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European Polymer Journal 43 (2007) 2491–2503
EUROPEAN POLYMER JOURNAL
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Miscibility and enzymatic degradation studies of poly(ε-caprolactone)/poly(propylene succinate) blends

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Received 14 December 2006; received in revised form 21 March 2007; accepted 30 March 2007
Available online 14 April 2007

Abstract

In the present study the miscibility behaviour and the biodegradability of poly(ε-caprolactone)/poly(propylene succinate) (PCL/PPSu) blends were investigated. Both of these aliphatic polyesters were laboratory synthesized. For the polymer characterization DSC, ^{1}H NMR, WAXD and molecular weight measurements were performed. Blends of the polymers with compositions 90/10, 80/20, 70/30 and 60/40 w/w were prepared by solution-casting. DSC analysis of the prepared blends indicated only a very limited miscibility in the melt phase since the polymer–polymer interaction parameter χ_{12} was -0.11. In the case of crystallized specimens two distinct phases existed in all studied compositions as it was found by SEM micrographs and the particle size distribution of PPSu dispersed phase increased with increasing PPSu content. Enzymatic hydrolysis for several days of the prepared blends was performed using *Rhizopus delemar* lipase at pH 7.2 and 30 °C. SEM micrographs of thin film surfaces revealed that hydrolysis affected mainly the PPSu polymer as well as the amorphous phase of PCL. For all polymer blends an increase of the melting temperatures and the heat of fusions was recorded after the hydrolysis. The biodegradation rates as expressed in terms of weight loss were faster for the blends with higher PPSu content. Finally, a simple theoretical kinetic model was developed to describe the enzymatic hydrolysis of the blends and the Michaelis–Menten parameters were estimated.

Keywords: Enzymatic hydrolysis; Biodegradable polymers; Aliphatic polyesters; Poly(propylene succinate); Poly(ε-caprolactone)

1. Introduction

In recent years, biodegradable polymers have attracted considerable attention as green materials and biomaterials in pharmaceutical, medical and biomedical engineering applications, including drug delivery systems, artificial implants and functional materials in tissue engineering. Aliphatic polyesters, due to their favourable features of biodegradability and biocompatibility, consist one of the most important classes of synthetic biodegradable polymers. They are nowadays available commercially

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doi:10.1016/j.eurpolymj.2007.03.051

in a variety of types used for artifacts production by injection moulding, extrusion processes as well as for films preparation. Additionally, several polyesters derived from different lactides and lactones, like polycaprolactone, can be used as drug carriers for controlled release devices and for biomedical applications. The advantage of these polyesters is their biocompatibility and higher hydrolysability in human body.

Poly(ε-caprolactone) (PCL) is an aliphatic polyester produced with very high molecular weight by ring opening polymerization of ε-caprolactone (ε-CL) and used extensively for the preparation of injection molding products, films, etc. Additionally, PCL due to its biocompatibility has gained increasing interest for uses such as tissue engineering and appropriate drug release carrier [1]. However, PCL has a high degree of crystallinity and thus its biodegradation rate is very slow. On the other hand, when PCL is copolymerized or blended with polymers that have a higher biodegradation rate, the produced materials can degrade faster compared to pure PCL [2,3]. Poly(ethylene glycol) (PEG), which is a hydrophilic, water soluble and fully biodegradable polymer, was extensively used in such block copolymer preparation [4]. PEG's lack of toxicity allows its usage in many biomedical and pharmaceutical applications [5,6]. Copolymers of ε-CL with other lactones can be synthesized by ring opening polymerization resulting in high molecular weight polyesters [7]. Such copolymers and especially those with relatively low molecular weights can be used as biodegradable drug delivery carriers. Their low molecular weight ensures good biocompatibility and ease in vivo degradation control [8]. Furthermore, from our previous study it was found that the incorporation of ε-CL content in the macromolecular chains of aliphatic polyester can improve the mechanical properties and the biodegradation rate of the copolymers compared with neat polymers [9]. An additional way to increase the biodegradability of a polymer is to mix it with other polymers having higher biodegradation rates. However, PCL is miscible only with a limited number of polymers like poly(4-vinyl phenol) [10], poly(vinyl chloride) [11], poly(vinyl alcohol) [12], poly(vinyl methyl ether) [13] and bisphenol-A-type epoxy resin [14], while most of its blends with other aliphatic polyesters are immiscible [15–17].

Poly(propylene succinate) (PPSu) is a relatively new polyester with very high thermal stability similar with other familiar polyesters like poly (butylenes succinate) (PBSu) or poly(ethylene succinate) (PESu) [18–20]. From our previous studies it was found that PPSu could be produced from its monomers at a relatively high molecular weight [21]. Additionally, its biodegradation rate is very high compared with other aliphatic polyesters with similar structure, like poly(ethylene succinate) and poly(butylene succinate) [22].

In the present study blends of PCL with PPSu were prepared and studied as a means of producing materials having high biodegradation rates. Introduction of a flexible PPSu domain, with melting temperature close to the body temperature, into the rigid PCL matrix, could generate a class of innovative materials used as drug carriers for pharmaceutical applications. Additionally, PPSu has a very slow crystallization rate, which could favour its miscibility with other aliphatic polyesters. Formerly, it was found that PPSu can form miscible blends in the amorphous phase with PESu [23]. Therefore, in this study, initially the thermal and morphological properties of PCL/PPSu polymer blends, consisted from minor phase of PPSu into PCL matrix are reported, in order to explore their miscibility behaviour. Subsequently, their enzymatic hydrolysis was studied and a simple theoretical kinetic model is introduced allowing estimation of the Michaelis–Menten parameters. Molecular and morphological characterization of the hydrolized samples was carried out using differential scanning calorimetry (DSC), polarized optical microscopy (POM), X-ray diffraction (WAXD) and scanning electron microscopy (SEM) techniques.

2. Experimental

2.1. Materials

Succinic acid (SA) (purum 99+%) and 1,3-propyleneglycol (PG) (99.6+%) were purchased from Aldrich Chemical Co. ε-Caprolactone (ε-CL) (99% Sigma–Aldrich) was dried over CaH_2 and purified by distillation at 130 °C under reduced pressure prior to use. Tetrabutyl titanate (TBT), used as catalyst, was of analytical grade and it was purchased from Aldrich Chemical Co. Polyphosphoric acid (PPA) used as heat stabilizer was supplied from Fluka. *Rhizopus delemar* lipase was purchased from BioChemika. All the other materials and solvents used for the analytical methods were of analytical grade.

