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Polymer 47 (2006) 553–560
polymer
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Thermoreversible gelation of biodegradable polyester (PHBV) in toluene

Andrij Pich [a,*], Nadine Schiemenz [a], Volodymyr Boyko [b], Hans-Juergen P. Adler [a]

[a] Institute of Macromolecular Chemistry and Textile Chemistry, Dresden University of Technology, D-01062 Dresden, Germany
[b] Institute of Physical Chemistry and Electrochemistry, Dresden University of Technology, D-01062 Dresden, Germany

Received 26 August 2005; received in revised form 21 November 2005; accepted 23 November 2005
Available online 13 December 2005

Abstract

In present paper we investigate thermoreversible gelation of biodegradable polyester poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) in toluene. Hot PHBV solutions became transparent gels after cooling to room temperature. This physical gelation process was followed by light scattering and viscosity measurements for solutions of different PHBV concentrations. It has been found that gelation temperature increases with increasing polymer concentration in toluene. PHBV films have been prepared by gelation process followed by solvent removal on solid substrates. It has been demonstrated that PHBV concentration in the solution influences the surface morphology of obtained films. Homogeneous PHBV films with increased surface roughness can be obtained by means of developed technique. Hydrolytic degradation studies indicate that surface morphology of obtained PHBV layers changes considerably with degradation time.

Keywords: Thermoreversible gelation; Biodegradable polymer films

1. Introduction

Recently biodegradable materials have received increased interest due to the ecological and recycling reasons. Among numerous bio-polymers microbial polyesters which belong to group of poly(hydroxyl alkanoates) (PHA) have been intensively investigated by numerous research groups. It has been established that a large number of different repeat units are found in these reserve materials, depending on the bacterial species and ingested substrate. Such polyesters are biocompatible and can be bio-degradated under environmental conditions. Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) found numerous applications in medicine (implants [1], drug delivery systems [2,3], tissue engineering [4,5]) due to its attractive properties.

The conformational properties of bacterial polyesters have been intensively investigated [6–8]. Mostly these studies include SANS [6], X-ray analysis [7], molecular modelling investigations [8] and have been focused on poly(3-hydroxybutyrate) (PHB). The solution properties of PHB have been studied by Marchessault et al. [9] by means of intrinsic viscosity, sedimentation analysis and optical rotary dispersion experiments in chloroform, ethylene dichloride, and trifluoroethanol. It has been found that the type of the solvent influences the chain conformation and it changes from coil to partially helical structure. Similarly to proteins a sharp helix-coil transition was observed by changing the solvent composition or temperature. Einaga et al. [10] investigated a series of PHB fractions in trifluoroethanol by means of light scattering and viscosity measurements. Authors reported a randomly coiled structure in dilute solution of good solvents. Baysal et al. [11] performed molecular dynamics simulation under different conditions for poly(3-hydroxy-5,8-decadienoate). They found strong persistence for rod-like helices in good solvent and sharp helix-to-coil transition was detected in going from good to poor solvent conditions.

The polymer conformational transitions mentioned above play an important role in formation of biopolymer gels and networks, which have been intensively investigated [12–14]. The crosslinking mechanism in such systems mostly involves physical bonds due to three main attractive interactions, i.e. hydrogen bonding, hydrophobic interactions, and electrostatic interactions. This is the reason why the mechanism of such crosslinking is difficult to understand. In present work we investigate thermoreversible gelation of PHBV in toluene. The big disadvantage of PHBV is bad solubility in common solvents, what complicates the processing. Usually chloroform or methylene chloride has been reported to be the best solvent for PHBV [15]. However, it has been found that PHBV can be

* Corresponding author. Tel.: +49 351 463 338 58; fax: +49 351 463 337 122.
E-mail address: andrij.pich@chemie.tu_dresden.de (A. Pich).

0032-3861/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2005.11.070

also soluble in toluene after heating. The interesting feature of such solutions is formation of physical gels after cooling to room temperature. This effect is reversible and can open new possibilities for design of biomaterials on PHBV-basis with defined dimensions and morphologies. This includes preparation of membranes, fibres or particles, which can be suitable for medical applications. Therefore, the scope of present work was detailed investigation of gelation process in PHBV-toluene system what should provide better understanding of network formation process. Herein we present also first results on formation of PHBV-layers by using gelation process and report some data concerning biodegradation process.

2. Materials and methods

2.1. Materials

PHBV was received with following characteristics: hydroxyvalerate (HV) content 7.5 wt%; $M_n = 1.257 \times 10^5$ g/mol, $M_w = 4.205 \times 10^5$ g/mol and $M_z = 7.106 \times 10^5$ g/mol; polydispersity index $d = 3.344$. Toluene was obtained from Biesterfeld and used as received.

