EFFECT OF HUMIDITY DURING ELECTROSPINNING OF BIODEGRADABLE POLYESTER NANOFIBERS

[1]Eva KUZELOVA KOSTAKOVA, [1]Vojtech HOMUTA, [1]Vera JENCOVA, [2]William J. A. HOMER,
[1]Lubos BEHALEK, [1]Kristyna HAVLICKOVA, [1]Sarka HAUZEROVA, [1]Maxim LISNENKO,
[1]Jan KOVACICIN, [1]Vít NOVOTNY, [1]Jana MULLEROVA, [1]David LUKAS

[1]*Technical University of Liberec, Liberec, Czech Republic, EU, eva.kostakova@tul.cz*
[2]*Aston University, Engineering for Health Research Group, Birmingham, UK*

Abstract

Among widely electrospun biodegradable polymer materials are aliphatic polyesters: polycaprolactone, polylactide acid or a copolymer of caprolactone and lactic acid (PLCL). Many different modifications of electrospinning can be used to change the fiber structure and, therefore, to set optimal, especially morphological, material effects for application, testing, or treatment with these means. Structural or shape changes include not only fiber diameters, their distribution, but also fiber orientation, their interconnection, their smooth or rough surface, and also the significant arrangement of the internal networks of polymer chains represented, for example, by the degree of crystallinity. The process and material parameters of the production of nanofibrous materials must still be studied to ensure the resulting properties of the materials lead to predictable effects during its application. A general understanding of the effect of five different solvent systems and especially the effect of air humidity (from 20 to 50% RH) during the needleless electrospinning process on the formation of nanofibrous materials from biodegradable polyesters copolymer (PLCL) is evaluated concerning the actual spinning process and the resulting morphology assessed by SEM. The internal chemical structure or arrangement evaluated using especially DSC, FTIR and GPC is necessary to ensure the reproducibility of the production of materials and thus their application results.

Keywords: Electrospinning, biodegradable nanofibers, ambient conditions, air humidity

1. INTRODUCTION

Nanofibrous materials are widely used in medical applications nowadays and are currently produced mainly by electrospinning [1]. There is a whole range of modifications of electrospinning technology leading to different arrangements of fibers, their shape, and especially surface changes. Morphology change is an essential factor for biomimetic mimicry of the intercellular mass. Mastering the possibilities of structural changes in nano and microfiber materials is important for the repeatability of production and predicting the resulting properties of tissue engineering scaffolds or wound dressings.

The PLCL copolymer belongs, like its homopolymers, to the group of biodegradable aliphatic polyesters. PLCL is characterized by its unique mechanical properties - relatively high flexibility compared to PLA homopolymer due to copolymerization with the much more flexible polycaprolactone. PCL, which has a very low glass transition temperature [2]. Of course, the mechanical properties and also biodegradation development will vary significantly depending on the chosen ratio of the monomers used. It was found that for statistical PLCL, crystallization occurs only in blocks of lactide chain segments, as they are long enough [3]. The glass transition temperatures T_g and melting temperatures T_m increase proportionally with a higher proportion of lactide units up to values similar to PLA alone. For comonomer ratio of 70:30 (L-lactide:ε-caprolactone), which is used in the study, T_g and T_m were around 16-35 °C and 137-158 °C, respectively.

In the research studies devoted to nanofibrous electrospun materials [4-6], the most used PLCL is Purac PLC 7015 with molar ratio L-lactide to ε-caprolactone 70:30 reported by the manufacture Corbion [7]. For the repeatable production of nanofibers using electrospinning technology, it is necessary to obtain a basic polymer source with repeatable properties, but it was found in [6] that the material may have different properties than those defined by the manufacturer (according to ^{1}H NMR, a real molar ratio of 82:18). The type of copolymer is still in the research stage, but it is intended to be used mainly in tissue engineering as biodegradable scaffolds [6], for example, the absorbable tubes for guiding nerves [8], rotator cuff augmentation [9]; the production of vascular bandages [4] or bypasses [6]. Nanofibrous PLCL materials for medical applications are electrospun from polymer solutions with different solvent systems, for example: mixture of chloroform and dimethylformamide [5] or mixture of chloroform, ethanol and acetic acid [6]. Both needle [5] and needle-less [4,6] direct current electrospinning methods and even alternating current electrospinning method [4] can be used for production of PLCL nanofibers. But PLCL is still an understudied material from the point of view of electrospinning and the connection of process and material parameters. This study demonstrates the influence of the used solvent systems and especially of air humidity on the resulting structure and morphology.

