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Near-to-Eutectic Mixtures as Bifunctional Catalysts in the Low-Temperature-Ring-Opening-Polymerization of ε-caprolactone

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Abstract

We have investigated the ring-opening polymerization (ROP) of ε-caprolactone using mixtures of methanesulfonic acid and the guanidine 1,5,7-triazabicyclo[4.4.0]dec-5-ene as the catalyst. Our interest in these mixtures is based on the capability of both acids and bases to behave as bifunctional catalysts; the former by the combined action of acidic hydrogens and basic oxygens, and the latter by the hydrogen-bond acceptor and donor features of, respectively, a basic nitrogen center and a ortho-hydrogen atom. We found that these compounds are capable to form eutectic mixtures for a certain molar ratio. Upon the use of these mixtures – e.g. with molar ratios near to the eutectic one – as catalysts, neither further solvents nor initiators were required to carry out the ROP of ε-caprolactone. The resulting PCLs were highly crystalline (more than 87%) and exhibited an excellent capability to support the growth of murine L929 fibroblasts. We consider that the preparation of biocompatible PCLs at physiological temperatures and in the absence of further reagents than the monomer and the catalyst offers an interesting alternative to both the self-cross-linked oligomers/macromers of acrylate-based PCL-derivatives and the pH/temperature-sensitive PCL copolymers used to date as injectable biomaterials.

Introduction

Polycaprolactone (PCL) is a hydrophobic and semi-crystalline polymer, that has become one of the most important synthetic biodegradable polymers investigated for a wide range of biomedical and pharmaceutical applications such as controlled drug delivery, resorbable sutures, medical implants, and scaffolds for tissue engineering because of its mechanical properties, miscibility with a large range of other polymers and biodegradability.[1] Particularly interesting has been the use of PCLs and PCL-derivatives as injectable biomaterials for drug delivery and tissue engineering.[2] Within this context, either self-cross-linked oligomers/macromers of acrylate-based PCL-derivatives[3, 4] or pH/temperature-sensitive PCL copolymers[5] have been widely used. Nonetheless, the development of novel forms of injectable PCL is yet challenging because there are certain aspects of both approaches – e.g. the uncontrolled rise of temperature at the surrounding environment of the PCL because of the exotermic nature of acrylic polymerizations in the former case and the uncontrolled tendency to form a gel and clog the needle as soon as the temperature rises to 37 °C in the latter one – that may limit their practical application. Thus, one may wonder, for instance, whether the design of a chemical process for PCL formation taking place under mild reaction conditions – e.g. at physiological temperatures and in the absence of harsh solvents – could offer an interesting alternative.

Two main pathways have been described in the literature to produce PCL: the polycondensation of 6-hydroxyhexanoic acid, and the ring-opening polymerization (ROP) of the cyclic monomer ε-caprolactone.[6] The former is typically performed under vacuum, without the addition of catalyst and is completed in 6 h at a temperature that gradually increases from 80 to 150 °C. However, emphasis has been put on the ROP pathway due to its prevalence in the literature and the superior polymer that is obtained. Actually, ROP was the method first described for PCL preparation, as early as the 1930s.[7] ROP of ε-caprolactone is typically catalyzed by the addition of organometallic compounds (e.g. tin octoate) and high temperatures are typically applied. Thus, none of these approaches is particularly carried out under mild conditions so, more recently, significant attention is paid to the use of green chemistry alternatives focussed on the use of low-temperature and metal-free synthetic strategies that helps enhancing the green and biocompatible features of the resulting

PCLs. Within this context, enzymes have been widely used to produce both homopolymers and copolymers.[8, 9, 10] Organic catalysts (sulfonic acids, N-heterocyclic carbenes and guanidines, just to mention some of the most popular) provide a suitable alternative to organometallic compounds as well,[11, 12, 13] with the additional advantage of being more economic than enzymes.

One step forward to enhance the green features of any organic synthesis is the use of solvent-less approaches. Molten salts (ionic liquids, ILs) and deep eutectic mixtures – DESs, first described by Abbott and coworkers in 2003 and typically formed through hydrogen bonding, with one molecule acting as hydrogen-acceptor and the second one as hydrogen-donor[14, 15, 16, 17] – with melting points near or below room temperature offer interesting alternatives in this regard.[18, 19, 20] DESs are considered a sub-group within conventional ILs as they actually share many properties (e.g. non-reactive with water, non-volatile and biodegradable). However, they have demonstrated a tremendous potential in number of synthetic processes.[21, 22, 23, 24, 25, 26, 27] For instance, eutectic mixtures can play an all-in-one role (e.g. acting simultaneously as solvent and as either the monomer itself or the catalyst) thus enhancing the greenness features – in terms of reagents economy – of different synthetic processes.[28, 29] In the particular context of ROPs, Coulembier *et al.* have demonstrated that the use of the eutectic mixture of L-lactide (L-LA) and 1,3-dioxan-2-one – in the presence of DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) as catalyst and benzylalcohol as initiator – allows the preparation of poly(L-lactide) homopolymers at room temperature without addition of any extra solvent.[30]