2.2. Synthesis of polyesters and characterization

Synthesis of poly(propylene succinate) was performed following the two-stage melt polycondensation method (esterification and polycondensation) in a glass batch reactor as described in our previous study [18]. Synthesis of PCL was performed by bulk polymerization of ε-CL in 250 mL round-bottomed flask equipped with a stirrer and a vacuum apparatus. The initiator was added as a solution in toluene at a concentration of 1×10^{-4} mol TBT/mol ε-CL. The polymerization mixture was purged with dry argon three times. Thereupon, the reaction was carried out at 180 °C for 2 h. Unreacted monomer was removed through distillation by applying a vacuum (≈5 Pa), slowly to avoid excessive foaming, over a period of 15 min. Polymerization was stopped by fast cooling to room temperature.

Gel permeation chromatography (GPC). GPC analysis was performed using a Waters 150C GPC equipped with differential refractometer as detector and three ultrastyragel (10^3, 10^4, 10^5 Å) columns in series. $CHCl_3$ was used as the eluent (1 mL/min) and the measurements were performed at 35 °C. Calibration was performed using polystyrene standards with a narrow molecular weight distribution.

Nuclear magnetic resonance (NMR). ^{1}H NMR spectra of polyesters were obtained with a Bruker AMX 400 spectrometer operating at a frequency of 400 MHz for protons. Deuterated chloroform ($CDCl_3$) was used as solvent in order to prepare solutions of 5% w/v. The number of scans was 10 and the sweep width was 6 kHz.

2.3. Polymer blends reparation and characterization

PCL/PPSu blends with concentrations 90/10, 80/20, 70/30 and 60/40 w/w were prepared by solution-casting. Proper amounts of both polymers were dissolved in chloroform as common solvent, at room temperature. Sonication was applied in order to achieve complete dissolution and fine mixing of the components. The blends in the form of thin films (200–250 μm) were set up after solvent evaporation at room temperature, under a gentle air stream. The following techniques were used to characterize the polymer blends.

Thermal analysis. A Perkin–Elmer, Pyris 1 differential scanning calorimeter (DSC), calibrated with Indium and Zinc standards, was employed. A sample of about 10 mg was used for each test, placed in sealed aluminum pan and heated to 35 °C above the melting point of PCL at a heating rate of 20 °C/min. The sample was held at that temperature for 5 min in order to erase any thermal history. After that it was quenched into liquid nitrogen and scanned again using the same heating rate as before. The glass transition temperature (T_g), the melting temperature (T_m) and the heat of fusion (ΔH_m) were measured.

Wide angle X-ray diffraction patterns (WAXD). X-ray diffraction measurements of the samples were performed by an automated powder diffractometer (PW 1050) with Bragg–Brentano geometry (θ–2θ), using Cu Kα radiation from 5° to 60°.

Scanning electron microscopy (SEM). The morphology of the prepared films before and after enzymatic hydrolysis was examined in a scanning electron microscopy (SEM) type Jeol (JMS-840) equipped with an energy-dispersive X-ray (EDX) Oxford ISIS 300 micro-analytical system. The films were covered with a carbon coating in order to have good conductivity of the electron beam. Operating conditions were: accelerating voltage 20 kV, probe current 45 nA and counting time 60 s.

Mechanical properties. Measurements of tensile mechanical properties were performed on an Instron 3344 dynamometer in accordance with ASTM D638 using a crosshead speed of 50 mm/min. From the films prepared, dumb-bell shaped tensile-test specimens (central portions 5 × 0.5 mm thick, 22 mm gauge length) were cut in a Wallace cutting press and conditioned at 25 °C and 55–60% relative humidity for 48 h. The values of elongation at break and tensile strength were determined. At least five specimens were tested for each sample and the average values are reported.

2.4. Biodegradation studies

Enzymatic hydrolysis. Polyesters in the form of films with 5 × 5 cm in size and approximately 0.5 mm thickness, prepared by solution casting, were placed in petries containing 50 mL phosphate buffer solution (pH 7.2) with 1 mg/mL *R. delemar* lipase (0.4 U/mg). The petries were then incubated at 30 ± 1 °C in an oven for several days while the media were replaced every 3 days. After a specific period of incubation (7 days), the films were removed from the petri, washed with distilled water and weighted until constant weight. The degree of biodegradation was estimated from the mass loss.

For all polymer blends, thermal properties together with SEM and WAXD analysis was performed as described above, at various time intervals of hydrolytic degradation.

3. Results and discussion

3.1. Polymer characterization

The PCL sample had a white color, while the PPSu sample was light yellowish-brown. The hardness of the polymer samples was quite different, meaning that PPSu was very soft compared to PCL. The PPSu sample had a number average molecular weight ($\overline{M}_n$) 23000 as was measured by GPC, while PCL a much higher value, of 72,580 g/mol (Table 1). The tensile strength and the elongation at break of PCL was much larger compared to PPSu. The ^{1}H NMR spectrum of PPSu was very simple, exhibiting a single peak at 2.63 ppm attributed to methylene protons of succinic acid, a triple peak 4.09–4.21 ppm attributed to b protons and a multiple peak between 1.9 and 2.02 ppm corresponding to c protons. For PCL, the peaks at 1.25–1.4 (multiple), 1.5–1.68 (double), 2.15–2.31 (triple) and 3.9–4.08 (triple) were attributed to methylene protons g, j, k and e, respectively, as assigned in Fig. 1.

From DSC thermograms of the as received samples it can be seen that PPSu and PCL have a melting point of approximately 45 °C and 65.2 °C, respectively. In a previous study it was found that the enthalpy of fusion for pure crystalline PPSu was 140 J/g [24]. For completely crystalline PCL the enthalpy of fusion has been reported to be 136 J/g [25]. Using these values the degree of crystallinity (X_c) for the studied polyester was calculated to be 32% and 62% for PPSu and PCL, respectively. Thus, PCL was found to be much more crystalline compared to PPSu. In order to obtain the polyesters in the amorphous phase, a low mass sample of each polymer was melted in the DSC and then quenched in liquid nitrogen. From the subsequent heating scans, it was found that PPSu shows a T_g at −35 °C. No melting peak was recorded. Due to its slow crystallization rate PPSu could not crystallize within the experiment time scale for heating scans by 20 °C/min. On the other hand, PCL although it was quenched in liquid nitrogen it was quite crystalline. Most probably crystallization of quenched sample should take place during loading in the DSC. Thus, in this second scan there was no clear cold-crystallization peak (T_{cc}), even though in the literature there are some studies referring that the T_{cc} of PCL is around −59 °C [26]. Furthermore, as can be seen the T_g of PCL at −62 °C was hardly detected.