2.   MATERIALS AND METHODS

A copolymer of L-lactide and ε-caprolactone in a 70/30 (LA 67-73 %; CL 27-33 %.) monomer molar ratio (Purasorb® PLC 7015, Corbion [7]) was used here. The supporting materials was spunbond Pegatex S (PFNonwoven) nonwoven fabric with an area weight of 20 gm^{-2}. The solvent dimethylformamide (DMF) was provided by VWR and formic acid (FA), 99.8% glacial acetic acid (AA), 96% ethanol (ETOH), acetone (Ac), dichlormethane (DCM) and chloroform (CF) were supplied by Penta. Five different solvent systems were used for electrospinning: (i) Ac/AA/FA 1:1:1 w/w (1:1:1); (ii) CF/ETOH/AA 8:1:1 w/w (8:1:1); (iii) CF/ETOH 8:2 w/w (8:2); (iv) CF/DMF 9:1 w/w (9:1); (v) DCM/DMF 7:3 v/v (7:3). Solutions of 10 wt% of PLCL were always freshly prepared immediately before electrospinning.

The electrospinning took place on NS 1S500U & NSAC150 Nanospider™ production line and precisely controlled air conditioning unit (Elmarco). The flow of incoming and outgoing air from the spinning chamber was set to 90 m^{3}h^{-1} and 110 m^{3}h^{-1}, respectively. The electrodes were 180 mm apart at an electric potential difference of 50 kV (40 kV on the spinning electrode in the form of a static wire with 0.2mm in diameter and -10 kV on the collector in the form of static wire too) and the withdrawal speed was 30 mm/min. Electrospinning was carried out at 20%; 30%; 40% and 50% (±2%) RH. The velocity of the solution carriage was set to 1 s (approximately 500 mm/s). Metal insert orifice 0.7 mm in diameter was used for control of polymer solution layer on the spinning electrode. All spinning was carried out at a temperature of 22 ± 1 °C. The final nanofibrous layers were removed from the supporting fabric and prepared for further analysis.

Basic characterization of electrospun materials is analysis of the fiber diameters, which represents the morphology and structure. An electrically conductive layer of gold approximately 10 nm thick (Quorum Q150R Plus) was deposited on all samples, and 5 images were taken for each sample at appropriate magnification using a scanning electron microscope (SEM; Vega S3B, Tescan). Fiber diameters were measured by FIJI ImageJ. FTIR analysis (Nicolet iZ10, Thermo Fisher Scientific, ATR technique on a diamond crystal) determined chemical composition and microstructure after the dissolution and electrospinning of the copolymer. GPC analysis (Dionex Ultimate 3000 HPLC) studied the molecular weight of the chains in the fibers and granules. GPC was performed in triplicate dissolved in chloroform and tetrahydrofuran. The mobile phase of tetrahydrofuran flowed through the Phenomenex Phenogel 1E5 column at a temperature of 30 °C at a flow rate of 1 ml/min. Detection of molecular weights was performed using UV scattering. The result of the DSC analysis (Differential Sensing Calorimeter DSC 1/700, Mettler Toledo) is the enthalpies of the individual samples, which makes it possible to compare the arrangement of the copolymer chains. However, due to the absence of an enthalpy value for 100% crystallinity of PLCL, it was not possible to convert the enthalpies to the percentage crystallinity of the samples. Triplets of samples in a weight of 4-6 mg were first left at -20 °C

for two minutes and then heated at 10 °C/min to 160 °C. Nuclear magnetic resonance (NMR) analysis was used to measure the actual ratio of monomer units in PLCL copolymer of three different PLCL production batches used during electrospinning tests. Bruker Advance NMR Spectrometer using a high-resolution dual (^{1}H, ^{13}C) gradients probe was used. Whole monomer signal was calculated according [10].