Herein, we aimed to prepare mixtures of methanesulfonic acid ($MeSO_3H$) and the guanidine 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), two organic catalysts frequently used in ROP reactions that are capable to form eutectic mixtures. $MeSO_3H$ has been described as an efficient and low-cost catalyst that provides an excellent control of the molecular weight – i.e. narrow dispersity – of the resulting PCL.[31] Meanwhile, TBD is considered one of most efficient catalysts described to date for the ROP of cyclic esters either by itself[32] or in form of salts.[33] Interestingly, recent reports have predicted that both acids and bases behave as bifunctional catalysts. Thus, acids (e.g. phosphoric and sulfonic type) work as proton shuttles by the action of the acidic hydrogen atom and basic oxygen atoms,[11, 34, 35] while bases (e.g. 4-

dimethylaminopyridine and TBD, among others) act both as a hydrogen-bond acceptor (through its basic nitrogen center) and a weak hydrogen-bond donor (through one ortho-hydrogen atom). [36] Considering that eutectic mixtures are molecular complexes formed between hydrogen-acceptors and hydrogen-donors, it seems reasonable to evaluate their performance as bifunctional catalysts. This approach actually imitates those using bifunctional catalysts in the ROP of cyclic esters and carbonates – e.g. (−)-sparteine and thiourea,[37] N,N(-dimethylamino)-pyridine and its protonated form, [38] or diphenyl phosphate and 4-dimethylaminopyridine[39] for L-LA, trifluoromethanesulfonic acid (TFA) and 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD) for TMC,[40] and sulfonic acids and their conjugated bases for both TMC and ε-caprolactone.[41, 42] Neither compound of these mixtures could polymerize cyclic esters/carbonates by themselves with reasonable reaction kinetics. However, in combination, they create an effective yet mild catalyst system based on two distinct forms of catalytic hydrogen bonding: electrophilic activation of monomer carbonyls and nucleophilic activation of propagating hydroxyls. Similar is also the more-recently-described-approach using ammonium betaines as catalysts in the ROP of L-LA and cyclic carbonates.[43] In our case, we have used mixtures of $MeSO_3H$ and TBD as bifunctional catalysts in the ROP of ε-caprolactone. Thus, ROPs were carried out at room temperature, over 2 hours, with or without initiator, and in the absence of any further solvent. The number-average molecular weight (M_n) of the resulting PCLs was determined by gel permeation chromatography (GPC) and ^1H NMR spectroscopy. Moreover, the crystalline character of the resulting PCLs was determined by differential scanning calorimetry (DSC) and X-ray diffraction (XRD). Finally, we performed some *in-vitro* studies to investigate the biocompatibility of the resulting PCLs to support the growth of mammalian cells.

Results and Discussion

As mentioned in the experimental part, the TBD:$MeSO_3H$ mixtures were formed with two different molar ratios – e.g. 0.5:1.5 and 0.1:1.5 (Fig. 1a). The formation of H-bond complexes in TBD:$MeSO_3H$ was confirmed by the upfield chemical shift of the signals ascribed to TBD and $MeSO_3H$ in the ^1H NMR spectra of the mixtures as compared to those of the individual components (Fig. S1). Furthermore, the

methylene groups of TBD in form of DES appeared as three distinguishable peaks as compared to the just two distinguishable peaks observed for TBD in solution. The DSC scans of the TBD:$MeSO_3H$ mixtures revealed that the melting point (T_m) was 37°C for the mixture with a 0.5:1.5 molar ratio and –3°C for the mixture with a 0.1:1.5 molar ratio (Fig. 2), this is below those of the respective components – e.g. 130 and 17°C for TBD and $MeSO_3H$, respectively. The glass transition temperature (T_g) of both mixtures was similar, in agreement with their similar viscosities – e.g. 45 and 12 cP for the mixtures with 0.1:1.5 and 0.5:1.5 molar ratios, respectively. It is worth noting that the eutectic mixture of TBD and $MeSO_3H$ was formed at a 0.05:1.5 molar ratio (Fig. 1a and Fig. S2), so our mixtures – named as the nearer-to-the and the farther-to-the eutectic one in Fig. 1a – should be considered as a solution of the component in excess (TBD in our case) in the liquid phase formed by the two components in the eutectic ratio. [44]

Thus, we first investigated the ROP of ε-caprolactone using benzyl alcohol as the initiator and the near-to-the eutectic mixture of TBD:$MeSO_3H$ (e.g. 0.1:1.5) as both the catalyst and the reaction medium. ROPs were carried out at 37 °C, over 2 hours, and in the absence of any further solvent. We prepared a first set of four PCLs resulting from the use of different benzyl alcohol and TBD:$MeSO_3H$ molar ratios (see PCL*x*I*y*_*z*:*w*, where *x* represents the catalyst concentration, *y* the initiator concentration and *z*:*w* the molar ratio of TBD:$MeSO_3H$, see Table 1). The resulting polymers were always obtained in high yields (ca. 99%, see Table 1). The dispersity (D) was around 1.5 for every sample as revealed by GPC analyses (Table 2, Fig. S3). The M_n of the resulting PCLs – also from GPC analyses – ranged from ca. 6120 to 8675 mol/g in the order PCL2I2_0.1:1.5 < PCL2I1_0.1:1.5 < PCL1I2_0.1:1.5 < PCL1I1_0.1:1.5 (Table 2). Thus, the PCL prepared using the lowest molar ratio – referred to ε-caprolactone – of both TBD:$MeSO_3H$ and initiator provided the highest M_n. The polymers were also identified as PCL by ^{1}H NMR spectroscopy (see Fig. 3, Fig. S4 and Table S1). [45, 46] The M_n calculated from the ^{1}H NMR spectra followed the same pattern than those obtained from GPC, with degrees of polymerization (DP) that ranged from 32 to 45 (Table 2). ^{1}H NMR spectroscopy also revealed the presence of both the α-ester from the initiating alcohol and the β-hydroxyl chain-ends as end-groups (Fig. S4). The presence of these groups was indicative of the formation of linear rather than cyclic PCLs. [47]