2.2. Preparation of gels

Gels were prepared by dissolving of appropriate amount of PHBV in toluene at ca. 90 °C. After cooling down transparent gels have been formed.

2.3. Preparation of films

PHBV films have been prepared by spin-coating (Specialty Coating Systems P6700) of PHBV solutions in toluene on glass substrates. Hot PHBV solutions were placed onto glass substrates (2 cm^2) at rotation speed 1500 rpm. After film-formation process samples were dried to remove any trace of toluene.

2.4. Hydrolytic degradation of PHBV films

For degradation tests prepared PVBV layers on glass substrates have been placed into 0.01 M phosphate buffer (pH = 7.4) and stored at 37 ± 1 °C. Every 3 weeks samples were rinsed with distilled water, dried and investigated by SEM.

2.5. Analytical methods

2.5.1. Dynamic light scattering

A commercial laser light scattering (LLS) spectrometer (ALV/DLS/SLS-5000) equipped with an ALV-5000/EPP multiple digital time correlator and laser goniometer system ALV/CGS-8F S/N 025 was used with a helium–neon laser (Uniphase 1145P, output power of 22 mW and wavelength of 632.8 nm) as the light source. PHBV solutions in toluene (2.5, 5, 7.5 and 10 g/l) have been investigated. Hot solutions were passed through 5 μm PTFE filter before measurements and placed into glass cuvettes. Measurements have been performed in temperature range 70–90 °C.

2.5.2. Scanning electron microscopy

SEM images were taken with Gemini microscope (Zeiss, Germany). PHBV films prepared on glass substrates have been dried under reduced pressure and coated with gold layer to improve the conductivity. Pictures were taken at voltage of 4 kV.

3. Results and discussion

3.1. Thermoreversible gelation

The thermoreversible gelation process of PHBV solutions in toluene was observed in concentration range from $c_{PHBV} = 2.5$ to 10 g/l. Transparent solutions prepared by dissolving PHBV in hot toluene became gels after cooling to room temperature (Fig. 1). It has been found that this process is fully reversible and system can be transformed into liquid state by heating.

The principal gelation schema is shown in Fig. 1. Based on studies of conformational properties of biopolyesters [6–8] and gelation of other biopolymer systems [16] it can be assumed that gelation process in PHBV-toluene system proceeds in following way. PHBV chains are in random-coil conformation at a given temperature and concentration (a); later on form partial helices after being cooled (b), and then helices

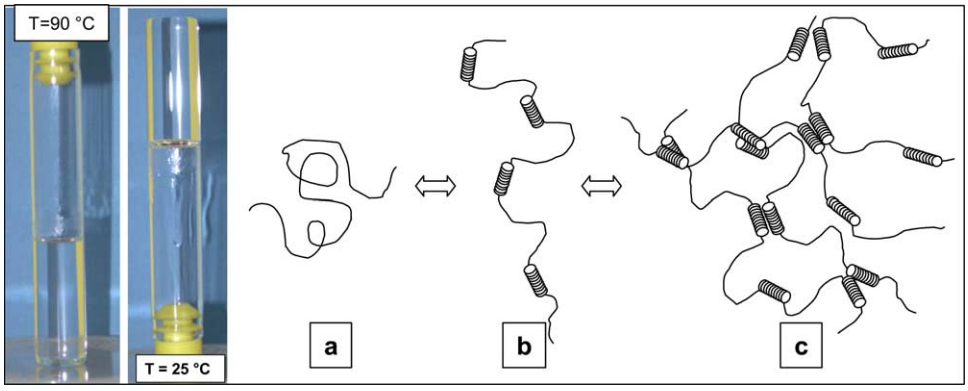


Fig. 1. Schematic representation of thermoreversible gelation in PHBV solutions (photograph shows PHBV solution in toluene at 90 °C and gel formed by cooling down to 25 °C).