3. RESULTS AND DISCUSSION

Fiber diameter differences and different complete structure of produced materials can be measured and observed from SEM images, see examples in **Figure 1**. Material produced from solvent system DCM/DMF 7:3(v/v) was not possible to separate from supporting nonwoven, thus it cannot be analysed completely.
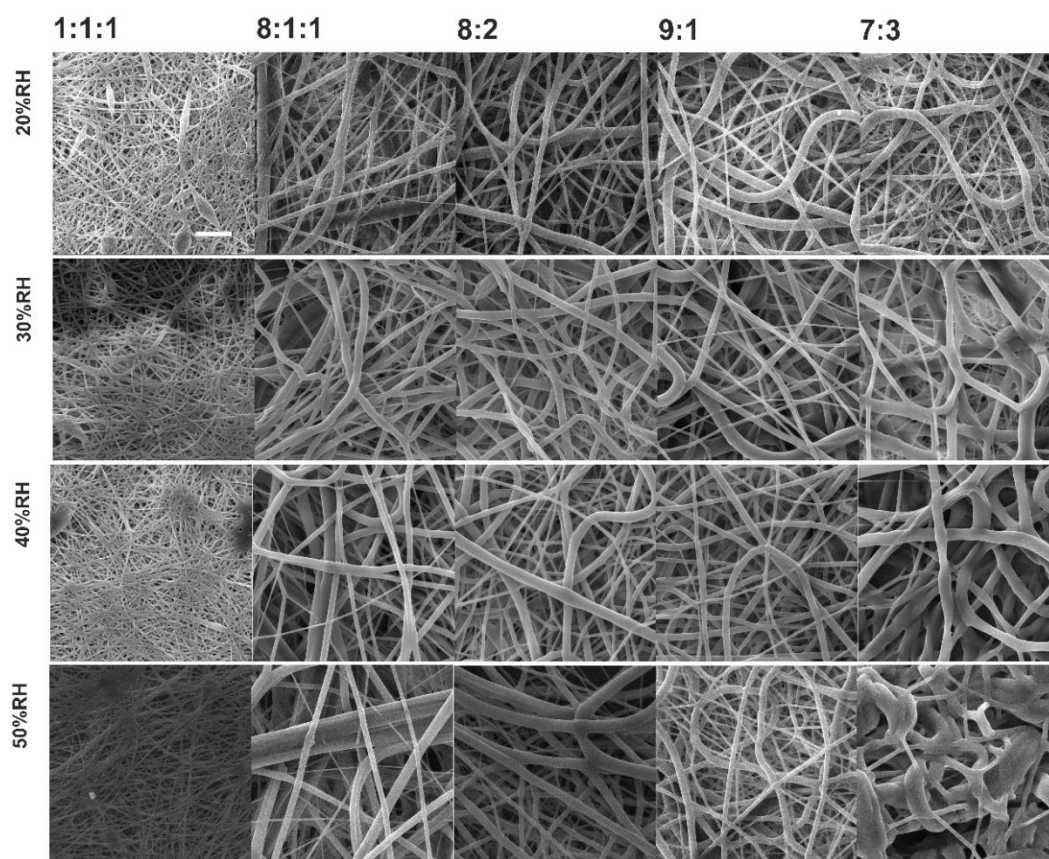

Figure 1 Scanning electrone microscope example images of fibrous materials electrospun from five different solvent systems at four different ambient air humidities The scale bar represents 10 micrometers.