As mentioned in the introduction, eutectic mixtures are formed between hydrogen-bond acceptors and hydrogen-bond donors. Thus, a plausible mechanism for the ROP of ε-caprolactone in presence of TBD:$MeSO_3H$ should resemble the mechanism reported for bifunctional catalysts where an acidic group capable of hydrogen bond donation and a Lewis basic atom capable of accepting a hydrogen bond interchange roles during reaction allowing proton transfer to occur between the catalyst, the initiator and the monomer (Scheme II). A thorough full description of the mechanism can be found elsewhere for bifunctional catalysts composed on both single organic acids – e.g. sulfonic acids – and their conjugated bases,[41, 42] and regular acid-base pairs – e.g. TFA and MTBD – with excess of base.[40] In our case, we had an acid-base pair with excess of acid so one could hypothesized a mechanism where $MeSO_3H$ acts as a proton shuttle via its acidic hydrogen atom and basic oxygen atoms – this is, the hydrogen-bond-acceptor role was played by not only TBD but also $MeSO_3H$. Nonetheless, it is worth noting that DESs differ from regular acid-base pairs – as well as from ILs – because of the wide range of stoichiometries in which the hydrogen-bond acceptor and the hydrogen-bond donor can combine. This is actually the case of TBD:$MeSO_3H$ mixtures, the eutectic of which is formed for a 0.05:1.5 molar ratio. Thus, the mixture of TBD:$MeSO_3H$ used as catalyst – with a 0.1:1.5 molar ratio – must be considered as a solution of TBD in the liquid phase formed by the components in the eutectic ratio and hence, we considered more plausible a mechanism based on excess of base than of acid (Fig. 1b).[40]

We subsequently performed the ROP of ε-caprolactone as described above – i.e. at 37 °C, over 2 hours, and in the absence of any further solvent – but without benzyl alcohol as initiator. In this case, we used both the eutectic mixture of TBD:$MeSO_3H$ (e.g. 0.1:1.5) and the non-eutectic one (e.g. 0.5:1.5), and we just focussed on the TBD:$MeSO_3H$ to ε-caprolactone molar ratio that provided the best results in the previous experiments. We also aimed to investigate the use of every catalyst in its single form to assess whether the combined action of the two catalysts was more effective. This was possible for $MeSO_3H$ – with a molar ratio of 0:1.5 according to the nomenclature used for eutectic and non-eutectic mixtures, see Fig. S2 – but not for TBD, the solubility of which in ε-caprolactone – for neither 0.1:0 nor 0.5:0 molar ratios, also according to the above-mentioned nomenclature – impeded ROP to

take place in a homogeneous fashion comparable to those studied before. Thus, this second set was finally composed of three PCLs (see PCL1I0_0:1.5, PCL1I0_0.1:1.5 and PCL1I0_0.5:1.5, in Table 1). The resulting polymers were obtained in high yield (ca. 99%) and the GPC analysis showed unimodal distributions with Ds of 1.5-1.6 in every case, this is similar to what was obtained in presence of initiator (Table 2, Fig. S3). The polymers were also identified as PCL by ^{1}H NMR spectroscopy (see Fig. 4, Fig. S5 and Table S2). The M_n calculated from the ^{1}H NMR spectra followed the same pattern than those obtained from GPC, with DPs that ranged from 42 to 57 (Table 2). As for the PCLs obtained with initiator, the presence of β-hydroxyl chain-ends as end-groups was indicative of the formation of linear rather than cyclic PCL (Fig. S5). Both GPC and ^{1}H NMR spectroscopy revealed that the three PCLs obtained in the absence of initiator experience a slight increase of the M_n as compared to those obtained in its presence. Actually, one could establish that M_n increases when the initiator concentration decreases in the order PCL1I2_0.1:1.5 < PCL1I1_0.1:1.5 < PCL1I0_0.1:1.5 (Table 2). Also in terms of M_n and among the different catalysts studied in the absence of initiator, the use of single $MeSO_3H$ proved to be less effective than any of the other mixtures (Table 1). Interestingly, the use of the non-eutectic TBD:$MeSO_3H$ mixture with the 0.5:1.5 molar ratio as the catalyst provided a slight increase in the M_n of the resulting PCL as compared to that obtained with the eutectic one (i.e. 9900 mol/g versus 8900 mol/g, see Table 2). As mentioned above, our mixtures can be considered as a solution of the component in excess (TBD in our case) in the liquid phase formed by the two components in the eutectic ratio.[44] Thus, the more efficient catalysis exerted by the farther-to the eutectic mixture – e.g. TBD:$MeSO_3H$ with the 0.5:1.5 molar ratio – could be ascribed to the presence of TBD in excess that is not participating in the formation of the eutectic and hence, it is more available for catalysing the reaction. Actually, we have previously observed the partial disruption of the H-bond complexes that form the DESs – either by dissolution and partial disruption of the H-bond complexes that form the eutectic mixture [48, 49, 50, 51] or by the use of an unbalanced molar ratio between the two components that form the eutectic mixture[52, 53] – enhances the monomers availability and hence, the overall efficiency of polycondensation.

At this stage, we investigated the crystalline character of the resulting PCLs by DSC and XRD. DSC scans provided the melting point (T_m) and heats of fusion (ΔH) (Fig.

5 and S6). The latter was used to calculate the degree of crystallinity (X_c) based on the enthalpy of melting of 100% crystalline PCL (ΔH^0 = 135.44 J/g).[54] In our case, the temperature used for synthesis was well below the T_m of PCL (e.g. 37 versus ca. 55°C). Thus, the as-synthesized PCLs exhibited an extremely high X_c (above 84% in every case, see Table 2) because of the well-known capability that bulk PCLs have to crystallize at temperatures within the 30 to 40°C range.[55, 56] X_c was particularly high (up to 98%) when ROP was carried out in the absence of initiator (Table 2).[57] The T_m of all the as-obtained PCLs was also well-above 55 °C (Table 2), in agreement with the high X_c described above. Nonetheless, the thermal treatment of the as-synthesized PCLs above their melting temperatures allowed the recovery of X_c and T_m values in range to those found in PCLs obtained in solution and/or using temperatures higher than 55°C (Table 2). The high crystalline character of the as-synthesized PCLs was also confirmed by XRD (Fig. 6 and Fig. S7).