Table 1
Molecular weights, mechanical and thermal properties of prepared polyesters

Sample	[η] (dL/g)	$\overline{M}_n$	Tensile strength (MPa)	Elongation at break (%)	T_m (°C)	T_g (°C)	ΔH_m (J/g)
PPSu	0.50	23000	1.6	3.7	45.0	−35.0	45.5
PCL	1.37	72580	25.2	860	67.2	−61.6	86.5

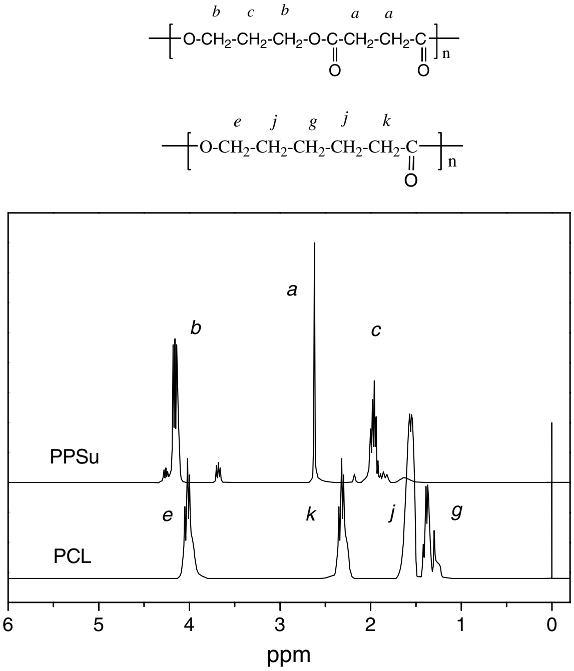

Fig. 1. ^{1}H NMR spectra of PCL and PPSu.

3.2. Miscibility study of the PCL/PPSu blends

Thin films of the blends were prepared, by solution casting and maintaining the solutions at a temperature of 25 °C until complete solvent evaporation. The formed thin films were structurally uniform, since no separated phases could be observed, indicating a fine mixing of the components. All films had a milky appearance like the neat PCL film, due to its high crystallinity.

As it can be seen in Fig. 2, all thermograms of the as received specimens, showed two distinct melting

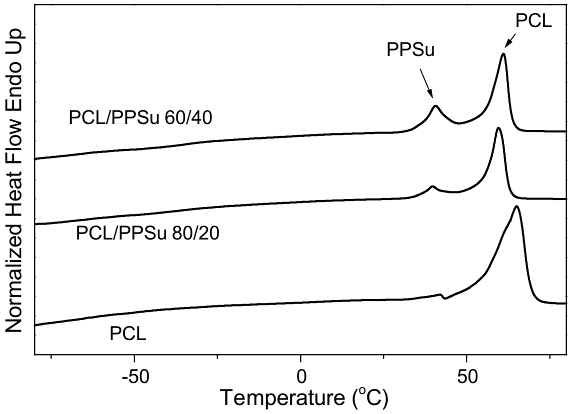

Fig. 2. Indicative DSC thermograms of neat PCL and PCL/PPSu polymer blends.

peaks over the entire composition range, indicating that these polymers should be classified as semicrystalline/semicrystalline polymer blends. Since always PPSu shows low crystallinity, its T_g in the blends was observed, but it was shifted slightly (1–2 °C) to higher temperatures, than the corresponding of the pure polymer. This is a result of phase segregation after PCL crystallization. The T_g of PCL is again hardly detected at temperatures close to that of the neat polyester [27]. Similar aliphatic polyester blends like PCL/PBSu were reported to be immiscible as evidenced by the composition independent glass transition temperature and the biphasic melt [28]. Therefore, in the studied PCL/PPSu blends the observation of two glass transition temperatures could be attributed to the immiscibility of the blends.

From an inspection of the melting temperature of each component in the blend, it was observed that the melting points of both polymers shifted to lower temperatures. A drop of approximately 4–5 °C, compared with the pure components was found. The depression of melting point of a crystalline polymer blended with other polymers provides information about their miscibility. Generally, in miscible blends, the melting point of the crystalline polymers is altered in comparison to pure component as the result of favourable thermodynamic interactions. In similar blends like poly(ethylene succinate) and poly(β-hydroxybutyrate) such shift was attributed to the miscibility of the two polymers [29]. However, in the studied PCL/PPSu blends the observations for the as received samples, did not allow to conclude for any clear correlation between melting point depression and polymer composition and thus in order to evaluate PCL/PPSu miscibility the equilibrium melting points should be taken into consideration, and not the apparent values.

The extent of melting point depression of the crystallizing component in polymer blends provides a measure of the interaction energy, as described by the Flory–Huggins theory of polymer miscibility [30]. Since reduction in melting temperature can be caused by both morphological and thermodynamic factors the effect of the high melting temperature polymer on the equilibrium melting temperature is examined next. The most popular method for the estimation of the equilibrium melting point of polymers is that of Hoffman–Weeks [31]. According to this procedure the measured melting temperatures (T_m) of samples isothermally crystallized at various temperatures (T_cs) are plotted against the crystallization temperatures. Linear extrapolation to the line $T_m = T_c$ gives an intercept equal to T_m^0. The associated equation is:

$$T_m = \frac{T_c}{2\beta} + T_m^0\left[1 - \frac{1}{2\beta}\right] \qquad (1)$$

where T_m^0 is the equilibrium melting temperature, and $\beta = l_c/l_c^*$ is the ratio of lamellar thickness of the mature crystal l_c at the time of melting to the thickness l_c^* of the nucleus. Therefore, β is supposed to be greater or equal to one. The factor 2 is because the thickness of the crystals undergoing melting is about double that of the critical thickness.

In Fig. 3 the Hoffman–Weeks plots for neat PCL as well PCL in its blends with PPSu are shown. Experimental factors such as scanning rate, crystallization temperature range and time of crystallization could affect the calculated values. Thus,

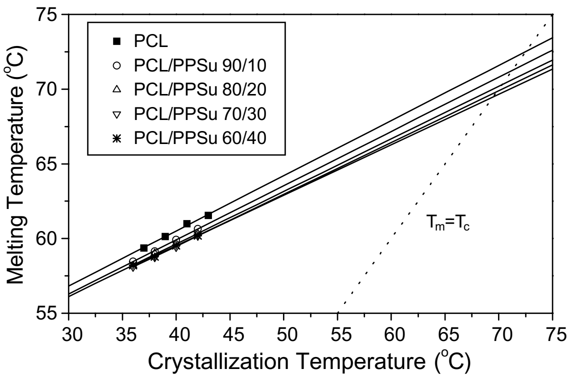

Fig. 3. Hoffman–Weeks extrapolation for the estimation of the equilibrium melting points of neat PCL and PCL in its blends with PPSu.

Table 2
Equilibrium melting points of PCL in its blends with PPSu obtained following the linear Hoffman–Weeks extrapolation

Blend composition PCL/PPSu (w/w)	T_m (°C)
100/0	72.57
90/10	70.81
80/20	70.30
70/30	69.84
60/40	69.50

similar conditions were used for the crystallizations and all melting temperatures were recorded at scanning at heating rate 20 °C/min. The T_m^0 values obtained for the blends are summarized in Table 2.