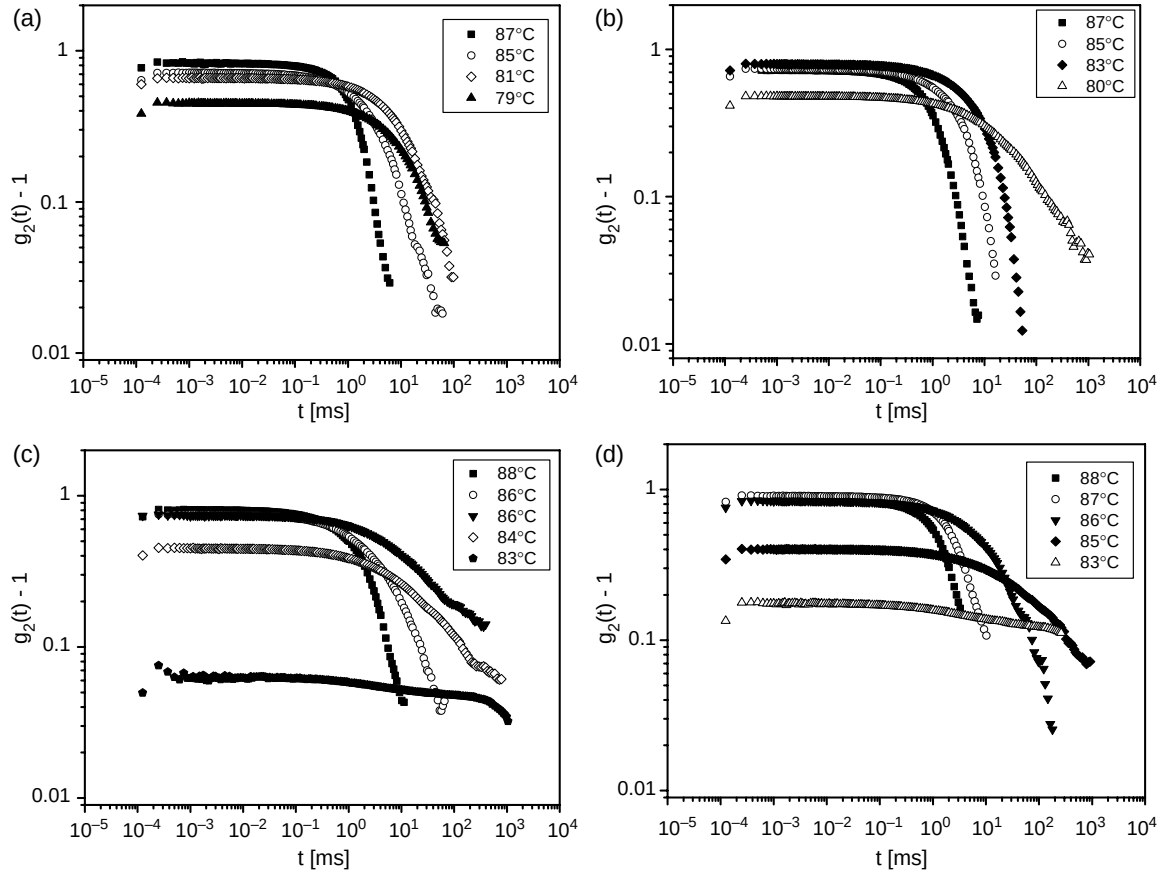


Fig. 2. Double-logarithmic plots of time correlation functions for different PHBV concentrations in toluene: (a) 2.5 g/l; (b) 5 g/l; (c) 7.5 g/l; (d) 10 g/l.

aggregate into multiple junctions (c) forming a three-dimensional network.

In present study the influence of PHBV concentration on gelation process was investigated by means of dynamic light scattering since this technique offers valuable information about gelation process. Fig. 2 shows time correlation functions (TCF) for four PHBV concentrations plotted double logarithmically against the decay time for different temperatures.

It can be seen that for all PHBV concentrations typical solution dynamics with nearly single exponentional TCF were observed at the high temperatures. Decreasing of the temperature leads to the shift of TCF to the longer decay times. This indicates the growth of polymer clusters due to the increasing interactions between PHBV polymer chains. The shift of TCF to the longer decay times is followed by the broadening of TCF and it can be no longer described by the single exponential function. To demonstrate clearly the influence of the PHBV concentration on the gelation process TCF curves could be fitted by stretched exponential function:

$$g_2(t) - 1 \sim g_1(t) = A \exp\left[-\left(\frac{t}{\tau}\right)^{\beta}\right] + B \tag{1}$$

with A as a constant which corresponds to the correlation strength and describes the initial amplitude of correlation function, B as the fitted baseline, τ as the relaxation time and β as the stretching parameter which decreases with broadening of decay time spectrum. Last two parameters characterize the mean relaxation time given by:

$$\langle\tau\rangle = \left(\frac{\tau}{\beta}\right)\Gamma\left(\frac{1}{\beta}\right) \tag{2}$$