To assess whether this approach could be suitable for preparation of injectable biomaterials based on PCL, we further studied some interesting features of the PCL1I0_0.1:1.5 sample as representative of the above-studied ones. For instance, Fig. 7 shows that the time frame of the starting fluid solution to turn into a viscous one and, eventually, become a gel was in range to those reported for, for instance, biomaterials based on acrylate-based PCL-derivatives – e.g. 30 min.[3] Also within the context of injectable biomaterials, the biocompatibility of PCL1I0_0.1:1.5 to support the growth of mammalian cells is critical as well.[3-5] For this purpose, we prepared thick films on which we cultured murine L929 fibroblasts and performed the following *in vitro* studies. These cells – commonly used to determine if a certain biomaterial is cytocompatible or cytotoxic[58] – are considered as slow-moving cells that typically display a fusiform and elongated morphology in control cultures – e.g. glass coverslips. Cells were cultured over 1, 4 and 7 days. Confocal laser scanning microscope (CLSM) images in Fig. 8 revealed that L929 fibroblasts properly adhered to the substrate since the very early stages of the culture and, afterwards, progressively proliferated and colonized the totality of the films surface – i.e. cultures reached confluence at 7 days – with high viability values (over 95 %). The preservation of the typical morphology L929 fibroblasts cultured on PCL was confirmed by scanning electron microscopy (see SEM micrographs in Fig. 9).

Conclusions

We have demonstrated the capability of two mixtures of $MeSO_3H$ and TBD in an either eutectic or non-eutectic form to catalyze the ROP of ε-caprolactone under physiological conditions, this is, at temperatures as low as 37 °C and in the absence of further solvents and even initiator. Because the polymerization was carried out at pseudo-high monomer concentrations and at temperatures below the melting point of PCL, the crystallinity of the as-synthesized PCLs was well above those typically found for PCLs synthesized in solution and at higher temperatures (e.g. up to 94 in the former case versus 66% in the latter one). Finally, we found that the PCLs synthesized in this work were highly suitable to support the growth of murine L929 fibroblasts. It is obvious that this behaviour must be ascribed to the intrinsic biocompatibility of PCL. Nonetheless, it is also worth noting that ROPs minimizing the presence of chemical reagents that may be potentially harmful (e.g., solvents, initiators, non-polymerized monomers, etc.) contribute to improve the biocompatibility of the resulting materials. This is indeed the situation with our DES-assisted ROPs that proceeded in the absence of solvents and initiators, and with nearly full monomer conversions. This result opens promising perspectives in the field of injectable biomaterials as an interesting alternative to both the self-cross-linked oligomers/macromers of acrylate-based PCL-derivatives and the pH/temperature-sensitive PCL copolymers used to date.

Experimental Part

Materials: 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD, 98%), methanesulfonic acid ($MeSO_3H$, 99.9%), ε-caprolactone, benzyl alcohol (BA) and deuterium water were purchased from Sigma Aldrich. Acetone was provided from Scharlab and deuterium chloroform was purchased from Tracer. Cell culture media and supplements were purchased from Lonza. All the reagents were used as received. Water was distilled and deionized.

Preparation of the Eutectic and Non-Eutectic Mixtures: TBD and $MeSO_3H$ were drop wise mixed in an ice bath to avoid acid-base/exothermic reactions. Two different mixtures with 0.1:1.5 and 0.5:1.5 molar ratios were prepared. Afterwards, the mixtures were thermally treated at 90 °C over 30 minutes under stirring until complete

formation of homogeneous and transparent liquid solutions. Once formed, the mixtures (both the eutectic one with a 0.1:1.5 molar ratio and the non-eutectic one for a 0.5:1.5 molar ratio) were cooled to 37 °C.

Polycaprolactone (PCL) Synthesis: In a typical synthesis, ε-caprolactone (1.712 g, 15 mmol) was placed in a 5 mL sealed container at 37 °C over 10 minutes. Then, the TBD:$MeSO_3H$ mixture (either the eutectic or the non-eutectic one) was added to e-caprolactone. The initiator was also added to this solution in those syntheses where it was used. The mixture was vortexed over five minutes and polymerization proceeded at 37 °C over the following 2 hours. The resulting PCLs were first washed in water (40 mL) for 24 hours to recover the eutectic and non-eutectic mixtures, and then in acetone to remove small oligomers. Dried PCLs were finally obtained after acetone evaporation – i.e. 48 hours at room temperature. The samples were obtained in form of both monoliths – when the synthesis was carried out in vials – or films – when the mixture containing all the components was cast onto glass coverslips.

Polymer Characterization: ^1H NMR spectra were recorded at 37 °C using a Bruker DRX-500 spectrometer. The neat eutectic and non-eutectic mixtures were placed in capillary tubes using deuterium chloroform as an external reference. The resulting PCLs (typically 20 mg) were dissolved in deuterium chloroform, placed in NMR tubes, and the spectra were recorded at room temperature. The spectra were performed before PCLs washing to calculate the polymer conversion, and after washing to calculate the DP and the M_n. The residue obtained after PCL washing was also studied by ^1H NMR spectroscopy. For this purpose, the residue was dissolved in deuterium water and placed in NMR tubes at room temperature. The M_n – as well as Ds – were also obtained by gel permeation chromatography (GPC) using Styragel (300×7.8 mm, 5 mm nominal particle size) Water columns and THF as the solvent. GPC analyses were carried out at 35 °C, at a flow rate of 1 mL min^{-1}, and using a RI detector. The resulting molecular weights were referenced to PS standards. DSC scans were performed in a DSC Q-100 calorimeter. Scans consisted of an initial cooling (at 5 °C min^{-1}) from room temperature to −90 °C, the temperature was maintained −90 °C over 10 min, then raised (also at 5 °C min^{-1}) to 100 °C, and finally cooled (at 5 °C min^{-1}) from 100 to room temperature. This cycle was repeated twice for DESs and three times for PCLs. The melting point (T_m) of DESs was obtained from the 1st run. The T_m and heat of fusion

(ΔH) of the as-synthesized PCLs were obtained from the 1st run while the 2nd run provided the T_m and ΔH of PCLs recrystallized after melting. X-ray diffraction (XRD) was carried out in a Bruker D8 Advanced diffractometer and using the CuK radiation (0.05° step size and 1.5 s counting time).