The complete comparison of average fiber diameters is introduced in **Figure 2a** and of areal weights is shown in **Figure 2b**. FTIR, DSC and GPC analysis was done only for nanofibrous material electrospun under the limit ambient humidity thus at 20 and 50% RH. FTIR analysis of electrospun samples and granules disproved the assumption that the dissolution of the copolymer in individual solvent systems and the subsequent electrospinning of the polymer solutions at different air humidities could significantly affect the internal structure or chemical composition. The results in **Figure 3** show that the chemical composition of the fibers electrospun at 20% and 50% RH was not significantly different from the PLCL granules. If the PLCL copolymer were to degrade in individual solvent mixtures or during electrospinning process, the molecular weight measured by GPC would decrease. Materials spun from the 8:2, 9:1 and 7:3 solvent systems had an average molecular weight M_w approximately the same as the PLCL granules at both relative humidities (**Figure 4a**). Thus the PLCL chains were not degraded in the given solvents. However, the molecular weight of the PLCL copolymer spun from mixtures 1:1:1 and 8:1:1 decreased significantly (**Figure 4b**).
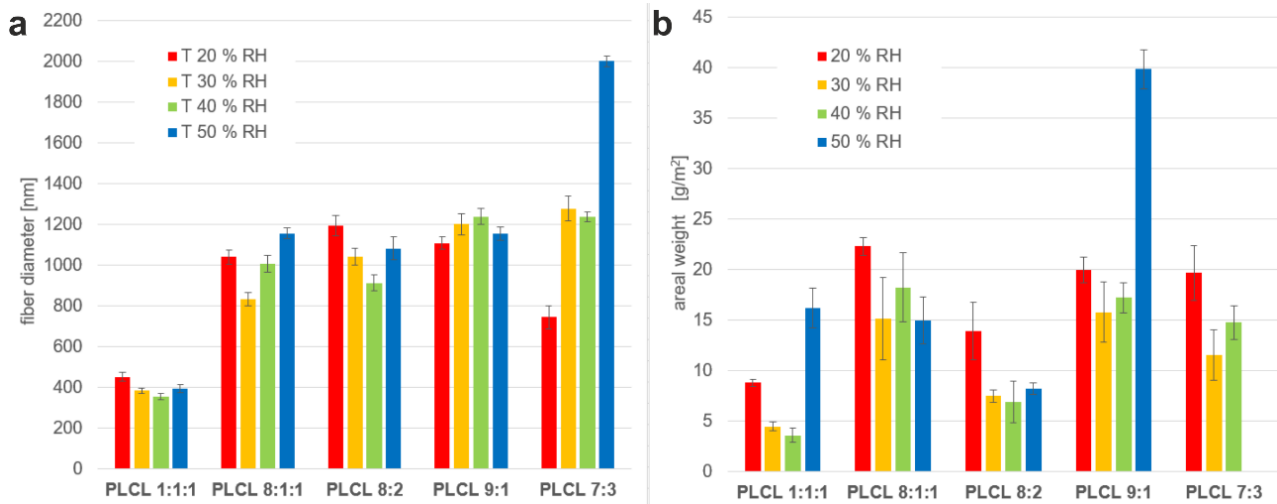
Figure 2 Average fiber diameters (a) and areal weights (b) of PLCL nanofibrous layers electrospun from different solvent systems at different ambient humidity
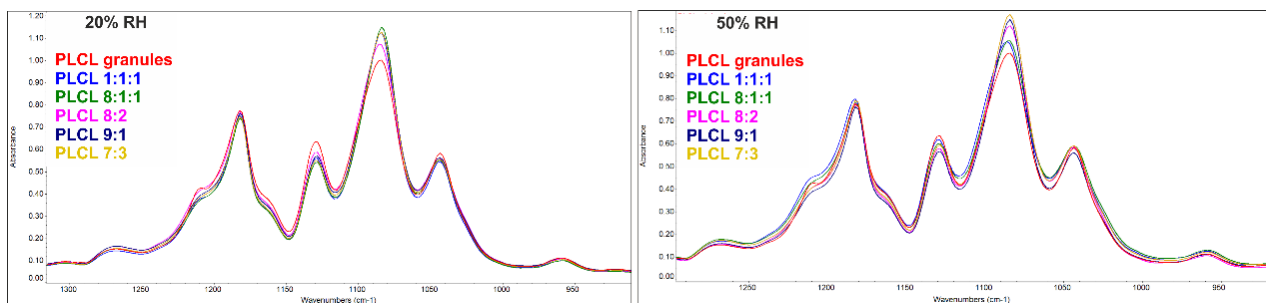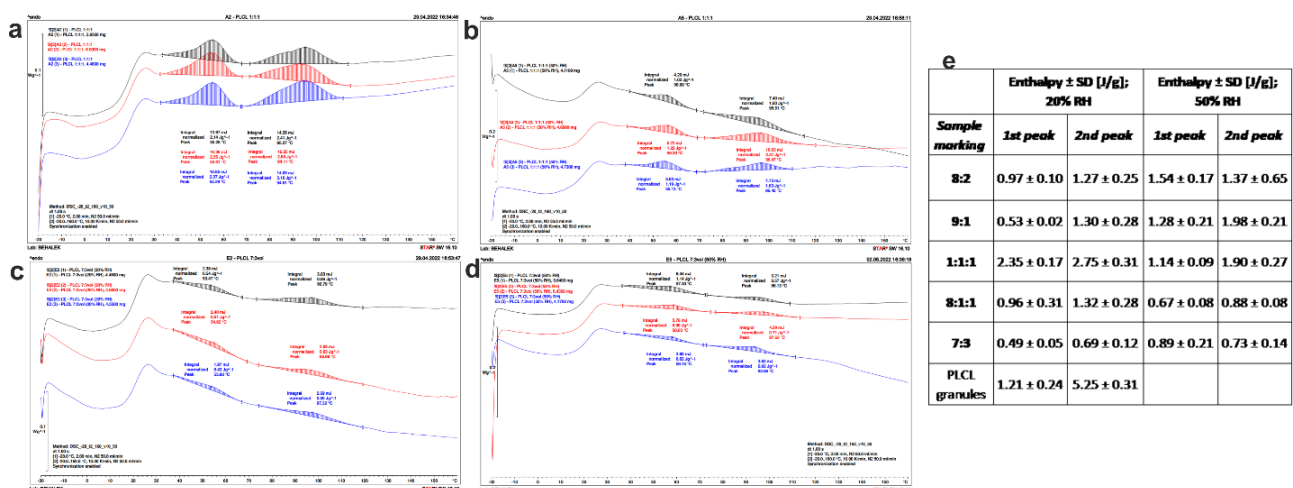

