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Poly(ethylene glycol)-poly(epsilon-caprolactone) block oligomers as injectable materials

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Abstract

A family of segmented low MW poly(ethylene glycol)-poly(epsilon-caprolactone) (PEG-PCL) oligomers was synthesized by reacting PCL-based macrodiisocyanates and diblocks comprising MPEG and PCL short segments. The oligomers have the following general structure: MPEG350-$(CL)_n$-HDI-PCL530-HDI-$(CL)_n$-MPEG350. The composition of the oligomers and their molecular weight was determined by ^1H and ^{13}C-NMR, FT-IR and GPC. Depending on the length of the $(CL)_n$ block, soft waxes to hard solids were produced. The isothermal crystallization process was performed at various temperatures and investigated by DSC, FT-IR and WAXS, while the mechanical behavior was determined using a compression test. All the oligomers investigated were able to crystallize at low temperatures (4 and 25 °C), while at body temperature, only oligomers comprising longer $(CL)_n$ blocks ($n = 5$ and above), were able to crystallize.

Keywords: Injectable materials; PCL-PEG block oligomers; Crystallization

1. Introduction

The development of injectable materials to be used in non-invasive surgical procedures, has triggered much attention in recent years. These materials are required to display low viscosity at insertion time, while a gel or solid consistency is developed in situ, later on.

Block copolymers comprising poly(ethylene glycol) (PEG) segments and biodegradable polyester blocks such as poly(lactic acid), poly(glycolic acid) and poly(caprolactone), have been described by various groups [1–3]. These polymers were used in various areas such as in drug delivery systems [4,5], in selectively biodegradable vascular grafts [6,7], in non-invasive surgical procedures [8,9] and in the tissue engineering field [10,11]. Poly(epsilon-caprolactone) (PCL) has been used in controlled drug delivery systems [12] and in orthopedic implants [13]. This substantially hydrophobic, semicrystalline aliphatic polyester, displays a glass transition temperature around −60 °C and a low melting point (60 °C) [14]. Poly(ethylene oxide) (PEG) is a highly biocompatible material that has been used in a diversity of biomedical applications [15].

Since their degree of crystallinity largely affects the mechanical properties and rate of degradation of PCL-PEG block copolymers, their morphology was extensively investigated [16–19]. Already in 1970, Yamashita et al. [20] reported that PCL fibers create an orthorhombic crystalline structure, similar to that of poly(ethylene). Other groups [21,22] investigated the influence of the molecular mass on the crystallization of different PEG-PCL block copolymers. In clear contrast to the comprehensive work done on high molecular weight PEG-PCL block copolymers, the study of their oligomeric counterparts was less explored [23,24].

A series of biodegradable poly(ether-ester-urethane)s, comprising PEG and PLA or PCL blocks, were developed at our laboratory [25,26] and used in various areas, most importantly in the prevention of post surgical adhesions [27,28]. The first step of this two-stage synthesis consisted in the ring-opening reaction of lactone, initiated by the hydroxyl terminal groups of the PEG chain. The second step involved the chain extension of these PLA-PEG-PLA or PCL-PEG-PCL triblocks with hexamethylene diisocyanate.

As part of a project aimed at developing biodegradable

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injectable materials, we have synthesized a family of low molecular weight PEG/PCL oligomers (1800–4200 Da). These low molecular weight polymers comprise a poly-(epsilon-caprolactone) (530 Da) central block and two lateral chains consisting of short poly(epsilon-caprolactone) (PCL) blocks of various lengths (230–1100 Da), end-capped with poly(ethylene glycol) methyl ether segments (350 Da). The middle PCL block and the MPEG-PCL segments were bound via a diisocyanate coupling agent. The general structure of the oligomers is MPEG350-$(CL)_n$-HDI-PCL530-HDI-$(CL)_n$-MPEG350, where n denotes the number of caprolactone repeating units. This study focuses on the morphological analysis of these materials, describes their mechanical behavior and assesses their potential as injectable materials.

2. Experimental

2.1. Materials

Poly(ethylene glycol) methyl ether 350 Da or MPEG350 (Aldrich) was dried 1 h before use, at 120 °C under vacuum. Poly(caprolactone) diol 530 Da (PCL530), epsilon-caprolactone (CL) and hexamethylene diisocyanate (HDI) (Aldrich) and Stannous (II) 2-hexyl-hexanoate (SnOct2) (Sigma), were used as received.

2.2. The synthesis

The syntheses were carried out under bulk conditions.

2.2.1. Synthesis of the MPEG350-$(CL)_n$ diblock

The MPEG350-$(CL)_n$ diblock was synthesized by a ring-opening polymerization reaction. The synthesis is exemplified hereby for a diblock containing in average 4.9 CL units. 40.1 g (0.11 mol) of MPEG350 were introduced in a 100 ml flask and dried as described above. Then, 62.7 g of epsilon-caprolactone (0.55 mol, 20% in molar excess) and 0.27 g SnOct2 [29] (7.10^{-4} mol, 1/800 molar ratio to CL) were added. The reaction proceeded at 145–150 °C for 2.5 h, with magnetic stirring, under dry N_2 (g) atmosphere. By varying the CL/MPEG350 ratios, caprolactone blocks of different length (n) were produced. Depending on the molecular weight of the $(CL)_n$ segment, soft waxes to hard solids were obtained.

2.2.2. Synthesis of the HDI-PCL530-HDI macrodiisocyanate

12.9 g (0.078 mol) of HDI (2% molar excess to PCL530) was weighed in a 250 ml three-necked flask. 20.1 g (0.038 mol) of the PCL530 were poured in a separation funnel and heated by hot air flow. The PCL530 melt was added dropwise to the HDI, over a 30 min period. The reaction was carried out at 75 °C under dry N_2 (g) flow and mechanical stirring (80–100 rpm) and continued for one additional hour after all the PCL was added. The material obtained was a soft white paste at room temperature.

