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Novel Biodegradable Poly(pentadecalactone-*co*-oxo-crown ether) Studied with Solid-State ^{1}H and ^{13}C NMR

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Summary: Two grades of a novel biodegradable copolymer of ω-pentadecalactone (PDL) and 2-oxo-12-crown-4 (OC), with respective molar compositions of 77/23 and 52/48, were characterized with 1D and 2D MAS ^{1}H and ^{13}C NMR spectroscopy. The results indicate that both copolymers are semi-crystalline with PDL divided over the crystalline and amorphous phase, and OC exclusively located in the amorphous phase. Proton T_2 and $T_{1\rho}$ relaxation confirm the existence of small crystalline domains.

Keywords: biomedical; copolyester; MAS; proton relaxation; WISE

Introduction

Aliphatic polyesters are biodegradable and therefore useful in several medical applications. Their stability under physiological conditions depends on the chemical properties of the specific monomers of which they are composed, as well as on the polymer nanostructure. Hydrophilic polyesters degrade more easily than hydrophobic ones, and water penetrates more readily into amorphous than crystalline domains.[1] Comparative degradation studies of structurally related systems, like poly(ε-caprolactone) and poly(1,5-dioxepan-2-one), suggested to us that the introduction of an ether function enhances the hydrolytic instability.[2] Rather than preparing *homo*-polyesters from monomers containing an ether function we have focused on *co*polymers of aliphatic lactones and oxo-crown ethers. The comonomer ratio in such copolymers offers a convenient tool for optimally tuning the biodegradability according to the specifications required for selected medical applications. We selected lipases as the catalyst of choice for the ring-opening polymerizations (ROP) of lactones. Lipases are convenient catalysts to obtain well-defined polyesters and provide random copolymers upon copolymerization of different lactones.[3] Our present study involves statistical copolymers of ω–pentadecalactone (PDL) and 2-oxo12-crown-4 (OC) synthesized with Novozym 435, *i.e.* Lipase B from *Candida antartica*, as the catalyst (Scheme 1).

In this communication we focus on solid-state NMR experiments of two grades of poly(PDL-*co*-OC) with molar PDL : OC ratios of ca. 3 : 1 and 1 : 1, respectively. Recently recorded high-resolution ^{1}H NMR spectra of the copolyesters dissolved in $CDCl_3$ are characteristic of a random copolymer.[4] DSC shows that the materials are semi-crystalline. The glass transition temperature of the amorphous phase, $T_g \sim -37 \pm 2\,^\circ C$, is fairly independent of the molar PDL/OC ratio. In contrast, the melting temperature T_m of the crystalline phase increases with increasing PDL content: 68 and 85 °C for molar PDL fractions of 0.52 and 0.77, respectively. These values are lower than the melting point of 97 °C for the homopolymer poly(PDL).[5] This could indicate that PDL and OC are both

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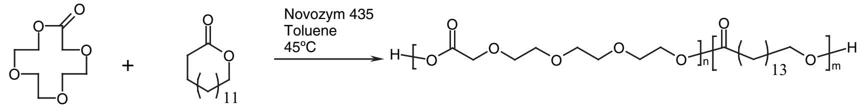
△ Footnote: Due to a mistake at the publishers Brahim Mezari's first name was misspelled in the pdf-file. We apologize for this error.



Scheme 1.
Lipase-catalyzed ring-opening copolymerization of ω-pentadecalactone (PDL) and 2-oxo-12-crown-4 (OC).

incorporated into the crystalline phase, and that the changing composition lowers T_m. It is hard to imagine, however, how random stretches of different monomers as PDL and OC units can be packed into a regular crystal structure. An alternative explanation would be that the crystalline domains in the copolymer consist of only PDL units, and are fairly small with sizes determined by the OC content. Obviously, an increased OC content lowers the probability of long homo-PDL sequences in the statistical copolymer. According to the Gibbs-Thomson relation small crystalline domains have size-dependent melting temperatures lower than those of large crystalline volume elements.[6]

We have tested this hypothesis by means of solid-state NMR spectroscopy. Our approach is based on the fact that at room temperature the chain mobility in the amorphous and crystalline phase strongly differs. Separately probing the mobility of the PDL and OC monomers in the copolymer by use of two-dimensional ^{1}H-^{13}C NMR thus yields the distribution of the two comonomers over the two phases. The results discussed below illustrate the use of solid-state NMR for investigating nanostructure and dynamics in heterogeneous polymer systems.[7]