Figure 3 FTIR spectra of electrospun PLCL nanofibrous materials from different solvent systems
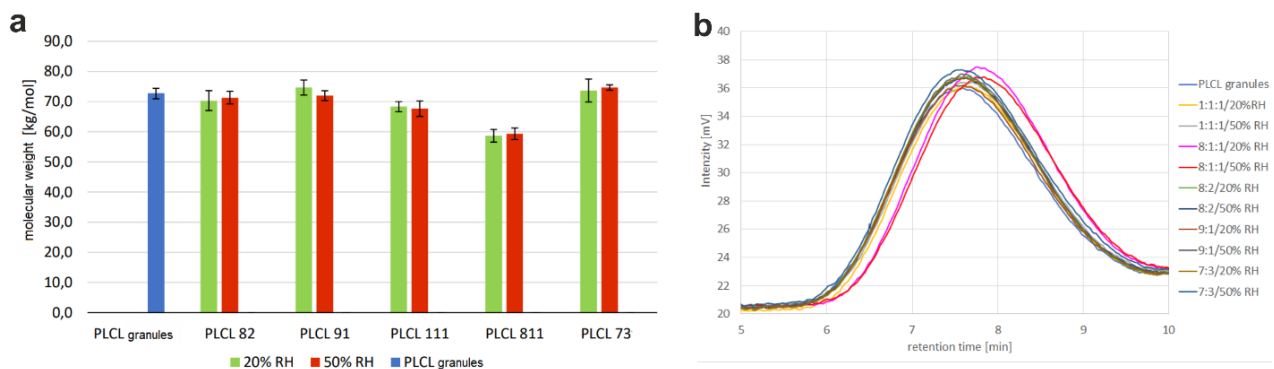

Figure 4 Average molecular weights of PLCL granules and electrospun materials at 50 and 20% RH measured by GPC (a); GPC average spectra for all measured materials (b)

DSC analysis revealed that all the spun samples as well as the initial PLCL granules contain two endothermic peaks (maxima) with different enthalpies in their internal structure, see **Figure 5**. The first peak appeared for all samples around 55 °C and the second around 96 °C. For the PLCL granules, the first peak also formed around 55 °C, but the second maximum only around 111 °C, which is perhaps due to the more rigid chains and the thermal history from the copolymer production. From the DSC results, it could be inferred that both the PLCL samples and granules contain long enough segments of both ε-caprolactone and lactic acid to be able

to crystallize. The first peak could belong to the crystalline phase of the ε-caprolactone segments and the second maximum to the crystalline sections from both types of segments, since T_m is between the melting point of PLA and PCL. The PLCL copolymer studied in this work obviously contains sufficiently long stretches of ε-caprolactone that crystallize. Thus, the copolymer is typically not statistical, but rather block. From the enthalpy values, it is possible to estimate the degree of order of the chains in the individual fibers. According the table in **Figure 5**, the most ordered chains in fibers spun at 20% RH were in 1:1:1 and the least in 7:3. At 50% RH, the most ordered chains are in 8:2, followed by 9:1 and 1:1:1, the least in 8:1:1. It should be noted here that the internal ordering in electrospun nanofibers may be related to diameters. It is noteworthy that the order of the ε-caprolactone sections (1st peak) does not necessarily mean the order of the lactic acid sections (2nd peak) and vice versa, which can be seen, when comparing the enthalpies for fibers prepared at 20% RH from solvent systems 9:1 and 8:1:1 For some samples, the fibrous layer is not homogeneous in terms of the arrangement of the internal structure. The higher the relative enthalpy error, the lower the homogeneity. This may be due to the different homogeneity of the distribution of comonomers in the copolymer within one layer caused by their different reactivity in the PLCL synthesis.