2.2.3. Synthesis of the MPEG350-$(CL)_n$-HDI-PCL530-HDI-$(CL)_n$-MPEG350 oligomer

The block oligomer was synthesized under the same conditions of the macrodiisocyanate synthesis. The required amount of diblock was weighed in a 30 ml syringe, added to the just synthesized macrodiisocyanate and the reaction was conducted for one hour. Depending on the length of the $(CL)_n$ segment, the final oligomer was a soft to brittle waxy material, at room temperature.

2.3. Characterization

2.3.1. NMR

^1H and ^{13}C-NMR spectra were performed at room temperature, from 15% (wt/v) $CDCl_3$ solutions, using a Bruker 300 MHz NMR apparatus.

2.3.2. MW analysis

The average molecular weight and polydispersity ($\bar{M}_w/\bar{M}_n$) were determined by GPC (Differential Separations Module Waters 2690 with refractometer detector Waters 410 and Millenium Chromatography Manager), using polystyrene standards. The elution solvent was chloroform (1 ml/min).

2.3.3. FT-IR spectroscopy

The samples were prepared by solvent casting from chloroform solutions or directly applied on NaCl crystals (Aldrich) and studied using a Nicolet Avatar 360 FTIR spectrometer.

2.3.4. Thermal analysis

The samples (9–11 mg) were sealed in 40 μl Al-cruicible pans and studied using a Mettler TA-400 differential scanning calorimeter. The oligomers were subjected to three consecutive DSC runs: first, they were heated up to 80 °C, then cooled down to −100 °C and finally they were heated up back to 80 °C. All runs were conducted at 5 °C/min heating or cooling rates. When the crystallization process was investigated, the samples were heated from the crystallization temperature to 80 °C at 5 °C/min. The enthalpy values reported are normalized to the correspondent block content of any given oligomer.

2.3.5. Wide angle X-rays

The X-Ray patterns at 37 °C were obtained in Fuji imaging plates, using a Searle camera equipped with Frank optics affixed to an Elliot GX6 rotating anode generator operating at 1.2 kW and producing copper radiation. The X-ray beam was Ni-filtered ($\lambda = 1.54$ Å) and was about 400 μm in diameter in the plane of the sample. The exposure time was 1.5 h. The imaging plates were scanned with a He–Ne laser (spectra physics) in conjunction with a

home-made reader based on an Optronics (Chelmsford, MA) densitometer and interfaced to a computer. The images were processed using image processing programs, NIH or ImageJ, both available on zippy.nimh.nih.gov. The histograms of the angular distribution rings were calculated using home-made software and fitted using Origin 4.1. (Microcal, Northampton, MA).

The crystallization process at RT was monitored both in the equipment previously described and in a Diffractometer PW 1710 tube; Cu, FF, 40 kV, 35 mA, wavelength:1.54060–1.54438, between 18 and 26°.

2.3.6. Small angle X-rays

The phase separation study was carried out by SAXS using a Ni-filtered Cu Kα radiation (0.154 nm) from Elliot GX6 rotating X-ray generator. Samples were introduced into quartz capillaries (1.5 mm diameter). The correlation length, which characterizes the size of the inhomogeneity, was estimated from the slope and the intercept in the linear section (at small q) of the graph $1/I^{1/2}$ versus q^2, following the Debye–Bueche equation [30], where I is the intensity and q is the scattering vector. Three specimens were tested and the results are expressed as the average. The standard deviation was 8–10%.

2.3.7. Rheological measurements

The viscosity was studied using a Brookfield Viscometer DV-II, with Bath/Circulator TC-500 and Wingather Software, using a T-F spindle at 0.05 rpm, at 37 °C. Aiming at minimizing shear effects due to the rotating spindle, the material was kept at 37 °C under static conditions for the required time period. Only then, the oligomer was transferred to the Brookfield apparatus and the viscosity was measured after 5 min.

2.3.8. Penetration tests

These measurements were carried out using a Universal Testing Machine (INSTRON 4500), by forcing a cylindrical probe into the sample formed in a cylindrical container (30 mm in diameter), at 37 °C [31,32]. The cross-head displacement rate was 0.1 mm/min and the penetration distance was 0.3 mm. The slope (S) of the curve describing the applied force as a function of penetration was used to calculate the apparent modulus (Y_e) from the equation:

$$Y_e = \frac{SL}{\pi r^2}$$

where L is the sample height (10 mm) and r is the diameter of the cylindrical probe (12 mm). A minimum of five specimens were tested and the results are expressed as the average. The standard deviation was between 8.8 and 12.4%.

2.3.9. Degradation tests

Samples were stored in PBS (pH = 7.4, 0.2 M) at 37 °C and the supernatant solution was renewed periodically. The solutions were dried at room temperature in vacuum and the MW was determined by GPC. The $\overline{MW}_t/\overline{MW}_0$ ratio was calculated, where $\overline{MW}_t$ and $\overline{MW}_0$ are the MWs at different times and the initial MW, respectively.

3. Results and discussion

3.1. The rationale

In light of the serious problems associated with fully invasive surgical procedures, increasing efforts are being devoted to the development of improved injectable polymers. These materials are required to display low viscosity at insertion time, while a gel or solid consistency develops at the site of implantation, later on. Our working concept focuses on the design of oligomeric molecules able to undergo sizeable morphological changes, resulting in a pronounced increase in the strength and stiffness of the material. The crystallization of the material is, therefore, the basic phenomenon harnessed to generate the increase in its mechanical properties. The oligomers will be injected in their non-ordered morphological state, characterized by low viscosity and enhanced flowability, while, in situ, the microstructural ordering phenomena taking place, will result in a marked increase in viscosity over time, attaining enhanced mechanical properties. Besides the inherent crystallizability of the oligomer, additional requisites need to be incorporated into the design of these molecules in order to achieve the desired changes at the required kinetics and at physiological temperature. Low molecular weight, high segmental mobility and biodegradability are among the key features required from these molecules.