Materials and Methods

Poly(PDL-*co*-OC) was synthesized by lipase-catalyzed ring-opening polymerization at 60 °C in toluene, similar as described previously.[8] Novozym 435 was obtained from Novozymes A/S. The synthesis of OC is described elsewhere.[9] All other chemicals were purchased from Aldrich. Relevant data for the two poly(PDL-*co*-OC) grades employed in the present study are summarized in Table 1.[4]

^{1}H-decoupled ^{13}C NMR spectra were recorded on a Bruker DMX500 spectrometer with a ^{13}C NMR frequency of 125.13 MHz. A 4-mm magic-angle-spinning (MAS) probehead was used with a sample-rotation rate 8 kHz. The radio-frequency power was adjusted to obtain 5-μs 90-degree pulses both for the ^{1}H and ^{13}C nuclei. All ^{13}C NMR spectra were recorded by use of standard ^{1}H-^{13}C crosspolarization with an amplitude-modulated contact pulse of 1 ms. For 2D ^{1}H-^{131}C CPMAS spectra we used the standard WISE pulse sequence with a ramped contact pulse, as well.[10] T_{2H}-filtered CPMAS spectra were obtained with the Hahn-echo proton pulse sequence $90^\circ - \tau/2 – 180^\circ - \tau/2$- preceding this contact pulse. $T_{1\rho}(^1H)$- filtered CPMAS spectra were measured with a proton lock pulse preceding the contact pulse. Static T_{2H} decays were measured with the $90^\circ - \tau/2 – 180^\circ - \tau/2$- sequence.

Results and Discussion

To determine the PDL and OC distribution over the crystalline and amorphous phase in the copolyesters we have carried out two-dimensional (2D) magic-angle

Table 1.
Comonomer molar ratio PDL/OC, molecular weight (M_n), glass transition temperature (T_g) and melting temperature (T_m) of poly(PDL-co-OC).

Polymer	PDL/OC (mol/mol)[a]	M_n (kg/mol)[b]	T_g (°C)[c]	T_m (°C)[c]
1	52/48	9.7	−39	68
2	77/23	12.2	−35	85

[a] from ^{1}H NMR; [b] from GPC, relative to polystyrene standards; [c] from DSC.

spinning (MAS) ^{1}H-^{13}C NMR. This technique, based on ^{1}H-^{13}C cross-polarization,[7] combines the high chemical resolution in ^{13}C NMR with the chain-mobility information in ^{1}H NMR. At intermediate sample rotation rates, the chain mobility is reflected by the ^{1}H NMR line width. Immobile polymer chains in the crystalline or glassy domains give rise to broad lines, whereas mobile polymer in rubbers have relatively narrow proton resonances.

As for the ^{13}C NMR dimension (vertical in Fig. 1), rotating the solid sample about the magic angle (54°) with respect to the magnetic field yields sufficiently narrow resonances to distinguish between PDL and OC signals. The resonance pattern in the aliphatic range below 40 ppm exclusively consists of PDL signals. The major signal at 31 ppm contains most of the $^{13}CH_2$ signals from PDL, except the one at ~27 ppm from the CH_2 next to the ω position, $-^{13}\underline{C}H_2CH_2OC(O)-$. The signal at 33 ppm is more intense than consistent with the liquid-state spectrum of the copolymer dispersed in $CDCl_3$. Similar to ^{13}C NMR spectra of semi-crystalline polyethylene[11] and polyamide,[12,13] we ascribe this additional signal to all-trans CH_2 stretches of PDL units densely packed within the crystalline phase. The signal at 71 ppm exclusively contains the CH_2 resonances from the OC units. The signal at 67 ppm contains both resonances from PDL and OC $^{13}CH_2$ moieties at the respective ω positions, $-\underline{C}H_2OC(O)-$, and is useless for separating mobility information. Comparing the individual ^{1}H NMR traces of the protons connected to the ^{13}C nuclei resonating at 31, 33 and 71 ppm (Fig. 1), we see that there is both mobile and immobile PDL present in copolymer **2**. Most protons connected to the carbons resonating at 33 ppm have a broad lineshape, as consistent with rigid polymer chains in the crystalline phase. The ^{1}H NMR lineshape associated with the 31 ppm is predominantly narrow, reflecting the high chain mobility in the amorphous phase. The lineshape separation is not complete. There is a minor narrow ^{1}H NMR signal visible in the crystalline trace, and broad foot in the amorphous trace (Figure 1), which could result from the overlap between 31- and 34-ppm signals in the ^{13}C NMR direction. Alternatively, there may be some amorphous-phase signals around 34 ppm, and some crystalline-phase signals around 31 ppm.[12] Irrespective of the detailed lineshape analysis, however, the association of both broad and narrow ^{1}H NMR lineshapes with the aliphatic ^{13}C NMR signals indicates that PDL is distributed over the amorphous and crystalline phase. In contrast the ^{1}H NMR line of the OC protons reflects highly