In vitro studies: Prior to cell culture, PCL thick films were placed on 24-well culture plates and sterilized under UV radiation for 20 minutes per side. After several washes in tissue grade water, samples were preconditioned in culture medium for 24 hours to promote serum protein adsorption to the surface. Murine L929 fibroblasts were then cultured at a density of $2.5 \cdot 10^4$ cells per well. The culture medium used was Dulbecco's Modified Eagle's Medium (DMEM) supplemented with fetal bovine serum (FBS, 10 %), streptomycin (100 UI mL^{-1}), penicillin (100 UI mL^{-1}), and L-glutamine (1 mM). Cultures were maintained at 37 °C in a sterile incubator under a CO_2 atmosphere (5 %) and media changed every other day. Glass coverslips were used as a control surface. Cell viability was assessed in cultured cells by using a Live/Dead® Viability kit (Invitrogen) based on the cellular uptake of two probes: calcein by live cells, and ethidium homodimer-1 (EthD-1) by dead ones. Samples were visualized by using a Leica SP5 confocal-laser scanning microscope (CLSM). The wavelength used for excitation was 488 nm – using an Argon laser. The emission was recorded from 505 to 570 nm for green fluorescence (calcein) and from 630 to 750 nm for red fluorescence (EthD-1). Light reflexion of the excitation wavelength was also recorded to visualize the material surface structure and the relative cellular location. The morphology of L929 fibroblasts cultured on PCLs was studied by scanning electron microscopy (SEM) in a Hitachi S-4700 microscope. For this purpose, cultured cells were rinsed in PBS twice and fixed with glutaraldehyde (GA, 2.5 % in phosphate buffer saline). After GA exposure over 30 minutes, samples were washed with abundant distilled water. Water was then replaced by different ethanol aqueous solutions of increased ethanol concentration. The treatment for each ethanol solution lasted 15 minutes and was repeated twice. The final treatment – with absolute ethanol – lasted 30 minutes. Samples were finally dried at room temperature over 24 hours, mounted in stubs and coated with gold under vacuum conditions for SEM visualization.

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Table 1: Summary of the components used in the synthesis, the molar ratio used for every one, the reaction time, and the yield in which PCL was obtained in every case.

Sample	TBD (mmol)	$MeSO_3H$ (mmol)	ϵ-caprolactone (mmol)	BA (mmol)	Time (hours)	Yield (%)
Set 1						
PCL1I1_0.1:1.5	0.1	1.5	15	0.15	2	99
PCL1I2_0.1:1.5	0.1	1.5	15	0.3	2	99
PCL2I1_0.1:1.5	0.05	0.75	15	0.15	2	99
PCL2I2_0.1:1.5	0.05	0.75	15	0.3	2	99
Set 2						
PCL1I0_0:1.5	0	1.5	15	0	2	96
PCL1I0_0.1:1.5	0.1	1.5	15	0	2	98
PCL1I0_0.5:1.5	0.3	1.5	15	0	2	98

Table 2: Summary of data – e.g. dispersity (D), degree of polymerization (DP), and the number-average molecular weight (M_n) – obtained from GPC and ^{1}H NMR for the different PCLs. Data coming from DSC with regard to the melting temperature (T_m), degree of crystallinity (X_c), and enthalpy of melting (ΔH) of the different PCLs (both as-synthesized – 1st run – and after thermal treatment and recrystallization – 2nd run) are also included.

Sample	D[a]	M_n (mol/g)[a]	DP[b]	M_n (mol/g)[b]	T_m (°C) 1st – 2nd run	ΔH (J/g) 1st – 2nd run	X_c (%) 1st – 2nd run
Set 1							
PCL1I1_0.1:1.5	1.5	8675	45	5250	60.6 – 48.5	116 – 87	86 – 65
PCL1I2_0.1:1.5	1.5	7760	40	4640	59.4 – 49.6	113 – 85	84 – 63
PCL2I1_0.1:1.5	1.5	7500	42	4910	60.6 – 51.5	113 – 83	84 – 61
PCL2I2_0.1:1.5	1.5	6120	32	3760	60.2 – 46.2	117 – 87	87 – 65
Set 2							
PCL1I0_0:1.5	1.6	8719	42	4799	66.5 – 49.1	132 – 85	98 – 63
PCL1I0_0.1:1.5	1.6	8960	54	6170	66.5 – 59.9	131 – 87	97 – 65
PCL1I0_0.5:1.5	1.5	9925	57	6480	63.5 – 53.5	118 – 83	87 – 61

[a] From GPC [b] From ^{1}H NMR

Fig. 1: (a) Left: Methanesulfonic acid ($MeSO_3H$) and the guanidine 1,5,7-triazabicyclo[4.4.0]dec-5-ene used for the preparation of mixtures. Right: Phase composition – in mol % TBD – diagram representing the melting points of the TBD:$MeSO_3H$ mixtures with molar ratios of 0.5:1.5 (cross), 0.1:1.5 (open triangle), 0.05:1.5 (solid square), and 0.02:1.5 (open circle). The melting points of $MeSO_3H$ (open diamond) and TBD (open square) are also included for comparison. The eutectic TBD:$MeSO_3H$ mixture was obtained for the 0.05:1.5 molar ratio. The arrows indicate the TBD:$MeSO_3H$ mixtures used as catalysts in this work. (b) The proposed mechanism – based on bifunctional activation – for the ROP of catalyzed by the TBD:$MeSO_3H$ mixtures with 0.5:1.5 and 0.1:1.5 molar ratios.