where Γ is gamma function. The inverse relaxation time being proportional to the translational diffusion coefficient at temperatures above the gel point can be also defined as cooperative diffusion coefficient of the gel mode below the gelling temperature. At the gel point it can be expected that $\langle\tau\rangle \to \infty$ or $1/\langle\tau\rangle = 0$ [17]. Fig. 3(a) shows the dependence of the mean relaxation time as a function of the temperature for PHBV solution 10 g/l. The mean relaxation time increases if temperature decreases. At the certain temperature the mean relaxation time riches the maximum value and decreases with further temperature decrease. Typically the initial amplitude is lower than unity for the non-ergodic medium (gels, glasses, etc). The total scattered intensity in this case is result of contributions of intensity component due to the thermal fluctuations (homodyne scattering) and non-fluctuating component (heterodyne scattering) due to the presence of frozen spatial inhomogeneities [18,19]. Therefore, abrupt decreasing of TCF (for example TCF at 85 °C for $c = 10$ g/l in Fig. 2(d)) is followed by decreasing of mean relaxation time and the temperature of this transition (or peak maximum on $\langle\tau\rangle$ vs temperature function) can be taken as the gelation point [18]. Similar behaviour of mean relaxation time with temperature has been observed for other PHBV solutions, however, if

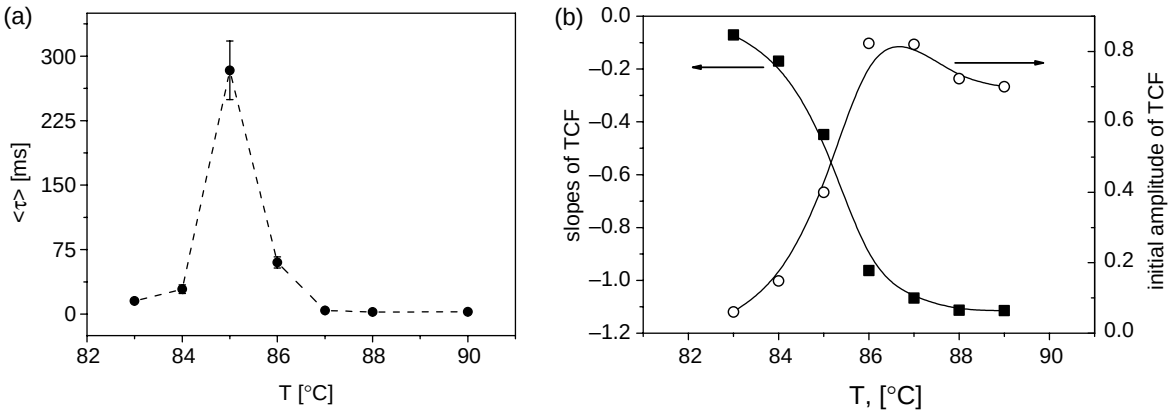


Fig. 3. Mean relaxation time (a); the initial amplitude of the TCF and slope of its linear part (b) as a function of temperature for PHBV concentration 10 g/l.

the concentration of biopolyester in toluene decreases the maximum is shifted to lower temperatures. Additionally the increase of $\langle\tau\rangle$ with temperature decrease was more step-wise for PHBV solutions 2.5, 5 and 7.5 g/l and obtained peak maxima were not so clear as compared to solution 10 g/l. For PHBV concentration 2.5 g/l the change of $\langle\tau\rangle$ with temperature is less pronounced. It has been also observed that gels obtained at $c_{\mathrm{PHBV}} < 2.5$ g/l were mechanically weak and separate clusters have been formed in toluene solution. These observations suggest that the gelation process in present system depends on the PHBV concentration and in concentrated solution gelling process is more efficient.

One can observe that the time correlation functions presented in Fig. 2 exhibit a linear part at the longer decay times. In Fig. 3(b) the slopes of these linear parts are plotted together with initial amplitude of TCF as a function of the temperature for sample with PHBV concentration 10 g/l. Close to the critical temperature there is a dramatic increase in slope and inflection point of fitted curves can be interpreted as the gel point [20]. In present case it is around 85 °C and it is the same temperature where the reduced initial amplitude of TCF was observed. All values presented in Fig. 3 calculated for the temperatures below the gel point are apparent due to the non-ergodic character of the sample. Comparing Fig. 3(a) and (b) it is obvious that the determination of the gel point by the change of the mean relaxation time and slopes of the TCF (or initial amplitude of TCF) lead to similar value of transition temperature which can be also compared with solutions containing different PHBV contents (Fig. 4). More detailed characterization of the gelation process (appearance of the power law behaviour of TCF and strong intensity fluctuations at the gel point) was not considered in this study. The cooling of PHBV several degrees below the gel temperature leads to practically complete disappearing of the TCF. This indicates the formation of very rigid network. The spectrum of the scattered light can be compared with those from glasses, i.e. the thermal fluctuations are absent due to the frozen structure. The similar rigid structure and dynamic properties were reported for agarose gel in water [21,22].