Based on the set of considerations delineated above, a family of biodegradable oligomers having the following general structure, were synthesized:

MPEG350-$(CL)_n$-HDI-PCL530-HDI-$(CL)_n$-MPEG350

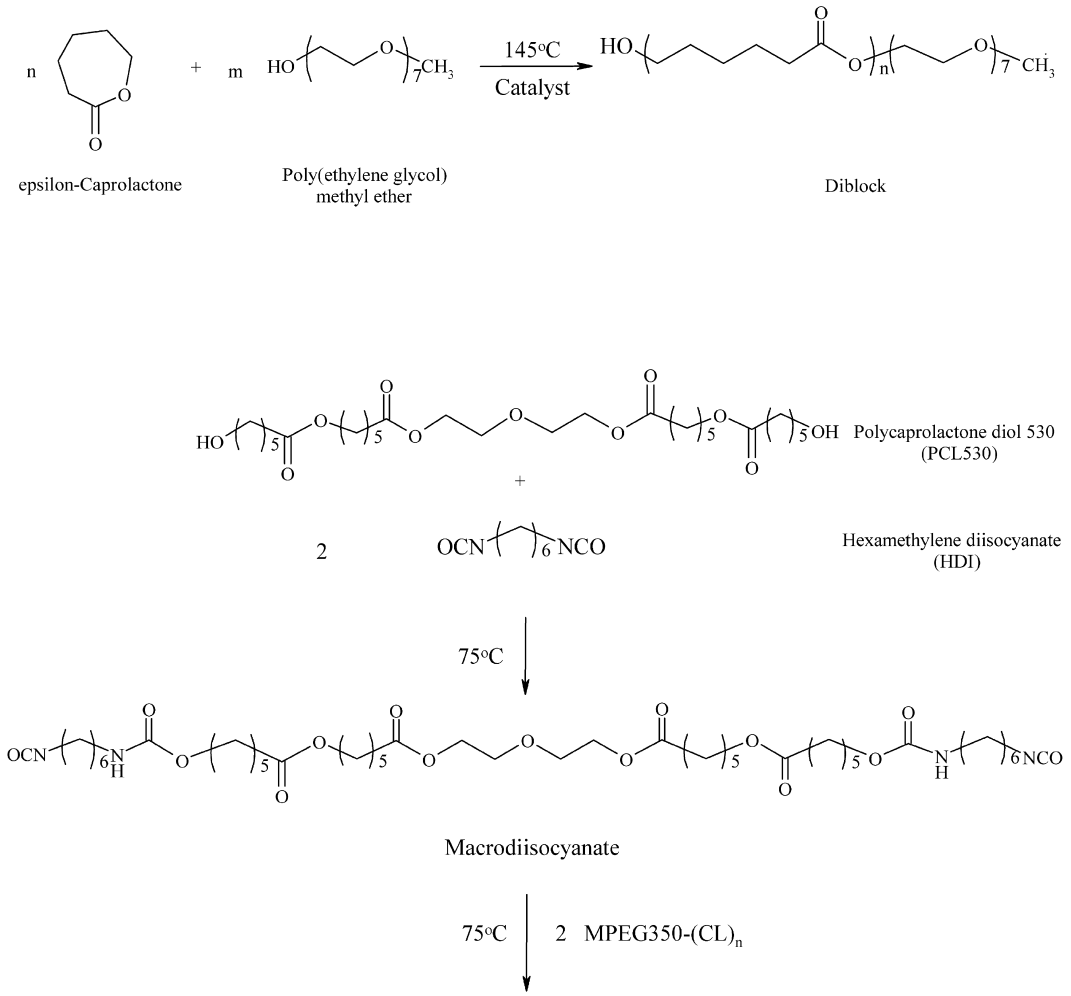
where MPEG350 is the poly(ethylene glycol) methylether (molecular weight 350) segment.

PCL530 [poly(caprolactone), molecular weight 530] and $(CL)_n$ are the middle and lateral hydrophobic segments of the molecule, respectively, and are responsible for the degradability of the oligomer. Evidently, the crystallizability of the PCL component will depend on the number of caprolactone units (n) present in $(CL)_n$.

HDI (hexamethylene diisocyanate) is a flexible bifunctional coupling agent which connects two MPEG350-$(CL)_n$ diblock units to the central PCL530 block.

3.2. The synthesis

The synthetic pathway is described in Scheme 1. The first step was the formation of the MPEG350-$(CL)_n$ diblock, by the ring-opening polymerization of epsilon-caprolactone. In the second stage, OH-terminated PCL530 was reacted with



Scheme 1. The synthetic pathway of the oligomers.

two HDI molecules, to form the HDI-PCL530-HDI macrodiisocyanate. In the final step, two hydroxy-terminated (MPEG350-$(CL)_n$) diblocks were coupled using the macrodiisocyanate, whereby urethane moieties were formed. GPC data revealed that the diblocks, the macrodiisocyanate and the oligomers produced have a monomodal molecular weight distribution displaying a low polydispersity (1.2–1.6).

