of nitrenes into the carbon hydrogen bonds of the polyester chains. Crosslinking degrees of 90% were reached within irradiation times lower than 1 min in mixtures containing 3 wt% of **BA**. The UV-induced crosslinking could be successfully employed in one- and two-step photolithographic processes (with reasonable resolution in the range of 50 µm) and for the finishing of films.

Currently, scl-**PHA**s with non-functionalized side-chains are the only **PHA**s commercially available in large quantities and are inherently the only congeners of this class exhibiting the potential to be used as novel materials in biodegradable products. These **PHA** biopolyesters can be produced from renewable resources in integrated production routines.[23–24] They are known for their low reactivity; nonetheless, they can successfully be crosslinked with the bisazide **BA** used in this study at room temperature. Furthermore, the method reported here has the following additional advantages: the UV-induced crosslinking showed (i) high reproducibility, following crosslinking according to the Charlesby-Pinner theory, with (ii) remarkably short irradiation times of less than 1 min to realize crosslinking degrees of 90%. The reaction yielded high crosslinking degrees with (iii) low to moderate concentrations of **BA**. UV-induced network formation was performed at room temperature, and, hence, (iv) chain scission reported for **PHA**s at elevated temperatures[27–32] does not occur in this (v) energy-saving process (compared to crosslinking strategies described in literature).[33–34]

Current and future investigations are and will be directed towards biodegradation studies of **PHB-HV** and related scl-**PHA**s.

Acknowledgements

This study was performed at the Polymer Competence Center Leoben GmbH (PCCL, Austria) within the framework of the Kplus-program of the Austrian Ministry of Traffic, Innovation and Technology, and at the Institute for Chemistry and Technology of Materials (ICTM) of the Graz University of Technology with fundings from the Laura Bassi Centre of Expertise “BRIC”, headed by A. Weinberg (managed by the Austrian Research Promotion Agency FFG). PCCL is funded by the Austrian Government and the State Governments of Styria and Upper Austria. The authors like to thank M. Koller and G. Braunegg (Institute of Biotechnology and Biochemical Engineering, Graz University of Technology) for the provision of **PHB-HV** and Jelena Mihailova (Faculty of Materials Science and Applied Chemistry, Riga Technical University) for the collaboration during her IAESTE internship at ICTM.