To analyze the effect of blending on the equilibrium melting temperatures the Nishi–Wang equation [27] based on the Flory–Huggins theory [30] can be used:

$$\frac{1}{T^0_{\mathrm{m(blend)}}}-\frac{1}{T^0_{\mathrm{m(pure)}}} = \frac{-R}{\Delta H^0}\frac{V_2}{V_1}\left[\frac{\ln\phi_2}{m_2}+\left(\frac{1}{m_2}-\frac{1}{m_1}\right)\phi_1+\chi_{12}\phi_1^2\right] \qquad (2)$$

where the subscripts 1 and 2 refer to the amorphous and the crystalline polymer, respectively. $T^0_{\mathrm{m(pure)}}$ and $T^0_{\mathrm{m(blend)}}$ denote the equilibrium melting points of the pure crystallizable component and that of the blend, respectively. V is the molar volumes of the repeating units of the polymers, R is the universal gas constant, ΔH^0 is the heat of fusion of the perfectly crystallizable polymer, m is the degree of polymerization, ϕ is the volume fraction of the component in the blend, and χ_{12} is the polymer–polymer interaction parameter. For high molecular weight polymers, both m_1 and m_2 are large and the related terms can be neglected:

$$-\frac{\Delta H^0 V_1}{RV_2}\left(\frac{1}{T^0_{\mathrm{m(blend)}}}-\frac{1}{T^0_{\mathrm{m(pure)}}}\right)=x_{12}\phi_1^2 \qquad (3)$$

If χ_{12} is assumed to be independent of the composition, a plot of the left-hand side of Eq. (3) versus ϕ_1^2 should give a straight line passing through the origin. In this study ΔH^0 for PCL was set equal to 136 J/g [25] and for PPSu 140 J/g [24]. The molar volumes of the components were calculated using the group contributions [32]. They were found to be $V_1 = 126.1$ cm^3/mol of the repeating unit of PPSu, and $V_2 = 104.3$ cm^3/mol of the repeating unit of PCL. The experimental data fit a line with a negative slope corresponding to $\chi_{12} = -0.11$, but not passing through the origin (see Fig. 4). The fact that the line does not pass through the origin is usually attributed to a residual entropy effect, which is neglected in the derivation of the equation [33,34]. Another explanation could be that the interaction parameter χ_{12} is composition dependent. Negative values of the interaction parameter have been reported for other crystalline/crystalline polymer pairs proving that these are thermodynamically miscible in the melt [35–37]. Thus, in the studied PCL/PPSu blends the result of equilibrium melting point determination showed some very limited miscibility.

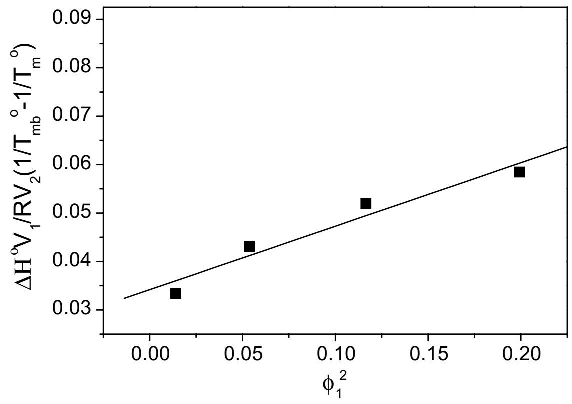

Fig. 4. Plot of $-\frac{\Delta H^0 V_1}{RV_2}\left(\frac{1}{T^0_{\mathrm{m(blend)}}}-\frac{1}{T^0_{\mathrm{m(pure)}}}\right)$ against ϕ_1^2 according to the Nishi–Wang analysis for melting point depression of PCL in the PCL/PPSu blends.

Furthermore, crystallization characteristics of the blends were investigated. All blends were isothermally crystallized at temperatures in the range between 36 and 42 °C. In such temperatures only PCL crystallizes. PPSu needs a large supercooling to crystallize. In practice, this happens below 20 °C. The tests showed that at the studied temperature range PCL crystallized at slower rates in the blends. The crystallization half times were estimated directly from the crystallization exotherms recorded as a function of time. Fig. 5 shows the variation of $t_{1/2}$ with crystallization temperature for the blends. It is obvious that $t_{1/2}$ increased with PPSu content for given temperature. It seems that crystallization of PCL is retarded because of the presence of the melt of the second component. This is in disagreement with the findings of PCL/PBSu blends, which were found to be immiscible [29]. In those blends, cold crystallization of PBSu was enhanced by the addition of PCL due to the positive effect of the previously formed PCL crystals on the PBSu nucleation. These differences with our PCL/PPSu blends could be attributed to the very low crystallization

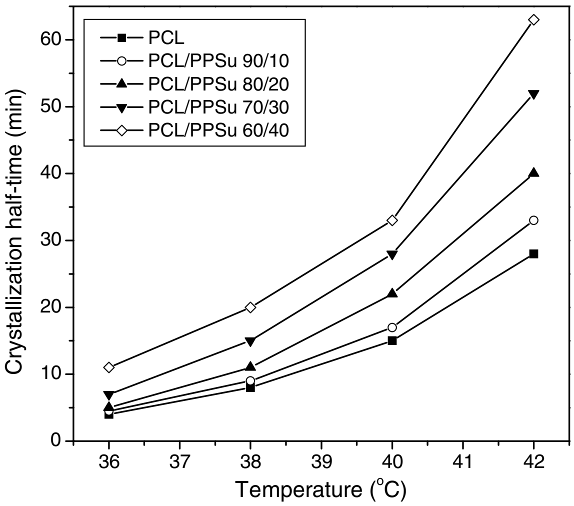

Fig. 5. Crystallization half-times for isothermal crystallization of PCL in the blends.

rate that PPSu has compared with PBSu or PCL [24].

POM observations showed that all blends create a homogeneous and transparent melt consisted from one phase. During cooling from the melt down to room temperature it was found that only PCL crystallizes. Pure PPSu needs extended time to crystallize at room temperature since its melting point is in fact a slightly higher (45 °C). In the blends crystallization of PPSu is confined due to the presence of PCL spherulites, which in practice cover a very high portion of the blend volume. Also, PCL was found to show decreased spherulite growth rates in the blends for crystallization at temperatures in the range of 35–45 °C.