The composition of the different diblocks was calculated from their ^{1}H-NMR spectrum, by integrating the area of the peaks due to the methoxy group of MPEG (3H, singlet, 3.5 ppm) and the methylene groups of the caprolactone segments (2H, triplet, 2.3 ppm) (see Fig. 1). In addition, the macrodiisocyanate synthesis was monitored using FT-IR analysis by observing the marked decrease of the isocyanate band at 2271 cm^{-1} and the concomitant increase in the urethane peak at 1732 cm^{-1}. MPEG350-$(CL)_n$ diblocks containing between 2.9 and 11.5 caprolactone units (average segmental length) were synthesized and then coupled using the PCL530 macrodiisocyanate. Finally, the chemical composition of the oligomers was studied by different techniques. The FT-IR spectra of the oligomers revealed the complete disappearance of the isocyanate peak and an additional increase in the urethane band. The presence of urethane groups in the oligomer was corroborated by ^{13}C-NMR, where the characteristic peak at 156.3 ppm was clearly observed, while the isocyanate band at 122.1 ppm totally disappeared. The assignment of the characteristic peaks and the determination of the molecular weight for the oligomers by ^{1}H-NMR spectrum was difficult because of the overlapping of peaks belonging to different blocks (for example, the methylene groups of the hexamethylene diisocyanate and those of the caprolactone repeating units). The molecular weight of the oligomer was then calculated assuming that two diblock units were chemically coupled via the macrodiisocyanate

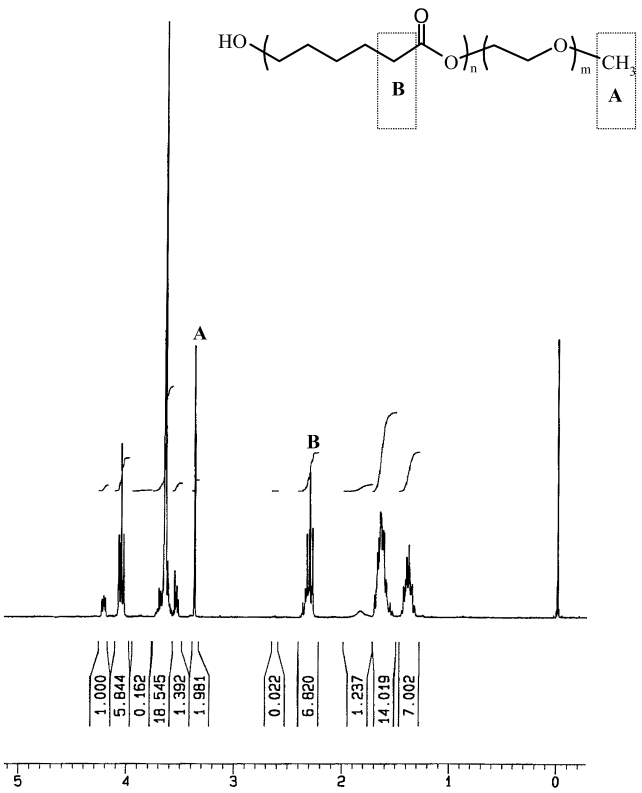


Fig. 1. ^{1}H-NMR of the MPEG350-$(CL)_{4.9}$ diblock.

(MW = 866). Thus, the molecular weight of the oligomers studied in this work span from 2300 to 4200 Da, for for n values rising from 2.9 to 11.5.

3.3. The crystallization process

The crystallization process displayed by the different oligomers was investigated at 4, 25 and 37 °C. Firstly, the thermal behavior of the building blocks of the oligomers, namely MPEG350, the various MPEG350-$(CL)_n$ diblocks and PCL530, were studied. MPEG350 exhibited a T_g at −86 °C and two overlapping melting endotherms at −5 and −30 °C with a total enthalpy of fusion of 58 J/g. PCL530, on the other hand, presented a T_g at −78 °C, a broad melting endotherm with peaks at 20 and 31 °C and an enthalpy of fusion of 42 J/g. As one would anticipate, the low molecular weight of both MPEG350 and PCL530, resulted in lower crystallinity levels, when compared to their high molecular weight counterparts, which attained 197 and 140 J/g values, correspondingly.

Once in the diblock molecule both the temperature of crystallization (T_c) and the melting temperature (T_m) of MPEG shifted steadily to lower values, for increasingly long $(CL)_n$ blocks. Also, MPEG's enthalpy of fusion dropped sharply, from 58 J/g for the free chain, down to 46, 18 and 5 J/g_{PEG} in diblocks containing 4.9, 7.7 and 11.5 CL units, respectively. Similarly, the presence of the MPEG segment affected the thermal behavior of the $(CL)_n$ block, causing both T_c and T_m to decrease, especially for the shorter CL blocks. This can be exemplified for the diblocks comprising 3.5 and 11.5 caprolactone units. While the former presented an ill-defined crystallization exotherm at 7 °C and two overlapping melting endotherms at 21 and 33 °C, the latter displayed a T_c at 25 °C and a T_m around 46 °C. The enthalpy of fusion of the $(CL)_n$ block followed the same basic trend, increasing with molecular weight.

Figs. 2 and 3 present the thermograms of five oligomers comprising increasingly long $(CL)_n$ blocks, obtained under non-isothermal conditions, during cooling and heating, correspondingly. As anticipated, the longer the poly(caprolactone) segment, the higher T_c and T_m, and the larger the enthalpy of fusion, that increased from 39 J/g_{PCL} for $n = 2.9$ to 71 J/g_{PCL} for $n = 11.5$. It is worth stressing that for all the composition, the temperature of crystallization observed was higher than the correspondent T_c of the MPEG segment in the diblock, suggesting that, once in the oligomer, the MPEG350 chain is unable to crystallize. The presence of more than one melting endotherm for PCL blocks was in fully agreement with the literature [19].

DSC, WAXS and FT-IR were used to investigate the crystallization behavior of the different oligomers, under isothermal conditions.