References

1 T. Hisano, K. I. Kasuya, Y. Tezuka, N. Ishii, T. Kobayashi, M. Shiraki, E. Oroudjev, H. Hansma, T. Iwata, Y. Doi, T. Saito and K. Miki, *J. Mol. Biol.*, 2006, **356**, 993–1004.
2 K. Numata, S. Sato, M. Fujita, T. Tsuge, T. Iwata, Y. Doi and H. Abe, *Polym. Degrad. Stab.*, 2007, **92**, 176–183.
3 J.-F. Lutz and H. G. Börner, *Prog. Polym. Sci.*, 2008, **33**, 1–39.
4 F. Siegmund, D. Veit and T. Gries, *Chem. Unserer Zeit*, 2009, **43**, 152–158.
5 D. Cunliffe, S. Pennadam and C. Alexander, *Eur. Polym. J.*, 2004, **40**, 5–25.
6 R. W. Lenz and R. H. Marchessault, *Biomacromolecules*, 2005, **6**, 1–8.
7 M. Lemoigne, *Bull. Soc. Chim. Biol.*, 1926, **8**, 770–782.
8 D. Y. Kim, H. W. Kim, M. G. Chung and Y. H. Rhee, *J. Microbiol.*, 2007, **45**, 87–97.
9 R. D. Ashby, H. L. Ngo, D. K. Y. Solaiman and G. Strahan, *Appl. Microbiol. Biotechnol.*, 2009, **85**, 359–370.
10 A. Steinbüchel and H. E. Valentin, *FEMS Microbiol. Lett.*, 1995, **128**, 219–228.
11 A. Ballistreri, G. Montaudo, G. Impallomeni, R. W. Lenz, Y. B. Kim and R. C. Fuller, *Macromolecules*, 1990, **23**, 5059–5064.
12 S. Taguchi, H. Matsusaki, K. Matsumoto, K. Takase, K. Taguchi and Y. Doi, *Polym. Int.*, 2002, **51**, 899–906.
13 K. Matsumoto, S. Nakae, K. Taguchi, H. Matsusaki, M. Seki and Y. Doi, *Biomacromolecules*, 2001, **2**, 934–939.
14 K. D. Gagnon, R. W. Lenz, R. J. Farri and R. C. Fuller, *Polymer*, 1994, **35**, 4358–4367.
15 M. S. Divyashree and T. R. Shamala, *Radiat. Phys. Chem.*, 2009, **78**, 147–152.
16 R. D. Ashby, A.-M. Cromwick and T. A. Foglia, *Int. J. Biol. Macromol.*, 1998, **23**, 61–72.
17 S. N. Kim, S. C. Shim, D. Y. Kim, Y. H. Rhee and Y. B. Kim, *Macromol. Rapid Commun.*, 2001, **22**, 1066–1071.
18 B. Hazer, S. I. Demirel, M. Borcakli, M. S. Eroglu, M. Cakmak and B. Erman, *Polym. Bull.*, 2001, **46**, 389–394.
19 G. J. M. De Koning, H. M. M. Van Bilsen, P. J. Lemstra, W. Hazenberg, B. Witholt, H. Preusting, J. G. Van, Der Galien, A. Schirmer and D. Jendrossek, *Polymer*, 1994, **35**, 2090–2097.
20 A. Dufresne, L. Reche, R. H. Marchessault and M. Lacroix, *Int. J. Biol. Macromol.*, 2001, **29**, 73–82.
21 M. Y. Lee and W. H. Park, *Macromol. Chem. Phys.*, 2000, **201**, 2771–2774.
22 J. Wang and H. Q. Yu, *Appl. Microbiol. Biotechnol.*, 2007, **75**, 871–878.
23 G. Braunegg, G. Lefebvre and K. F. Genser, *J. Biotechnol.*, 1998, **65**, 127–161.
24 R. V. Nonato, P. E. Mantelatto and C. E. V. Rossell, *Appl. Microbiol. Biotechnol.*, 2001, **57**, 1–5.
25 M. Koller, P. Hesse, R. Bona, C. Kutschera, A. Atlic and G. Braunegg, *Macromol. Biosci.*, 2007, **7**, 218–226.
26 M. Koller, R. Bona, E. Chiellini, E. G. Fernandes, P. Horvat, C. Kutschera, P. Hesse and G. Braunegg, *Bioresour. Technol.*, 2008, **99**, 4854–4863.
27 M. L. Di Lorenzo, P. Sajkiewicz, A. Gradys and P. La Pietra, *e-Polymers*, 2009, 27.
28 E. Chiellini, E. G. Fernandes, M. Pietrini and R. Solaro, in *Macromolecular Symposia, Vol. 197: Recent Advances in Biodegradable Polymers and Plastics*, ed. E. Chiellini and R. Solaro, WILEY-VCH, Weinheim, 2003, pp. 45–56.
29 N. Grassie, E. J. Murray and P. A. Holmes, *Polym. Degrad. Stab.*, 1984, **6**, 47–61; N. Grassie, E. J. Murray and P. A. Holmes, *Polym. Degrad. Stab.*, 1984, **6**, 95–103; N. Grassie, E. J. Murray and P. A. Holmes, *Polym. Degrad. Stab.*, 1984, **6**, 127–134.
30 H. Tsuji, Y. Echizen and Y. Nishimura, *Polym. Degrad. Stab.*, 2006, **91**, 1128–1137.
31 Y. Aoyagi, K. Yamashita and Y. Doi, *Polym. Degrad. Stab.*, 2002, **76**, 53–59.
32 H. Ariffin, H. Nishida, Y. Shirai and M. A. Hassan, *Polym. Degrad. Stab.*, 2008, **93**, 1433–1439.
33 T. M. Scherer, R. C. Fuller and R. W. Lenz, *J. Environ. Polym. Degrad.*, 1994, **2**, 263–269.
34 B. Fei, C. Chen, S. Chen, S. Peng, Y. Zhuang, Y. An and L. Dong, *Polym. Int.*, 2004, **53**, 937–943.
35 L. Schlegel and W. Schnabel, in *Radiation Curing in Polymer Science and Technology, Volume 1: Fundamentals and Methods*, ed. J. P. Fouassier and J. F. Rabek, Elsevier Science Publisher Ltd, New York, 1993, pp. 119–192.
36 A. Charlesby and S. H. Pinner, *Proc. R. Soc. London, Ser. A*, 1959, **249**, 367–386.
37 B. Ivan, T. T. Nagy, T. Kelen, B. Turcsanyi and F. Tüdös, *Polym. Bull.*, 1980, **2**, 83–88.