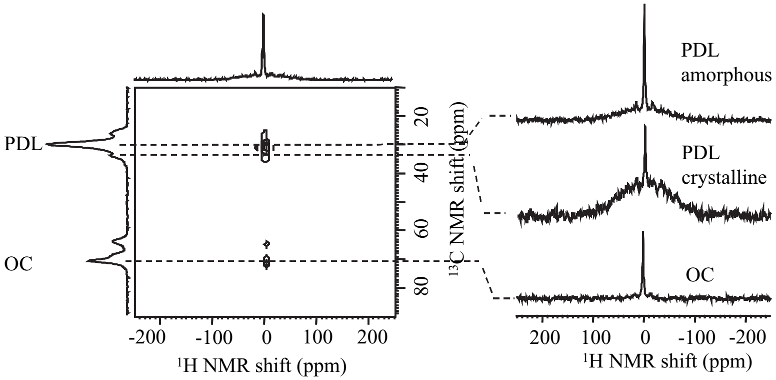


Figure 1.
(left) 2D MAS ^{1}H-^{13}C NMR spectra of **2** to separate chain-mobility information for the PDL and OC units in the copolymer. (right) relevant ^{1}H NMR traces reflecting the respective mobilities.

mobile polymer chains only, indicating that OC is just present in the amorphous phase.

Rigid polymers have shorter proton transverse-relaxation times T_{2H} than mobile polymers. In fact, for polymer ^1H NMR lineshapes dominated by ^1H-^1H dipole coupling the line width $\Delta\omega_H$ in the frequency domain and the relaxation time T_{2H} in the time domain are Fourier conjugates. Detailed insight into the chain dynamics of the different comonomers in the copolymer can thus also be obtained from T_{2H}-filtered ^1H-^{13}C cross-polarization. Recording a few 1D spectra at relevant T_{2H}-filter times is more time-efficient than obtaining a full 2D data set. Figure 2 shows the effect of T_{2H} filtering on the cross-polarization spectra from the two copolymers. For comparison the spectra have been normalized to the peak height at 31 ppm.

Three types of chain mobility can be recognized. The PDL signal at 33 ppm marked with an "S" in Figure 2 is practically eliminated at a filter time of 240 μs, as consistent with rigid polymer chains in the crystalline phase and their short T_{2H} value. The OC signal at 71 ppm (marked L) decays only slowly, reflecting the high mobility of these polymer chains in the amorphous phase well above T_g. There is no sign of any quickly decaying OC signal, as could, in principle, arise from any OC in the crystalline phase. The PDL signal marked M at 31 ppm shows intermediate T_{2H}-relaxation behavior. The relaxation difference between the medium and long component is most evident for copolymer **2** (Figure 2), where the height of the OC signal clearly increases with respect to that at 31 ppm at increasing filter time. The observed effect for copolymer **1** is similar, but smaller. The different T_{2H} relaxation of PDL and OC in the amorphous phase indicates that their respective chain mobilities are not the same.

The above 1D and 2D NMR spectra are powerful in *qualitatively* illustrating the different mobilities of PDL and OC in the semi-crystalline copolymer. Since they are based on ^1H-^{13}C cross-polarization, however, a reliable *quantitative* analysis is not trivial. Cross-polarization tends to overemphasize the rigid polymer components in the ^{13}C NMR spectra. Thus, one cannot, for instance, simply deconvolute the aliphatic region to obtain the exact distribution of PDL over the two phases. Other ^{13}C NMR techniques, based on direct excitation instead of crosspolarization, suffer from the long ^{13}C NMR spin-lattice relaxation time of the crystalline phase (not shown). Apart from that, magic-angle spinning also modulates the effective T_{2H} behavior, from which we estimate the chain mobility. At sample rotation rates >20 kHz (faster than the 8-kHz MAS rate used in our experiments) the dipole inter-

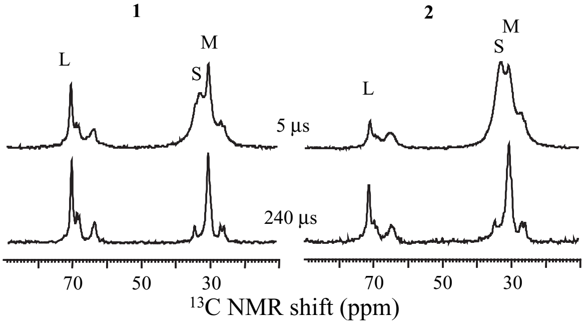


Figure 2.