From the above analysis it was verified that PCL/PPSu blends might have a limited miscibility in the melt state while after crystallization of each one component they are phase separated. In order to find out if these blends are compatible their mechanical properties were measured. The stress–strain behaviour of these blends, as shown from the respective characteristic curves, was intermediate of those of PPSu and PCL neat polymers. The break stress and strain of the blends varied with the composition, ranging between those of PCL and PPSu. The values decreased with increasing PPSu content, probably because for high PPSu content the dispersed PPSu rich phases within the PCL matrix were larger, resulting in discontinuity. PPSu due to its lower molecular weight has a very low tensile strength of almost 1.6 MPa and breaks before yielding. Thus in fact, its elongation at break is lower than 10%. On the other hand, PCL membranes cast after dissolving in chloroform have a break stress of 25.2 MPa and an elongation at break of 800–900%. These high values are the targeted properties for the blends. The variation of tensile strength and elongation at break of the prepared polymer blends with different compositions are presented in Fig. 6. As can be seen tensile values of blends are decreasing by increasing the PPSu amount. Similar is also the trend in elongation at break. For immiscible or incompatible blends tensile strength as well as elongation at break takes a minimum value, which in most cases appears at blend containing equal amounts (50/50 w/w) of each polymer. Furthermore, this minimum value could be even lower than the tensile strength values of both neat polymers. This is because especially for equal amounts of the components in the blends the different formed phases are quite large, resulting in brittleness. In the studied PCL/PPSu blends the absence of a minimum in both mechanical

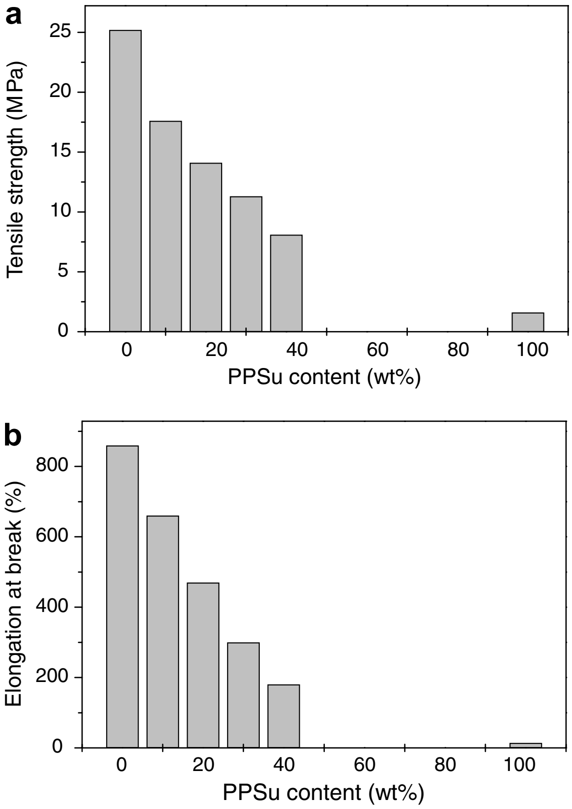

Fig. 6. Variation of mechanical properties with the PPSu content for neat polymers and all PCL/PPSu blends. (a) Tensile strength and (b) elongation at break.

properties may be an indication that the two polymers are partially miscible or compatible.

3.3. Biodegradability studies

3.3.1. Kinetics of enzymatic hydrolysis

Biodegradability of a certain polymer in the form of enzymatic hydrolysis is controlled by several factors. The most important is its chemical structure and the occurrence of specific bonds along its chains, like those in groups of esters, ethers, amides, etc. which might be susceptible to hydrolysis [38,39]. For aliphatic polyesters, hydrolysis rates are affected by the temperature, molecular structure, ester group density as well as by the species of enzyme used [40]. The degree of crystallinity may be a crucial factor, since enzymes attack mainly the amorphous domains of a polymer. Copolymers with low crystallinity show increased hydrolysis rates [9,41]. Biodegradation of PCL is caused by the hydrolytic cleavage of the ester groups and the primary factor for digestion is of course the molecular weight and the degree of crystallinity. As it can be seen in Fig. 7a the enzymatic hydrolysis of PCL is low at the conditions of the specific experiments. In the literature much higher enzymatic hydrolysis rates were reported [42–44]. The slower rates in the studied samples should be attributed to the different used enzyme – *R. delemar* lipase instead *Pseudomonas* lipase – and to the lower temperature (30 °C instead 37 °C). For the PCL/PPSu blends the weight loss is proportional to the PPSu content, with blends containing larger amounts of PPSu to exhibit higher enzymatic hydrolysis rates. Thus, the blend with 40 wt% of PPSu shows the highest rate and loses almost 11 mg/cm^2 after 5 weeks of enzymatic hydrolysis. This behaviour could be attributed to the highest biodegradation rate of PPSu compared to PCL. From our previous study, on PPSu enzymatic hydrolysis it was found that it shows high rates. PPSu is a semicrystalline polymer with very low degree of crystallinity [24]. Furthermore, PPSu compared with PCL has a larger number of ester groups per unit mass and thus cleavage of ester bonds is more probable than in PCL. This was also verified by measuring the absolute weight loss of neat polyesters without the use of lipase. Neat PPSu showed a weight loss less about 3 wt% and seemed to stabilize after 3 weeks of hydrolysis at 30 °C, while PCL had a negligible weight loss, less than 0.28 wt% at the same period (Fig. 7a). Such a small hydrolysis rate for PCL was also reported by Huang et al. [4].

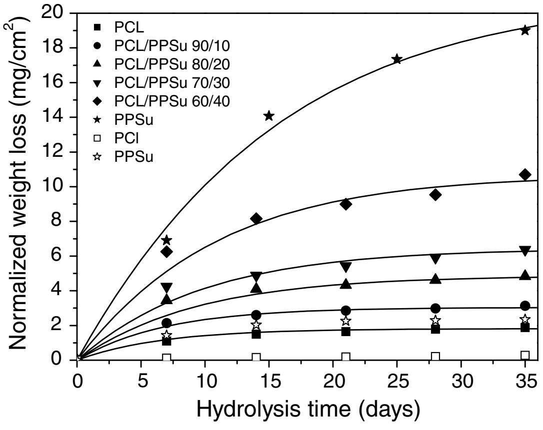

Fig. 7a. Weight loss versus time of PCL, PPSu and PCL/PPSu blends during enzymatic hydrolysis (filled symbols) and hydrolysis without the use of enzymes (open symbols).

In order to quantify these results a simple theoretical kinetic model was developed to predict the time evolution of the polymer weight loss. It should be pointed out that the derivation of a detailed mathematical model is rather complicated, since mass transfer phenomena (between the solid-polymer phase and the enzyme) occur in parallel to the chemical reaction. This model is formally based on the kinetic mechanism of enzymatic hydrolysis according to the Michaelis–Menten scheme:

$$\mathrm{E}+\mathrm{S} \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} \mathrm{ES} \tag{4}$$

$$\mathrm{ES} \xrightarrow{k_2} \mathrm{P}+\mathrm{E} \tag{5}$$

where E and S represent the enzyme and substrate-polymer, respectively and ES is the enzyme/substrate complex, finally, P is used to denote the hydrolysis reaction products.