3.3.1. DSC

Findings for three oligomers ($n = 4.1$, 4.9 and 7.7) are presented below. The samples were initially brought to 80 °C for one hour, to erase their previous thermal history and then cooled down to temperature of study. Table 1 presents the ΔH_f of the three oligomers versus time, for isothermal treatments performed at 4 and 25 °C. It is apparent from the data presented, that the longer the $(CL)_n$ block, the faster the oligomer crystallized and the higher the degree of crystallinity it attained. The melting endotherm was affected as well, gradually shifting to higher temperatures. When the treatment was conducted at 25 °C, T_m raised from 34 to 46 °C, as the number of CL units increased from 4.1 to 7.7. Expectedly, oligomers containing shorter $(CL)_n$

Table 1
Enthalpies of fusion of three oligomers with *n*s 4.1, 4.9 and 7.7 versus time at 4 and 25 °C, by DSC

Time	Polymer					
	$n = 4.1$		$n = 4.9$		$n = 7.7$	
	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
	$\Delta H_{melting}$ (J/g_{PCL})					
1 day	29	0	28	7	38	41
2 days	31	3	41	11	42	46
3 days	31	12	42	16	52	51
6 days	34	14	42	16	61	54
40 days	46	19	52	18	75	57
76 days	48	19	59	18	80	58

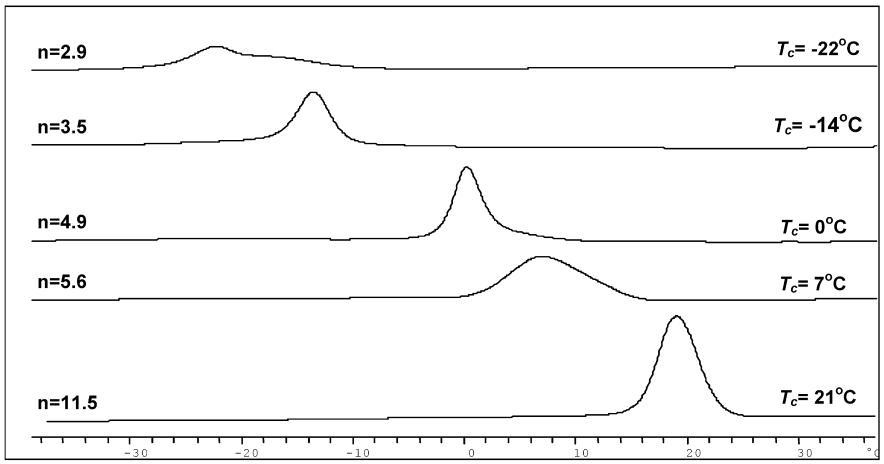


Fig. 2. The cooling stage thermograms of five oligomers with increasingly long $(CL)_n$ blocks by DSC.

blocks ($n = 2.9$ and 3.5) were unable to crystallize to any considerable extent.

Because of its obvious clinical significance, the isothermal treatment was also conducted at 37 °C. At this temperature, materials containing short $(CL)_n$ blocks ($n = 4.1$ and 4.9) remained basically amorphous, regardless of the length of the treatment. Only oligomers comprising longer $(CL)_n$ blocks were able to crystallize significantly. The oligomer containing 7.7 CL units, for example, displayed a T_m at 44 °C after being allowed to crystallize at body temperature. Having said that, the crystallizability of this oligomer at body temperature was rather limited, and longer $(CL)_n$ blocks were required (for example, $n = 11.5$), for the oligomer to be able to crystallize effectively.

3.3.2. WAXS

The appearance and development of PCL's typical crystalline pattern shown by different oligomers and their respective C_r values, was determined using WAXS. The oligomer with 4.9 CL repeating units, at 25 °C, showed PCL's characteristic signal at 23.80° after 6 h (belonging to a 200 Miller index), with additional peaks appearing at 19.24, 20.60 and 21.44°, as time elapsed. After six months this material reached a 7.5% degree of crystallinity. In accordance with the DSC findings discussed previously, oligomers comprising longer $(CL)_n$ blocks ($n = 7.7$ and 11.5), achieved significantly higher degrees of crystallinity. For example, the oligomer containing 11.5 CL units attained a $C_r = 29\%$ after six months, a degree of crystallinity almost four times higher than that developed by the molecule comprising 4.9 CL repeating units. When the isothermal treatment was carried out at 37 °C, the oligomer containing 4.9 CL units remained essentially amorphous for five months. In contrast, oligomers with $n = 7.7$ and even more so $n = 11.5$, crystallized much faster and attained higher degrees of crystallinity. The X-ray pattern versus time at 37 °C of the oligomers containing 7.7 and 11.5 CL units, are shown in Fig. 4. It was only after 10 h that the oligomer with $n = 7.7$ started to crystallize ($C_r = 4\%$) and after six days the oligomer achieved a modest 9% degree of crystallinity. The oligomer having $n = 11.5$ presented significant crystallinity levels already after 2 h, increasing up to 17% after 6 h.

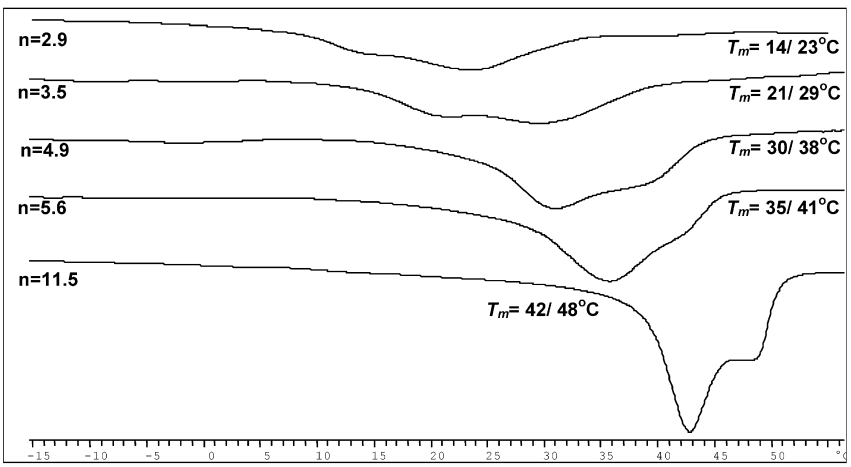


Fig. 3. The heating stage thermograms of five oligomers with increasingly long $(CL)_n$ blocks by DSC.