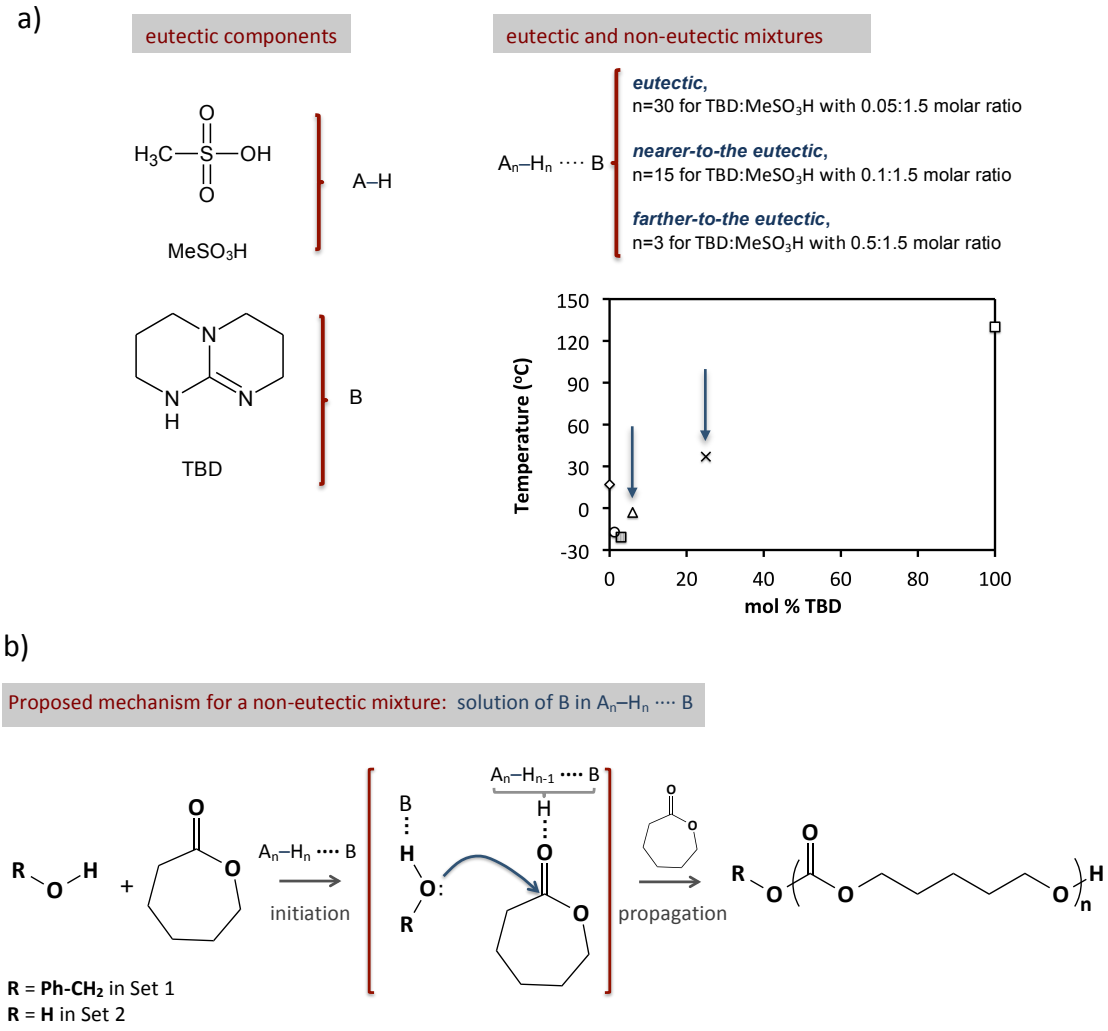


Fig. 2: DSC scans of the TBD:$MeSO_3H$ mixtures with (a) 0.5:1.5 and (b) 0.1:1.5 molar ratios.

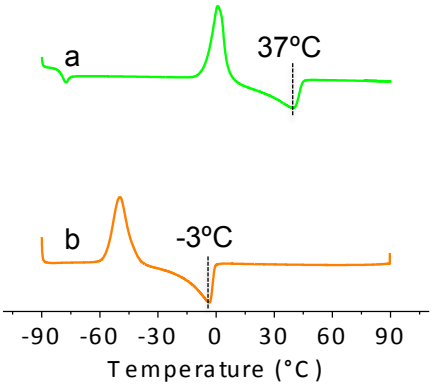
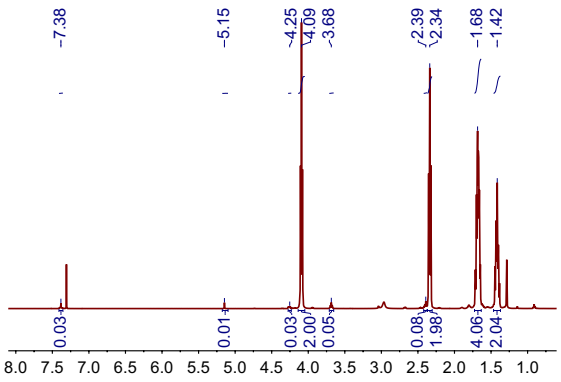


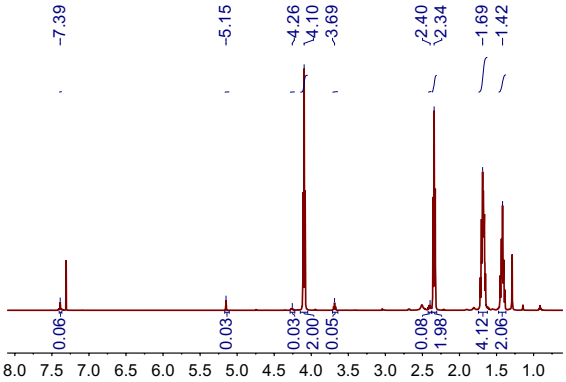
Figure 3: ^{1}H NMR spectra of the PCLs obtained with initiator as described in Table 1.