A summary of gelling temperatures extracted from DLS measurements in the way shown in Fig. 3 is presented in Fig. 4. It is obvious that gel point increases in linear order with increasing PHBV concentration in the system. These results are in agreement with proposed gelation mechanism since increase of the PHBV concentration leads to more pronounced intermolecular interactions at certain temperature. Therefore, gelation process occurs at higher temperatures for concentrated solutions. Obtained results should be proved by rheological measurements, which provide also exact gel point determination and such investigations are in progress.

3.2. Preparation of PHBV-layers

In addition to the investigations of gelation process PHBV films have been prepared on glass substrates. In this case similar PHBV solutions have been used as studied by DLS and after heating above gelation temperature applied by spin-coating on glass substrates. This means that gel formation process takes place on glass substrate due to the temperature decrease caused by spinning process followed by removal of toluene and formation of PHBV layers. For comparison PHBV solutions in chloroform prepared at similar concentrations have been used for film preparation. Fig. 5 shows SEM images of PHBV films at high and low magnifications prepared from solutions with different polymer concentrations. The thickness

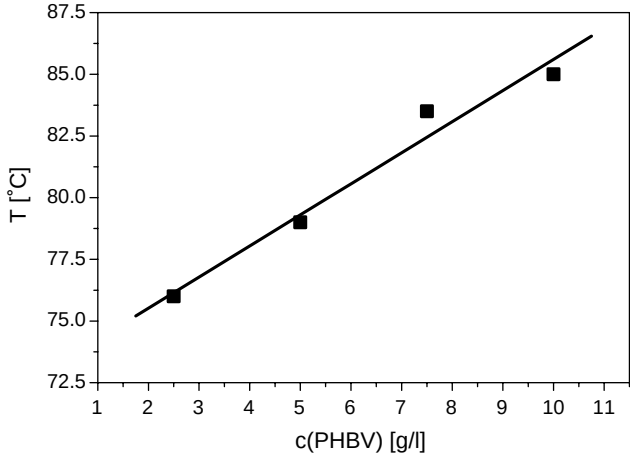


Fig. 4. Gel point as a function of PHBV concentration in toluene.