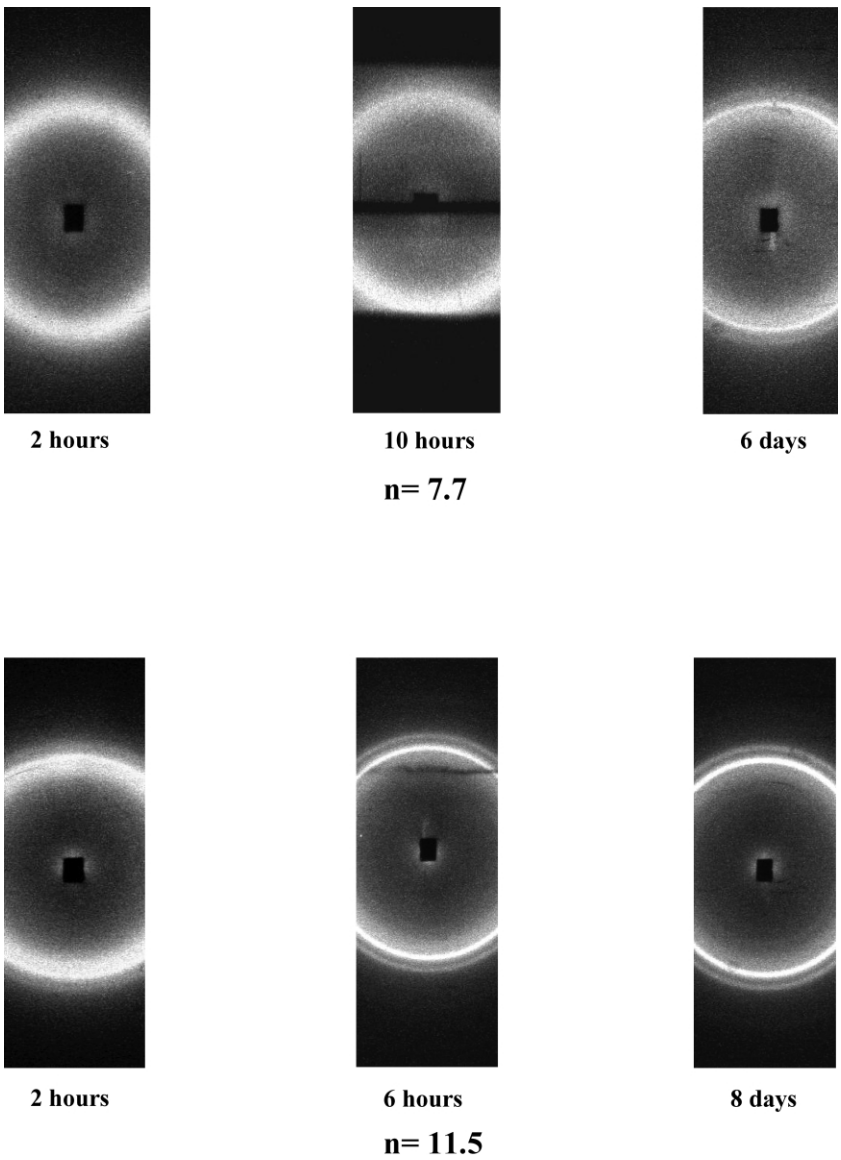


Fig. 4. WAXS pattern versus time of the oligomers containing 7.7 and 11.5 CL units, at 37 °C.

3.3.3. FT-IR

The crystallization study was also carried out by comparing the carbonyl bands characteristic of the amorphous and crystalline PCL blocks, appearing at 1732 and 1726 cm^{-1}, respectively [33]. This is exemplified in Fig. 5, which presents the FT-IR spectrum of MPEG350-$(CL)_{4.9}$-HDI-PCL530-HDI-$(CL)_{4.9}$-MPEG350, at 25 °C, as a function of time. Firstly, a steady shift of the peak at 1732 cm^{-1} towards 1726 cm^{-1}, indicative of an increasingly crystalline PCL block, was observed. Supporting evidence was provided by the increase of the small, although significant peak at 1295 cm^{-1}, distinctive of PCL's crystalline phase. It worth stressing that this peak was larger, the longer the $(CL)_n$ block. Also, a gradually increasing peak was apparent between 1685 and 1690 cm^{-1}, that can be attributed to hydrogen-bonded urethane carbonyl groups [34,35].

Two additional oligomers, comprising a shorter ($n = 4.1$) and a longer ($n = 7.7$) PCL block, were treated at 25 °C. While the former showed the 1295 cm^{-1} band only after 75 days (with the concomitant shift of the carbonyl peak, from 1732 to 1726 cm^{-1}), the material containing 7.7 CL units displayed a faster crystallization process, with changes being detectable already after two hours. When the latter was treated a 37 °C, the various spectral features indicative of an increasingly crystalline content, were apparent already after 20 h.