According to this kinetic scheme the mass balance of the substrate – polymer can be expressed from:

$$\frac{\mathrm{d}C_\mathrm{S}}{\mathrm{d}t} = -k_1 C_\mathrm{E} C_\mathrm{S} + k_{-1} C_\mathrm{ES} \tag{6}$$

However, since reaction (4) involves a solid surface it is better to transform Eq. (6) in surface area terms:

$$\frac{\mathrm{d}(A(1-\theta))}{\mathrm{d}t} = -k_1 C_\mathrm{E} A(1-\theta) + k_{-1} A\theta \tag{7}$$

where A is the substrate surface area, θ is the fraction of substrate occupied by the ES complex,

$(1-\theta)$ is the "free" surface fraction and C_{E} is the enzyme concentration. The same terminology has been also used in the literature [45].

Assuming that the substrate surface area, A, is proportional to the free surface fraction powered to a constant value, α, according to $A = A_0(1-\theta)^a$, Eq. (7) can be derived as:

$$(1+\alpha)\frac{\mathrm{d}(\theta)}{\mathrm{d}t} = k_1 C_{\mathrm{E}} - (k_1 C_{\mathrm{E}} + k_{-1})\theta \tag{8}$$

Finally, assuming C_{E} to be constant, Eq. (8) can be integrated to give:

$$\theta = \frac{k_1 C_{\mathrm{E}}}{k_1 C_{\mathrm{E}} + k_{-1}}\left\{1 - \exp\left[-\left(\frac{k_1 C_{\mathrm{E}} + k_{-1}}{1+\alpha}\right)t\right]\right\} \tag{9}$$

The experimentally measured normalized weight loss in mg/cm^2, $M_{\mathrm{S0}} - M_{\mathrm{S}}$ can be correlated to θ according to the following equation:

$$M_{\mathrm{S0}} - M_{\mathrm{S}} = \frac{N_{\mathrm{S0}}}{A_0} - \frac{N_{\mathrm{S}}}{A} \tag{10}$$

where the symbol N is used to denote the substrate mass and the subscript 0 refers to initial conditions (i.e. time equal to zero).

Using the expression for the surface area A reported previously, Eq. (10) is transformed to:

$$M_{\mathrm{S0}} - M_{\mathrm{S}} = \frac{N_{\mathrm{S0}}}{A_0}\left[1 - (1-\theta)^{1-a}\right] \tag{11}$$

If a is set equal to zero a constant surface area during the reaction is assumed, furthermore, if α is set equal to 1 a constant normalized weight loss is obtained. In this study the arithmetic mean value was considered, i.e. $\alpha = 0.5$, since also this value led to better experimental data fitting.

Table 3
Kinetic and physical parameters determined by fitting equations (9) and (11) to the experimental data of enzymatic hydrolysis of the blends studied

Polymer sample	k_1 (mL/mg days)	k_{-1} (days^{-1})	N_{S0}/A_0 (mg/cm^2)
PCL	0.021	0.210	39
PCL/PPSu 90/10	0.030	0.195	44
PCL/PPSu 80/20	0.033	0.135	47
PCL/PPSu 70/30	0.039	0.123	50
PCL/PPSu 60/40	0.054	0.097	54
PPSu	0.055	0.055	72

Eqs. (9) and (11) can then be used to fit the experimental data of the normalized substrate weight loss as a function of time for the polymer blends used. In these equations three different fitting parameters were identified, namely, k_1, k_{-1} and N_{S0}/A_0. It was decided to keep the initial normalized substrate mass, N_{S0}/A_0, as a fitting parameter in order to have a fitting estimation of the initial polymer mass, which actually is biodegradable. A non-linear curve-fitting algorithm was used based on the Levenberg–Marquardt method and the best fitting values for the parameters are presented in Table 3. From the results reported in this Table it was obvious that as the amount of PPSu in the PCL/PPSu blends was increased an increase in the right-direction kinetic rate constant, k_1 was obtained with the reverse effect observed in the left-direction rate constant, k_{-1}. This means that increased amounts of PPSu in the blends leads to polymers more susceptible to enzymatic attack. Furthermore, the best fitting value of the initial normalized substrate mass, N_{S0}/A_0, taking part in the enzymatic hydrolysis was found to increase with the amount of PPSu in the blends. In order to test if the values of N_{S0}/A_0 could be correlated with the % amount of the amorphous phase in the PCL/PPSu blends, initially the % crystallinity of the blends was estimated. This task was accomplished by taking the values of 62% and 32% for the neat PCL and PPSu reported in Section 3.1 and assuming a linear correlation with the % amount of each polymer in the blend. Following, a Figure was constructed (Fig. 7b) by correlating the estimated N_{S0}/A_0 value for each blend with the corresponding calculated % amount of the amorphous phase (% amorphous phase = 100 – % crystalline phase). Very surprisingly an almost linear correlation was observed. Hence the assumption that the effective material mass taking place in the

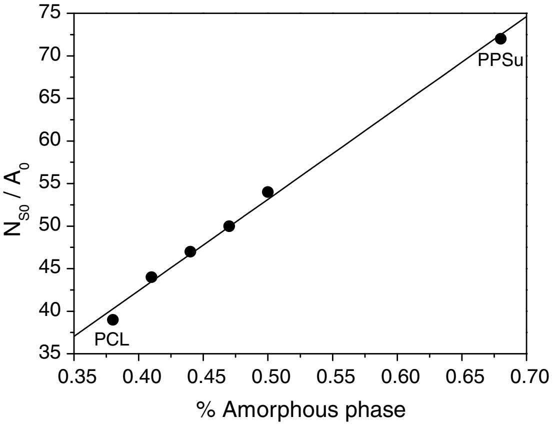

Fig. 7b. Initial normalized substrate mass taking part in the enzymatic hydrolysis as a function of the % amount of the amorphous phase in the PCL/PPSu blends.

degradation, is that of the amorphous polymer was confirmed.

3.3.2. Morphological features of the enzymatic hydrolyzed blends as observed with SEM

Weight loss offers a general trend about the rate of enzymatic hydrolysis but information on how the hydrolysis proceeds is not provided. For this reason morphological examinations are necessary. In Fig. 8a, SEM micrographs of PCL during enzymatic hydrolysis at various times are presented. As can be seen the extent of hydrolysis is very small. Only at specific areas a few holes were created and cracks were obvious on the film surface. However, some other parts still remained unaffected. As it is well known enzymatic hydrolysis is a heterogeneous process. Enzymes are attached on the surface of an insoluble substrate and hydrolysis takes place via surface erosion. In general, the interior of polyesters specimens is not attacked until extended holes are created on the surface allowing the enzymes to enter and attack the main body. For similar aliphatic polyesters like PBSu and its copolymers, the surface initiated enzymatic degradation occurs in a maximum depth of 0.22 μm [46]. Thus, hydrolysis rates decreased after consumption of the amorphous material of the surface. Afterwards, a layer of crystalline domains remains, where only slow degradation may occur. This was also verified for neat PCL, though its crystalline zone can also be degraded by lipase [43]. As can be seen in Fig. 8a(iii) the crystallites can be very well separated from the