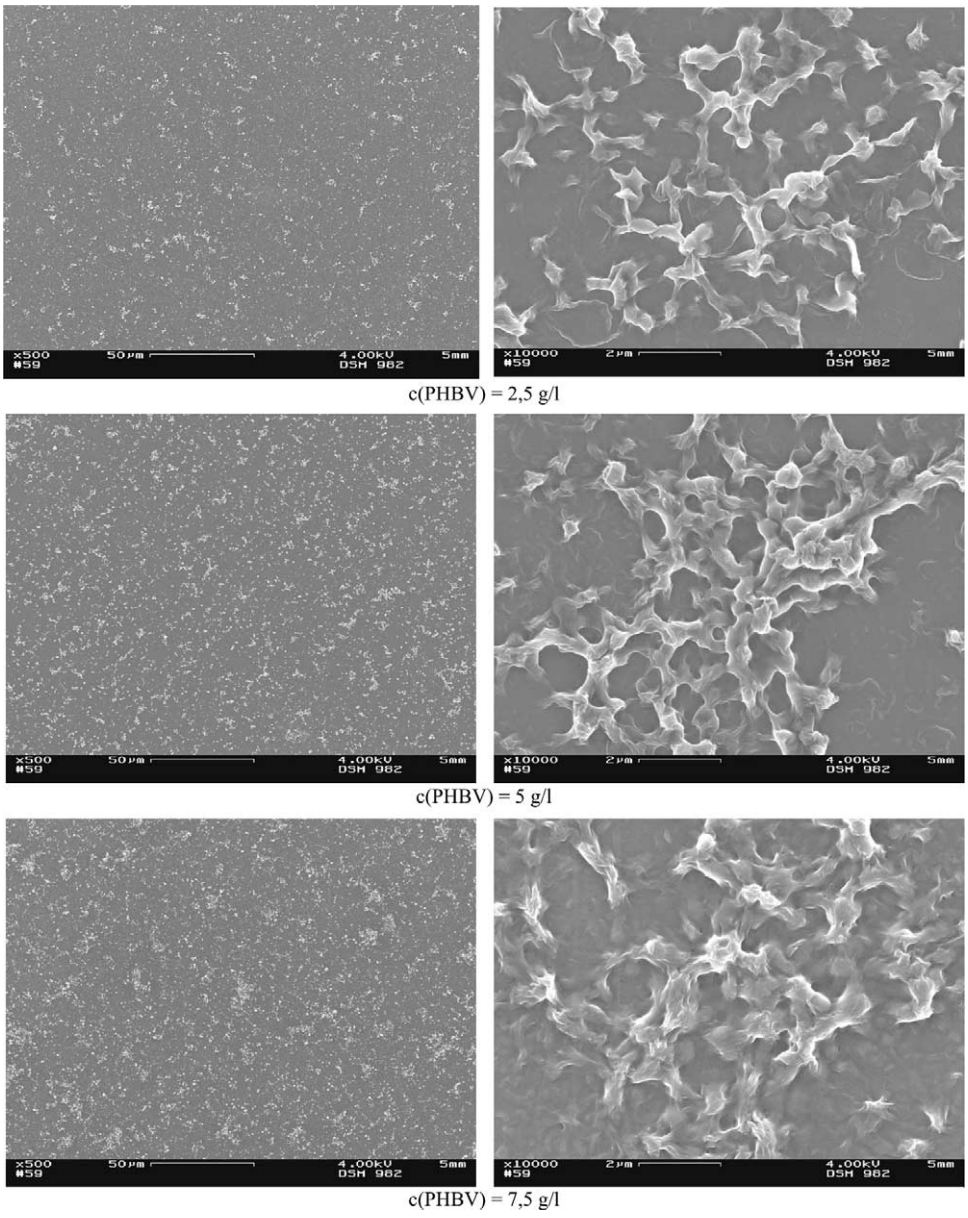


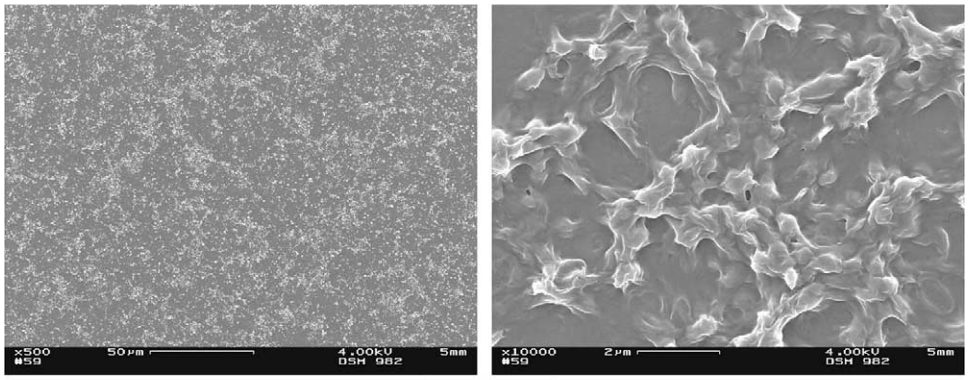
Fig. 5. SEM images of PHBV layers prepared from toluene solutions.

of obtained PHBV films increases with PHBV concentration in toluene (film thicknesses change from 700 nm to 5 μm by varying PHBV concentration from 2.5 to 50 g/l). Obtained PHBV films are semi-transparent mechanically stable materials, which can be easily removed from the glass substrate. The surface morphology of obtained PHBV films was investigated by SEM.

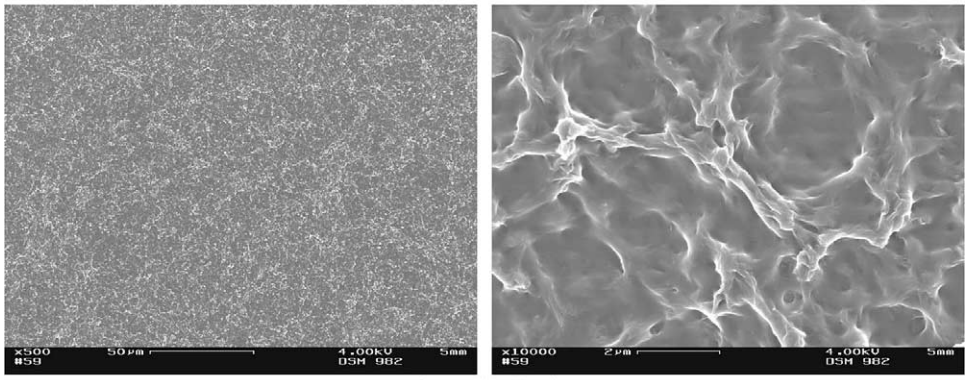
Scanning electron microscopy images indicate that at lowest PHBV concentration (2.5 g/l) a polymer film is formed on glass substrate after solvent evaporation consisting of separate PHBV domains connected with each other. In this case no dense layer has been formed and glass substrate is visible through the porous polymer film. With increase of PHBV concentration in solution the surface morphology of polymer films changes considerably. It is obvious that starting from 7.5 g/l dense polymer layers have been formed.

It is interesting to note that obtained films possess rough surface which is probably a consequence of shrinkage of polymer layer during toluene evaporation from gel-like coating. The bright domains visible in low-magnification SEM images are PHBV clusters, which are located on the surface of the films and increase the surface roughness. The amount of such clusters increases with PHBV concentration in toluene and they are homogeneously distributed on the film surface.