3.4. Rheological properties

The increase in the viscosity of the oligomer as a function of time constitutes a key issue in order to assess the potential of these oligomers as injectable biomaterials. The measurement of the viscosity of the material at 37 °C was, therefore, of special interest. Aiming at minimizing shear effects due

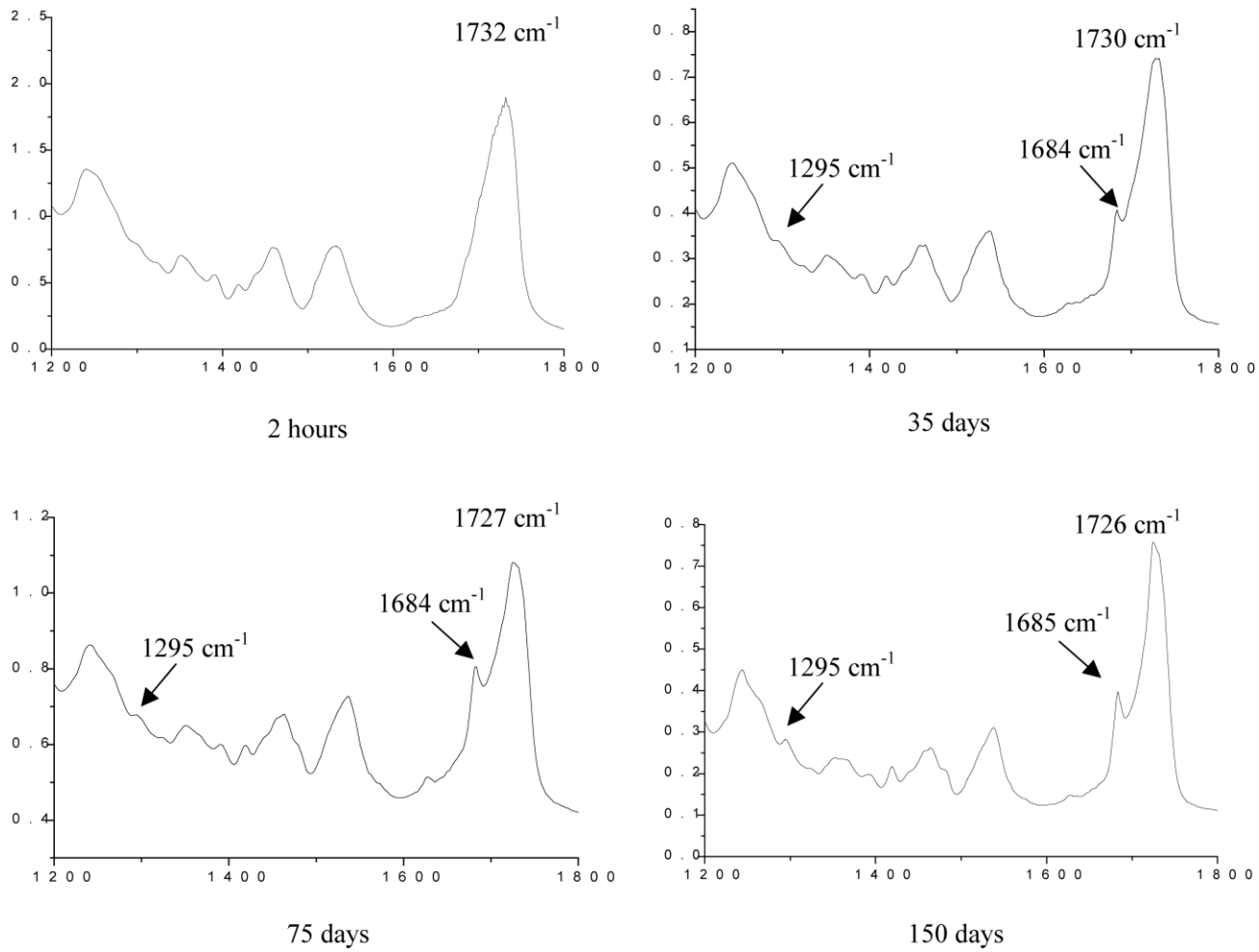


Fig. 5. FT-IR spectrum versus time of MPEG350-$(CL)_{4.9}$-HDI-PCL530-HDI-$(CL)_{4.9}$-MPEG350, at 25 °C.

to the rotating spindle during the viscosity measurement, the material was kept at 37 °C under static conditions for the required time period. Only then, the oligomer was transferred to the Brookfield apparatus and the viscosity was measured after 5 min. The viscosity versus time behavior of the oligomers is exemplified in Fig. 6, for MPEG350-$(CL)_{4.9}$-HDI-PCL530-HDI-$(CL)_{4.9}$-MPEG350. Its is apparent from the curve shown, that the viscosity of the oligomer increased remarkably over the first 12 days of treatment, leveling off thereafter. The mechanical behavior of the oligomers was assessed by a compression test. Fig. 7 decribes the increase in the stiffness of the oligomer material, as a function of time by plotting the apparent modulus (Y_e) versus time (t) curves of MPEG350-$(CL)_{4.9}$-HDI-PCL530-HDI-$(CL)_{4.9}$-MPEG350, at three different temperatures. In full agreement with the morphological

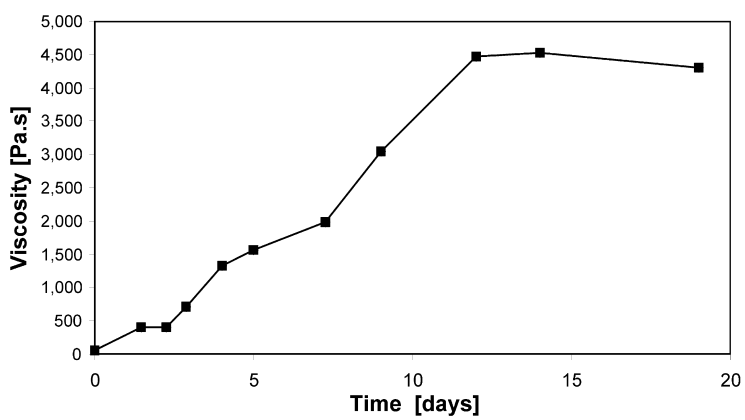


Fig. 6. The viscosity profile of the oligomer with $n = 4.9$ with time, at 37 °C.