PCL1I1_0.1:1.5



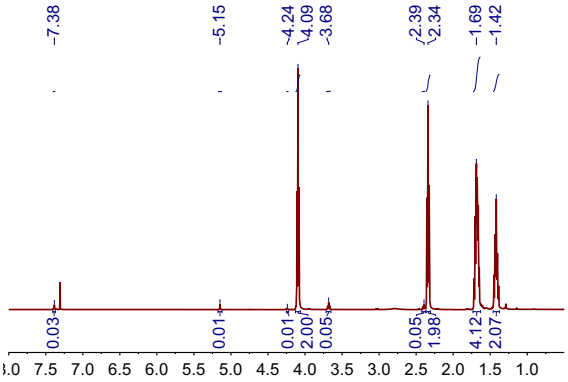
δ (ppm)

PCL1I2_0.1:1.5



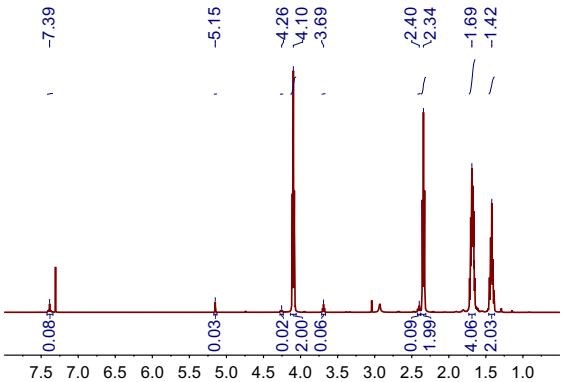
δ (ppm)

PCL2I1_0.1:1.5



δ (ppm)

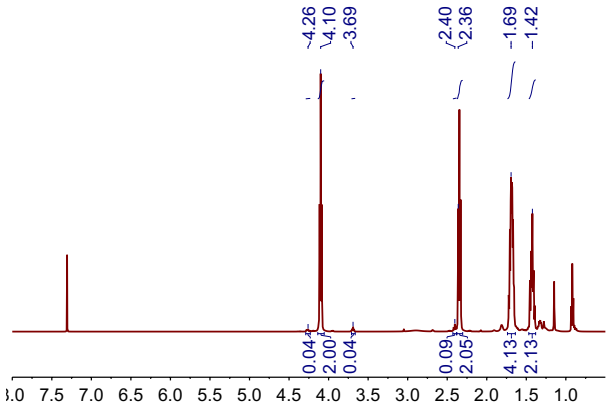
PCL2I2_0.1:1.5



δ (ppm)

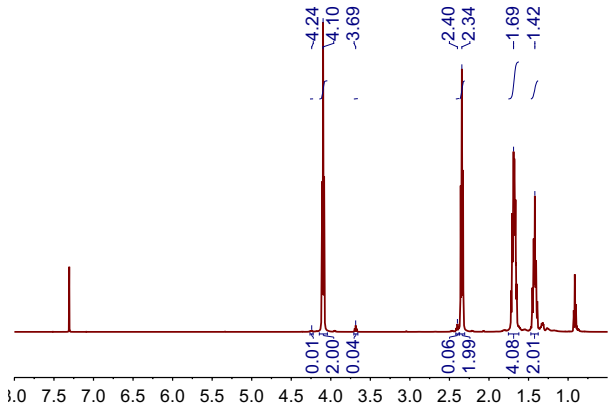
Figure 4: ^{1}H NMR spectra of different PCLs obtained without initiator as described in Table 1.

PCL1I0_0.1:1.5



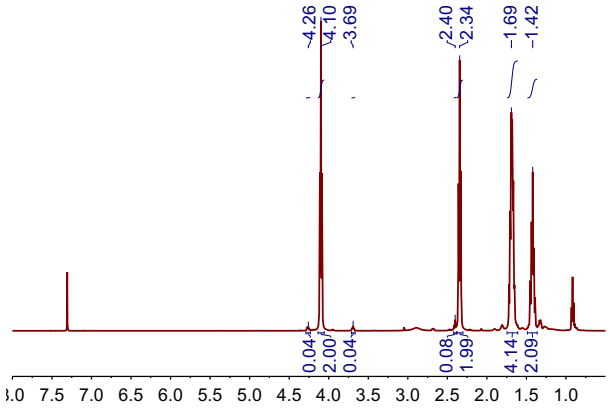
δ (ppm)

PCL1I0_0.5:1.5



δ (ppm)

PCL1I0_0:1.5



δ (ppm)

Fig. 5: DSC scans of PCL1I0_0.1:1.5 (a) as synthetized – i.e. 1st run – and (b) after melting and recrystallization – i.e. 2nd run.

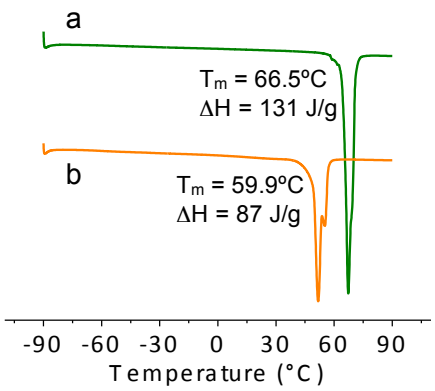


Fig. 6: XRD patterns of PCL1I0_0.1:1.5 (a) as synthetized and (b) after melting and recrystallization – recovered from 2nd run of DSC experiment.