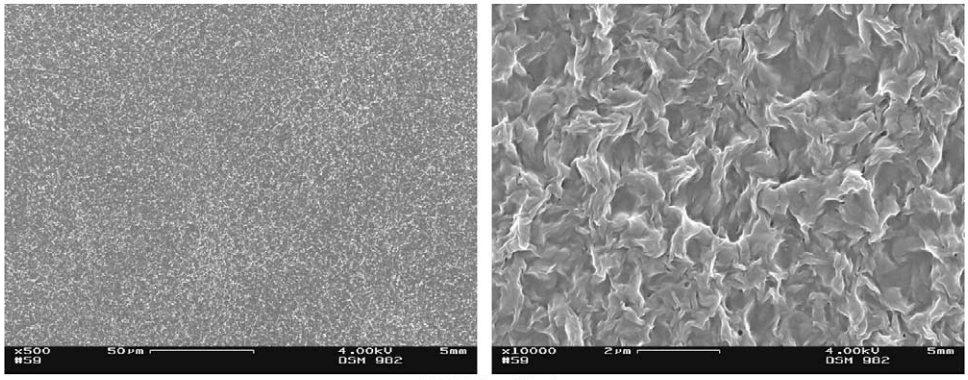
PHBV films prepared under similar conditions by casting from chloroform solutions exhibit totally different morphology as compared to their analogues formed from toluene solutions. As it is shown in Fig. 6 obtained layers are smooth



c(PHBV) = 10 g/l



c(PHBV) = 30 g/l



c(PHBV) = 50 g/l

Fig. 5 (*continued*)

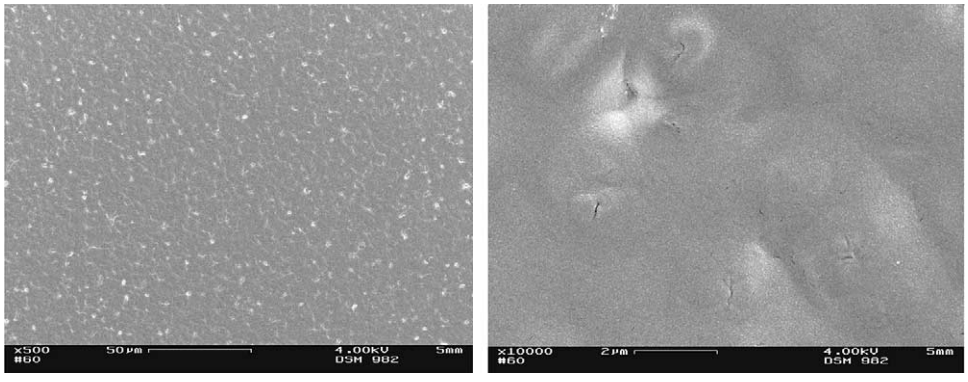


Fig. 6. SEM images of PHBV layers prepared from chloroform (c_{PHBV} = 50 g/l).