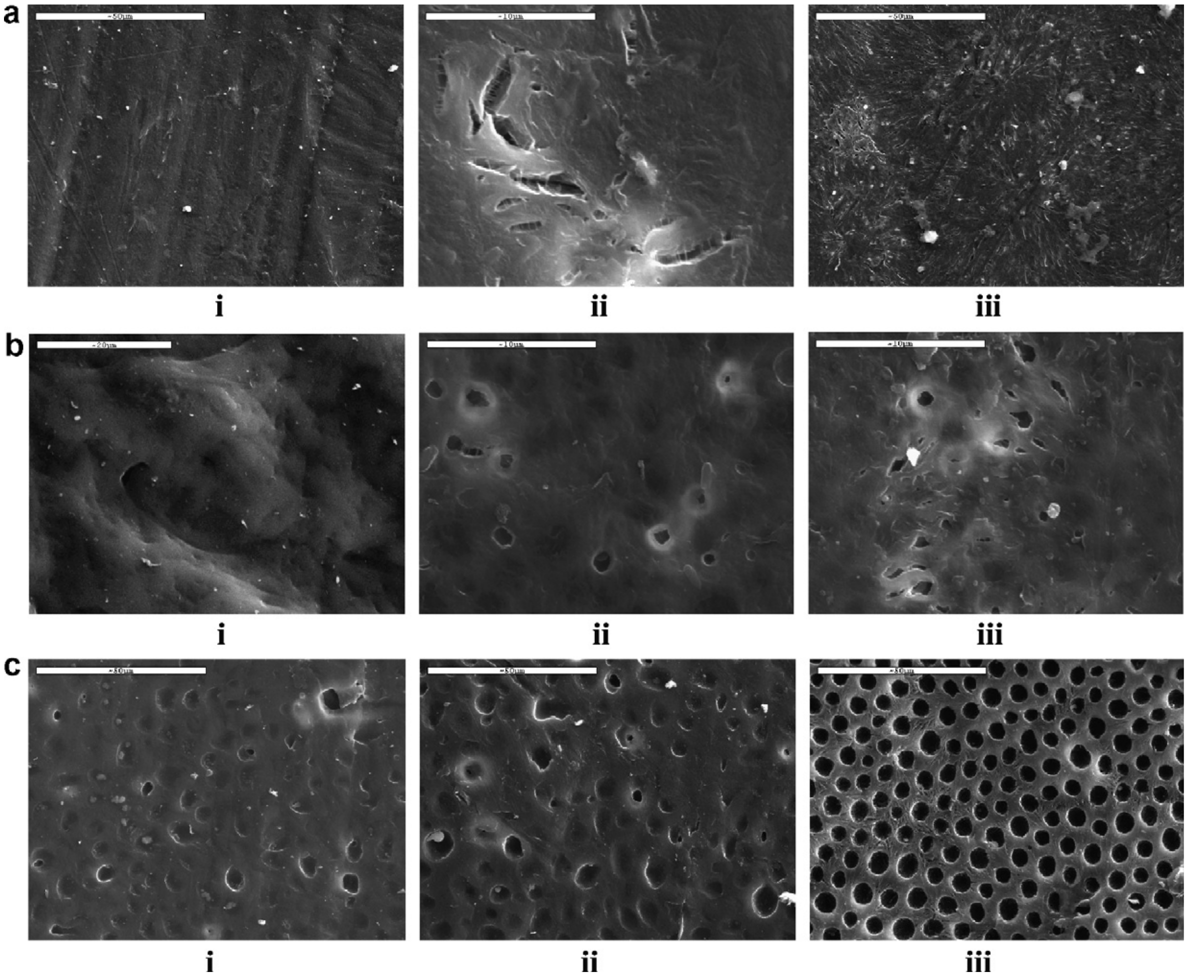

Fig. 8. (a) SEM micrographs of PCL during enzymatic hydrolysis at several times. (i) 0 weeks, (ii) 3 weeks and (iii) 5 weeks. (b) SEM micrographs of PCL/PPSu blend containing 20 wt% PPSu during enzymatic hydrolysis after (i) 0 weeks, (ii) 1 week and (iii) 5 weeks. (c) SEM micrographs of PCL/PPSu blend containing 40 wt% PPSu during enzymatic hydrolysis after (i) 0 weeks, (ii) 1 week and (iii) 5 weeks.

other parts after 5 weeks of enzymatic hydrolysis and remain almost unaffected. In principle, hydrolysis affected the amorphous material surrounding the spherulites and numerous holes were then created in the respective.

In the PCL/PPSu blends the behaviour was somewhat different. Spherical holes appeared in contrast to the irregular shapes formed in the case of neat PCL. This was also a proof that PPSu degrades faster than PCL, as has already been concluded from weight loss measurements. It has been reported that degradation is favoured in the case of low crystallinity [47]. By increasing the amount of PPSu, which is the component with lower crystallinity in the PCL/PPSu blends, the signs of degradation on the film surfaces became more obvious (Figs. 8b and c). The created holes due to the subtracted material progressively increased. This removable material corresponds to oligomers produced during enzymatic hydrolysis. Most probably, the appearance of holes is the result of the removal of hydrolysed PPSu, which in the blends corresponds to the dispersed phase. This is more clearly illustrated in Fig. 8c where the micrographs of PCL/PPSu blend containing 40 wt% PPSu are presented. Also, weight loss measurements for the specific blend showed the highest biodegradation rate. However, in this blend some holes can be also observed in the initial sample, due to the higher phase separation and solvent evaporation. Similarly, appearance of holes was also mentioned for poly(L-lactide)/poly(ε-caprolactone) blends [43]. To verify that the PPSu is more rapidly degraded than PCL ^{1}H NMR studies of the film surfaces were taken place. As can be seen in Fig. 9, where the PCL/PPSu blend containing 20 wt% PPSu is presented, by increasing the time of enzymatic hydrolysis the PPSu peaks at 1.9–2.03, 2.63 and 4.29 ppm are progressively decreased.

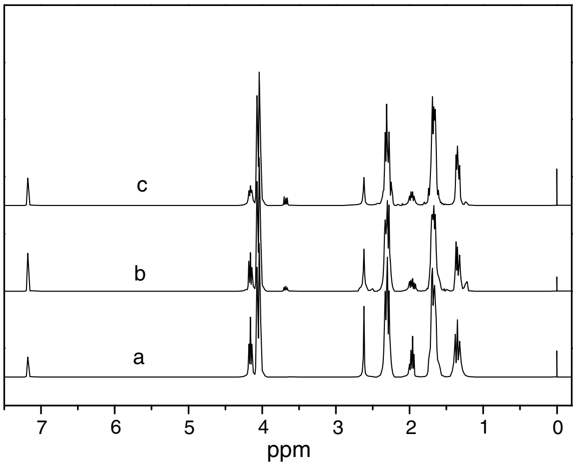

Fig. 9. ^{1}H NMR spectra of PCL/PPSu 80/20 w/w blend at different enzymatic hydrolysis times. (a) 0 weeks, (b) 3 weeks and (c) 5 weeks.