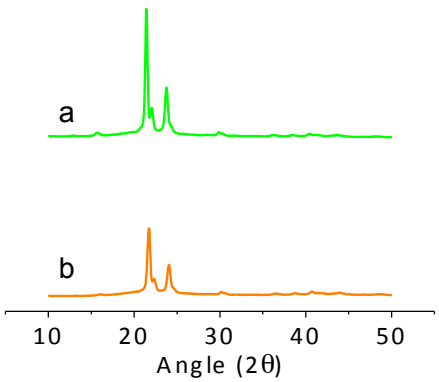


Fig. 7: The pictures reveal the change in viscosity – from a fluid solution to a viscous one in less than 20 min and up to a gel in less than 40 min – that takes place during the ROP of ε-caprolactone for the preparation of PCL1I0_0.1:1.5 – i.e. at 37 °C and in the absence of any further solvent.

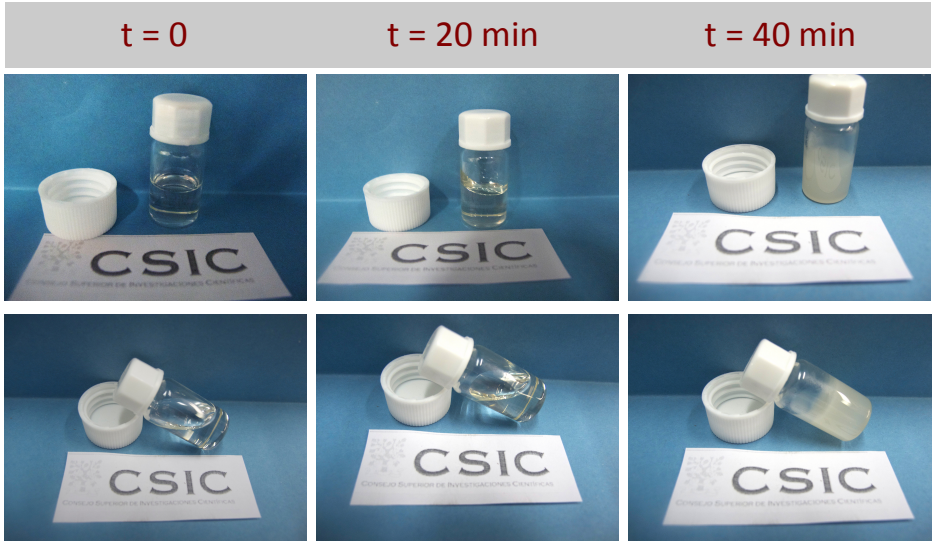


Fig. 8: Fluorescent confocal micrographs of mouse L929 fibroblasts cultured on glass (used as control) and PCL1I0_0.1:1.5. Cells were cultured over 1, 4 and 7 days. Every micrograph was taken at the same magnification.

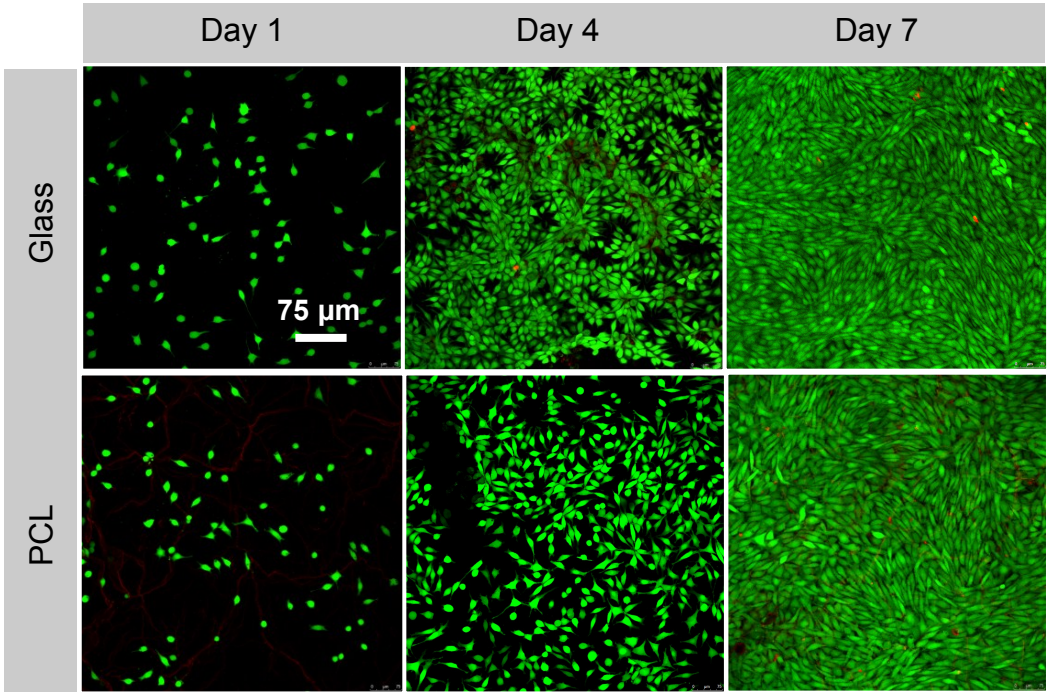


Fig. 9: SEM micrographs of mouse L929 fibroblasts cultured on glass (used as control) and PCL1I0_0.1:1.5. Cells were cultured over 1, 4 and 7 days. Every micrograph was taken at the same magnification.