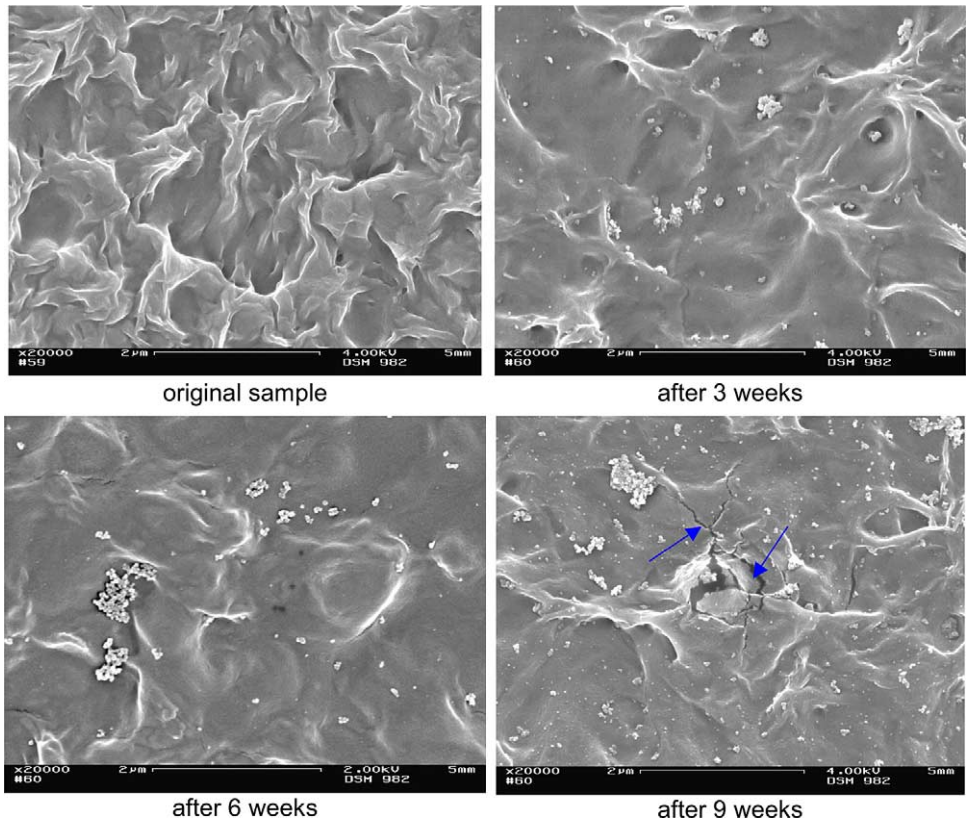


Fig. 7. SEM images of PHBV film after hydrolytic decomposition (c(PHBV) = 50 g/l) (arrows indicate cracks formed in polymer layer).

and do not exhibit leaf-like structure as compared with Fig. 5(2) (see sample 50 g/l). It has been also noted in other studies that PHBV films prepared by casting from chloroform or chloroform:dichloromethane mixtures possess smooth surface and compact structure [4].

In summary, it can be concluded that preparation of PHBV films from hot toluene solutions can lead to preparation of biodegradable layers with interesting morphology. Gelation process combined with solvent removal gives a possibility to prepare structured films, which probably possess also higher porosity comparing to films cast from chloroform. The morphology of PHBV layers could have considerable effect on degradation process or cell adhesion what should be the scope of future investigations.

Herein we present first results of hydrolytic degradation of formed PHBV layers. In this case the morphology changes have been followed at different time intervals by means of electron microscopy. Fig. 7 shows microscopy images of PHBV layers prepared from toluene solution (50 g/l) after storage in buffer solution. Following morphology change of polymer films with time one can realize two effects. Firstly, film surface becomes smoother and secondly small spherical domains appear on the surface. This effect can be explained by partial degradation of PHBV layer and formation of oligomers after decomposition of polyester macromolecules. They can form aggregates on the film surface due to bad solubility in water and remain strongly attached to the outer layer. After 9 weeks of storage in buffer solution the formation of cracks has been detected which indicates surface erosion and probably the degradation process proceeds not only on the surface but also inside of the polymer layer.

In contrast to our observations Doi et al. [23] investigated hydrolytic and enzymatic degradation of microbial polyesters and it has been mentioned that after 48 days of hydrolytic degradation the surface of PHBV films has been not changed. But authors determined considerable loss of PHBV molecular weight with time and simultaneously increase of the PHBV film thickness. It has been suggested that the degradation process occurs mostly in the inner part of the PHBV films and, therefore, water penetrates into the layer due to considerable changes in the film microstructure leading to the film swelling. In case of PHBV films prepared from toluene solutions by gelling followed by solvent removal the degradation process changes the surface morphology and leads to crack formation. This effect can be explained by higher porosity of the PHBV layers prepared from hot toluene solution as compared with PHBV films cast from chloroform solution. Therefore, films possess larger surface area and better possibilities for diffusion of water molecules inside of PHBV films leading to more effective degradation. In future degradation process should be investigated more in detail at different pH values and probably for longer time period. Additionally measurements of weight loss of PHBV films can help to follow the kinetics of degradation process more precisely.

4. Summary

In present paper we investigated thermoreversible physical gelation process of bacterial polyester PHBV in toluene. Light scattering measurements indicate that gelation temperature increases with increasing PHBV concentration in toluene solution. It is believed that this interesting phenomenon can be useful for preparation of biodegradable materials with interesting properties and morphologies. As one particular example we showed formation of PHBV layers on solid substrate by spin coating of hot PHBV solutions in toluene. In this process the ultra-thin gel layer is formed on the substrate followed by solvent evaporation. This complex mechanism determines eventually the PHBV film morphology and properties. It has been shown that uniform PHBV films can be obtained with leaf-like morphology if the PHBV concentration in toluene is higher as 7.5 g/l. It has been demonstrated by microscopy measurements that PHBV concentration in solvent influences considerably the morphology of the film surface. First degradation results indicate that obtained films can be gradually decomposed in aqueous medium and this process is followed by considerable morphological changes on the surface.

Acknowledgements

The authors are thankful to Mrs E. Kern for SEM measurements; Deutsche Forschungsgemeinschaft (DFG, Sonderforschungbereich 287 'Reactive Polymers') and European Graduate School, 'Advanced Polymer Materials' (EGK 720-1, DFG) for financial support.

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