Sample marking	Enthalpy ± SD [J/g]; 20% RH		Enthalpy ± SD [J/g]; 50% RH	
	1st peak	2nd peak	1st peak	2nd peak
8:2	0.97 ± 0.10	1.27 ± 0.25	1.54 ± 0.17	1.37 ± 0.65
9:1	0.53 ± 0.02	1.30 ± 0.28	1.28 ± 0.21	1.98 ± 0.21
1:1:1	2.35 ± 0.17	2.75 ± 0.31	1.14 ± 0.09	1.90 ± 0.27
8:1:1	0.96 ± 0.31	1.32 ± 0.28	0.67 ± 0.08	0.88 ± 0.08
7:3	0.49 ± 0.05	0.69 ± 0.12	0.89 ± 0.21	0.73 ± 0.14
PLCL granules	1.21 ± 0.24	5.25 ± 0.31		

Figure 5 Selected examples of DSC spectra in triplet measurements for 1:1:1/20%RH (a); 1:1:1/50%RH (b); 7:3/20%RH (c); 7:3/50%RH (d) and table of enthalpies for all electrospun samples and PLCL granules
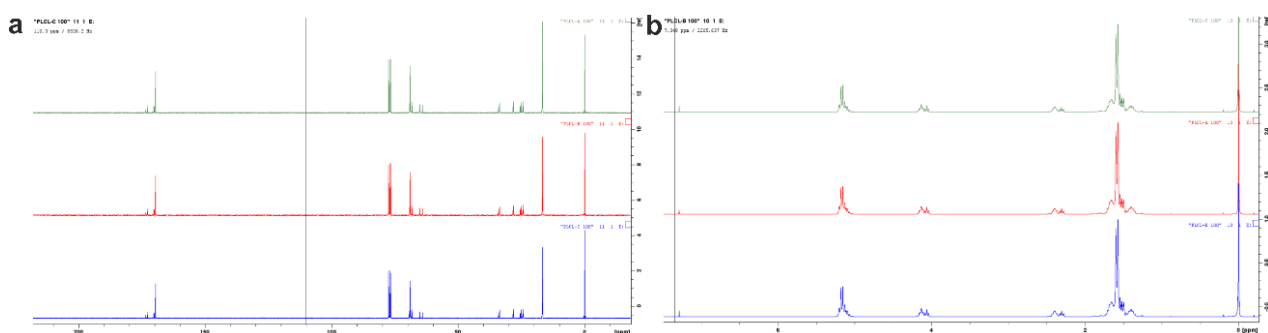
Figure 6 H NMR spectra (a) and C NMR spectra (b) for PLCL granules from three different batches

Side by side comparison of the three samples of PLCL by H NMR (**Figure 6a**) shows near identical spectral for all three materials, indicating a high level of consistency between production runs (batches). These result in a calculated monomer percentage for PLCL granules sample of 69.43 % L-lactide and 30.57 % caprolactone, within manufacturer specified ranges. C NMR (**Figure 6b**) of the 3 granules samples, which

have consistent spectra, with the only small variation around chemical shift of 78 ppm, attributed to the chloroform solvent.

4. CONCLUSION

The electrospinning of PLCL is very significantly influenced by the used solvent system and the ambient air humidity. It is obvious that the choice of solvent system, while maintaining constant process parameters, can have an effect on all fiber diameters; the productivity and internal structure of the resulting nanofib. The study of the influence of air humidity clearly indicates that some polymer solutions (especially 7:3) are more sensitive to its changes and some less so (for example 1:1:1). FTIR, DSC and GPC analyzes pointed to very interesting facts that need to be followed up in the development of electrospun materials from PLCL. The rate of degradation in solutions containing carboxylic acids needs to be further studied, but it was clearly demonstrated by GPC analysis that PLCL chains degrade in these solutions. DSC analysis suggests a block copolymer arrangement, which is probably due, among other things, to the different reactivity of the incoming comonomers during production. NMR analysis demonstrated the repeatability of the properties of PLCL Purasorb 7015 input granulate. The PLCL is interesting material mainly in medical applications in the form of electrospun nanofibers. Its use in this area is wide ranging from vascular bandages and replacements to wound coverings and scaffolds for the regeneration of many tissues. The internal polymer structure in the nanofibers, their morphology, representation of defects, porosity and other parameters can significantly influence the resulting unique properties for PLCL, especially mechanical properties, wettability, biocompatibility and biodegradability. These properties give sufficient reason for this copolymer to be further investigated.

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