T_{2H}-filtered ^1H-^{13}C CPMAS spectra of the two copolymers **1** and **2** at filter-times of 5 and 240 μs. The signals at 31, 33 and 71 ppm have been labelled according to their relative T_{2H} relaxation times: M(edium), S(hort) and L(ong), respectively.

actions between the protons become suppressed even for the rigid polymer parts, resulting in longer apparent T_{2H} values which could be misunderstood as a sign of high chain mobility.

For a quantitative analysis we have therefore carried out static proton-observed T_{2H} measurements. Since this technique does not involve cross-polarization or magic-angle spinning, the results are truly quantitative. The trade off, however, is the lack of spectral resolution, and the ambiguity in the interpretation of multi-component decays. Static ^{1}H NMR lineshapes consist of a relatively narrow line with a width of ~10 kHz on top of a broad foot with a half-height width of ~50 kHz (not shown). Like in MAS ^{1}H NMR traces (Figure 1), the first arises from mobile polymer chains in the amorphous phase, while the latter reflects the rigid chains in the crystalline phase. Figure 3 illustrates the T_{2H} decays of the total spectral integral for the two copolymers. Copolymer **2** shows faster relaxation than **1**, indicating that **2** has less overall chain mobility, in accordance with its higher PDL content, and resulting higher crystallinity. The curves cannot be described by simple mono- or bi-exponential decays. Models with three exponential components fit well to the relaxation data, but suffer from big correlation errors in the fit parameters. In addition to the spectral integral, however, we may use the lineshape information. Since the wings of the static ^{1}H NMR line ($\Delta\nu > 25$ kHz) purely arise from the crystalline phase, we are able to determine its relaxation component separately (Figure 3 inset). The corresponding wing integral shows fast dipolar oscillations, typical of the strong CH_2 proton pair interaction in the crystalline phase. As an emperical Ansatz we use a function of the form:

$$D(t) = A\exp(-a\,t) + B\exp(-b\,t)\sin(c\,t)/(c\,t) \qquad (1)$$

with phenomenological fit parameters A, B, a, b and c ($A + B = 1$) to describe the decays of the wing intensity. With "fixed" D(t), we then fit the total-integral decays by a tri-component model:

$$R(t) = f_S D(t) + f_M \exp(-t/T_{2M}) + f_L \exp(-t/T_{2L}). \qquad (2)$$

By assumption, the *central* part of the crystalline line component decays in the same way as its wings, or hardly contributes to the total intensity. Table 2 shows the resulting fractions f_S, f_M, and f_L, as well as the medium and long T_{2H} components T_{2M} and T_{2L} together with their error margins.

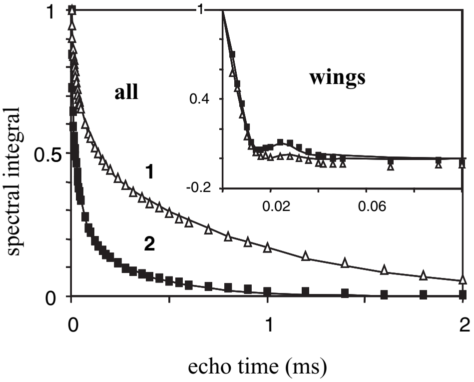


Figure 3.
Proton transverse-relaxation decays of the total spectral intensity for copolymers **1** and **2** without sample spinning as a function of the Hahn-echo time. The inset shows the Hahn-echo decay for the spectral wings (frequency offset $|\Delta\nu| > 25$ kHz), which is modulated by dipolar oscillations at a timescale of ~25 μs.

Table 2.
Fit of a tri-component decay model to the T_{2H} decays of **1** and **2** (Figure 3).

Component	**1**		**2**	
	fraction ± 0.05	T_{2H} (ms) ± 15%	fraction ± 0.07	T_{2H} (ms) ± 20%
S	0.19	–	0.47	–
M	0.33	0.063	0.33	0.042
L	0.48	0.87	0.20	0.32

Despite the good data quality and the incorporation of lineshape information, the possible errors are still quite substantial as a result of correlation effects. Within the indicated error margins, the model still fits within 130-% of the standard deviation of the best fit. In addition, inaccurate determination of D(t) could result in a systematic error.