After hydrolysis of PPSu the remaining holes are an indication for the extent of mixing or phase separation in the crystallized blends. PPSu, which is the minor component, is dispersed into PCL matrix in the form of micrpspheres. The dispersed phase size increased gradually with PPSu content, from less than 1 μm in the blend with 10 wt% PPSu, to more than 2 μm in diameter in the case of 40 wt% PPSu. Thus the compatibility between PCL and PPSu, even though they are both aliphatic polyesters, decreases with increasing PPSu content.

3.3.3. *Thermal and WAXD characterization of the hydrolyzed blends*

Physical characterization of the samples subjected to enzymatic hydrolysis was further investigated using DSC and WAXD. In the DSC thermograms two well-defined melting peaks were recorded for all samples, as well as for the initial polymers. As it can be seen in Table 4 the melting

Table 4
Variation of the melting points (°C) during enzymatic hydrolysis of PCL/PPSu blends

PCL/PPSu (w/w)	Melting points (°C)											
	0 Weeks		1 Weeks		2 Weeks		3 Weeks		4 Weeks		5 Weeks	
	T_{m1}	T_{m2}	T_{m1}	T_{m2}	T_{m1}	T_{m2}	T_{m1}	T_{m2}	T_{m1}	T_{m2}	T_{m1}	T_{m2}
100/0	–	67.2	–	67.7	–	68.3	–	69.2	–	70.4	–	70.3
90/10	44.5	65.6	46.1	65.3	47.9	66.2	49.2	66.8	51.3	68.3	52.6	68.2
80/20	44.8	66.3	46.5	66.7	48.1	67.2	51.9	66.3	52.4	67.5	53.9	68
70/30	44.2	65.8	46.6	66.2	48.0	66.1	50.7	66.5	52.9	67.1	54.2	67.8
60/40	45	65.5	47.5	65.3	48.8	66.2	51.3	66	53.2	66.5	55.5	67.2

Subscripts 1 and 2 denote the two components PPSu and PCL, respectively.

points of both polymers PCL and PPSu used for blends preparation were shifted to higher values when the time of enzymatic hydrolysis was increased. This behaviour could be attributed to the crystallinity increase in accordance with previous studies reported for PCL blends [4]. Furthermore, it is associated with the annealing that takes place at the temperature of enzymatic hydrolysis. PPSu has a melting point almost 15 °C above this temperature, while PCL approximately 37 °C higher.

Furthermore, as revealed from SEM micrographs, amorphous parts degraded first. Thus, the crystallinity of the samples is expected to increase with enzymatic hydrolysis time due to the consumption of the amorphous material of each polymer specimen. This could be supported from measurements of the heat of fusion of the samples. A slight increase was found in the heat of fusion of PCL in the blends. In contrast the heat of fusion for the PPSu component decreased showing that mainly PPSu was degraded.

Further evidence that crystallization increases during enzymatic hydrolysis was supported from WAXD measurements (Fig. 10). Pure PPSu showed an amorphous background, due to its low crystallinity. Strong diffraction peaks at angles $2\theta = 19.26°$ and 22.19°, as well as weak diffraction peaks of $2\theta = 18.3°$, 20.24°, 25.72°, and 34.13° were observed. In the case of pure PCL patterns several diffraction peaks at angles $2\theta = 21.31°$ (strong), 21.9° (weak) and 23.67° were recorded. Examining the WAXD patterns of PCL/PPSu blends it was evident that they were consisted from the corresponding diffraction peaks of both polymers. In general, in crystalline/crystalline blends, the components are crystallized separately [22] and the peak intensities depend on the amount of each polymer in the blend. It should be noted, that new diffraction peaks or some shifts of them were not observed. This is an indication that co-crystallization between the two components or any modification of the unit cell parameters of the neat polymers should be excluded. From the WAXD patterns of all blends, it was clear that the characteristic peaks of each component were increased during enzymatic hydrolysis, meaning that the degree of crystallinity of both PCL and PPSu components was also increased (Fig. 10). This is in full agreement with the DSC data reported previously.

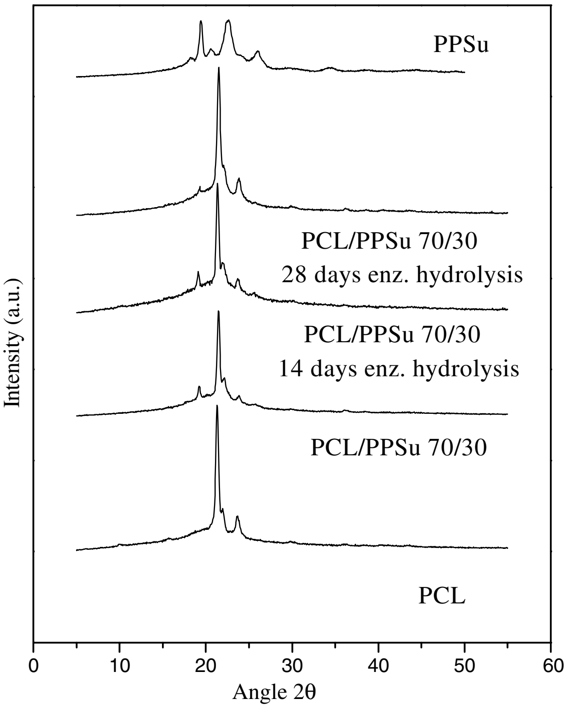

Fig. 10. X-ray patterns of the studied PCL/PPSu 70/30 w/w blend during enzymatic hydrolysis.

4. Conclusions

PCL/PPSu crystalline/crystalline blends were found to show only a very limited miscibility, as the melting point reduction indicated. Both components crystallized in the blends. However, there is large difference in their crystallization rates, meaning that PCL crystallizes much faster and the PPSu can only crystallize in a confined environment. This fact results in phase segregation in the crystallized samples. POM study showed that the spherulitic growth rates decreased with increasing PPSu content. PPSu spherulites could not be observed at all. SEM pictures revealed phase segregation in the crystalline specimens, with the average dispersed PPSu phase size to increase with PPSu content in the blends. The enzymatic hydrolysis rates of the blends increased with increasing the PPSu content, which is the component with lower crystallinity and faster hydrolysis rates. A simple kinetic model was developed to analyse the hydrolytic behaviour of the blends on the basis of the Michaelis–Menten scheme. The degradation rates showed a straight dependence on the crystallinity of the samples. On the other hand, the tensile properties were found to decrease with increasing PPSu content. After all, blends with up to 20 wt% PPSu show sufficient degradation rates and at the same time retain the high values of the tensile properties of PCL.

Acknowledgment

This work was funded by the Greek Ministry of Education (EPEAEK Pythagoras II 80922).

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