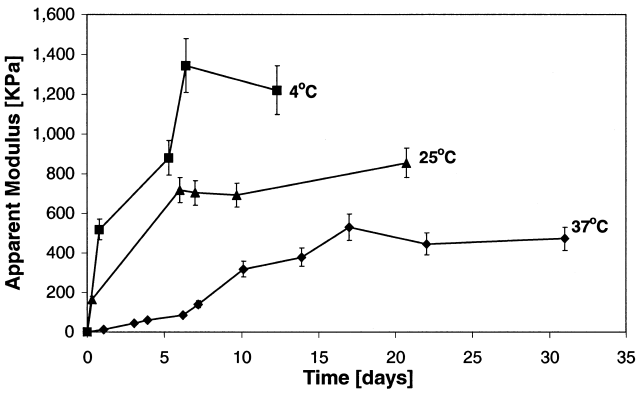


Fig. 7. Y_e versus time of MPEG350-$(CL)_{4.9}$-HDI-PCL530-HDI-$(CL)_{4.9}$-MPEG350 at three different temperatures.

findings discussed above, the lower the temperature, the faster the Y_e increase and the higher the maximum stiffness attained. The rigidity developed by this oligomer after one day at 4 and 37 °C was 500 and 12 kPa. Also, the maximum stiffness achieved by the material increased with decreasing temperature, with Y_e maximum values of around 1300, 700 and 500 kPa being measured at 4, 25 and 37 °C, correspondingly. It is important to stress the negligible Y_e values for $t = 0$, underscoring the enhanced injectability of this oligomer.

Due to its clinical relevance, its worth emphasizing that Y_e increased significantly at 37 °C, during the first 14 days. Since both DSC and WAXS findings demonstrated that MPEG350-$(CL)_{4.9}$-HDI-PCL530-HDI-$(CL)_{4.9}$-MPEG350 did not crystallize at 37 °C during this short time interval, crystallization phenomena cannot explain the increase in Y_e. An alternative phase separation mechanism was suggested and the oligomer was investigated by applying the Debye–Bueche equation [30,36]. Since the oligomers synthesized comprise two clearly different domains—a hydrophobic $(CL)_n$-HDI-PCL530-HDI-$(CL)_n$ central block and two MPEG hydrophilic lateral segments—it was reasonable to expect that a phase segregation mechanism could take place. Fig. 8 graphically presents the increase in the correlation length, as defined by the Debye–Bueche (see Section 2.3) at

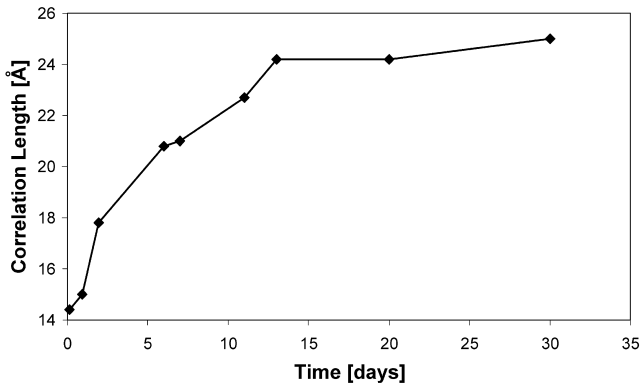


Fig. 8. The correlation length versus time of the oligomer containing 4.9 CL units at 37 °C.

37 °C, measured for the MPEG350-$(CL)_{4.9}$-HDI-PCL530-HDI-$(CL)_{4.9}$-MPEG350 oligomer. The noteworthy raise in the correlation length, increasing from about 14 to 24 Å over the first 13 days, is indicative of a significant phase segregation process taking place in the oligomeric matrix. These findings fully concur with the mechanical data presented, suggesting that this process is probably the responsible for the stiffness increase displayed by the oligomer over time.

Based on the data previously described, selected oligomers (4.9, 7.7 and 11.5) were thermally treated, aiming at combining enhanced injectability with an increase in stiffness at 37 °C over time. Typically, the oligomers were placed in a syringe, then they were kept at 80 °C for an hour, to erase any crystallinity, and then quenched by immersing the syringe in liquid nitrogen for 30 min. Finally, the material was brought to 37 °C and its syringability was qualitatively assessed. The oligomer with $n = 4.9$ was successfully quenched, remaining fully amorphous, was easily syringed and its rigidity increased slowly over time. In clear contrast, the material with $n = 11.5$ crystallized so rapidly, that our present quenching procedure failed to prevent its crystallization. Expectedly, therefore, once brought to 37 °C, the material was a stiff opaque solid that could not be syringed. In between these two extreme cases, the oligomer with $n = 7.7$ combined rather encouraging features. Once quenched, a transparent glassy material was obtained, indicative that it basically remained in its amorphous state, and once heated to 37 °C, the oligomer could be syringed. Furthermore, the material became increasingly stiff, as time elapsed.

3.5. Degradation tests

In vitro degradation tests performed in PBS (pH = 7.4, 0.2M) at 37 °C showed that the molecular weight of the oligomers changed only marginally during the first year, with a gradual decrease being detectable afterward.

4. Conclusions

A family of segmented PEG-PCL oligomers was synthesized and thoroughly characterized.

The composition of the MPEG350-$(CL)_n$-HDI-PCL530-HDI-$(CL)_n$-MPEG350 oligomers was tailored by fine tuning the length of the $(CL)_n$ blocks, resulting in materials differing in their morphological behavior. The hydrophilicity of the different segments, their degree of crystallinity and their respective molecular weights played a key role in determining both their crystallizability as well as their mechanical and rheological properties. Even though materials that combine syringability with an increase in stiffness at 37 °C over time, were generated, further work is required. Based on the findings reported hereby, work currently underway focuses on further fine tuning the

composition of the oligomers, concentrating on molecules comprising $(CL)_n$ blocks with n values close to 7.7. Also, efforts are being devoted to improve the efficiency of the quenching technique.

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