It may seem reasonable to identify the fast, intermediate and slow components in the static T_{2H} decays with the corresponding ones in the T_{2H}-filtered ^{13}C NMR spectra (Figure 2), and accordingly assign these to crystalline PDL, amorphous PDL and amorphous OC, respectively. Indeed, there is a nice correlation between the longest-T_{2H} fractions, 0.48 and 0.20, and the OC content, 0.48 and 0.23 for **1** and **2**, respectively. However, these numbers must be corrected by a factor 2, because of the different proton numbers per OC and PDL monomer. This reduces the correspondendence, and indicates that a simple three-phase picture with a crystalline PDL phase, a mobile amorphous OC phase, and another less mobile, amorphous PDL phase does not apply. A more realistic model should also include mobility gradients along the polymer backbone. For instance, the local rotational mobility of PDL CH_2 moieties directly flanking the ester function may be quite different from those farther away in the sequence.

To estimate the size of the crystalline domains we have measured proton spin-lattice relaxation in the rotating frame, $T_{1\rho}\{^1H\}$. The protons in a polymer material form a dipolarly coupled network. As a result of this coupling, a perturbation of the proton magnetization in one volume element will diffuse into the surrounding area. This phenomenon is called spin diffusion. It is characterized by a diffusion coefficient D, which for rigid aliphatic polymers typically is in the order of 1 nm^2/ms,[7] but is much lower for mobile, or less proton-containing polymers. As a result, proton relaxation measurements tend to yield mobility information averaged over a diffusion length scale $\sim\sqrt{6DT}$, where T indicates the relaxation-time value of the specific NMR relaxation under study. The above mentioned T_{2H} relaxation, e.g., tends to be fast on the ms time scale, and is thus a highly localized mobility probe. The generally slower $T_{1\rho}\{^1H\}$ relaxation (1–10 ms) allows more spatial averaging (up to typically 10 nm), and can thus be used to study phase separation in semi-crystalline polymers. At temperatures between T_g and T_m there is a big contrast between polymer chain mobility in the two phases, which, in principle, causes a large difference between the intrinsic $T_{1\rho}$ values. If, however, the crystalline domains are small as compared to spin-diffusion length scale, the intrinsic relaxation differences average out, and all the protons in the polymer show the same average relaxation behavior. This is actually the case for the two copolymers, as well. Figure 4 shows the effect of $T_{1\rho}\{^1H\}$ filtering via cross-polarization to the ^{13}C nuclei. All signals show the same scaling corresponding to $T_{1\rho} \sim 4$ ms, indicating that crystalline PDL (33 ppm), amorphous PDL (31 ppm) and amorphous OC (71 ppm) are close together. Several factors (chain mobility, magic angle spinning) complicate a precise calculation of the spin diffusion coefficient D. It is therefore difficult to estimate even a reliable upperlimit for the average size of the crystalline domains from the present results. Taking the rigid-poly-

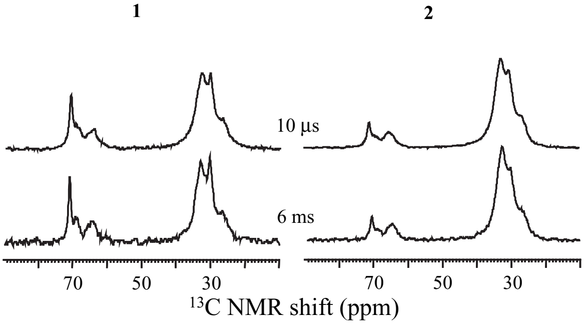


Figure 4.
Proton-$T_{1\rho}$ filtered CPMAS ^{13}C NMR spectra of the two copolymers. The spectra show the same scaling of all signals (factor 0.2 corresponding to $T_{1\rho} \sim 4$ ms), and thus reflect small crystalline domain sizes, and close distances between OC and PDL comonomers in the amorphous phase.

mer value 1 nm^2/s as an upperboundary, we believe that the lamellar thickness of the crystalline domains is less than ~5 nm. Small crystalline-domain sizes in the copolymers would indeed be consistent with their relatively decreased melting points compared to the homopolymer poly(PDL).

Conclusions

^{1}H and ^{13}C NMR spectroscopy and relaxometry show that the crystalline phase of the novel poly(PDL-*co*-OC) copolymers **1** and **2** exclusively consists of PDL. Apparently PDL and OC have no tendency to co-crystallize in these novel materials. In contrast, the amorphous phase is composed of both PDL and OC at a molar PDL/OC ratio lower than the overall value. Proton spin-diffusion controlled NMR relaxation measurements show that all protons in the semi-crystalline copolymer decay at the same rate. This indicates that the crystalline domains are fairly small (<5 nm). The fact that PDL and OC are not equally distributed over the crystalline and amorphous phase is relevant for the biodegradation properties of the two polymers, and thus for their potential medical use.

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