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Kimikako Gradua

GRADU AMAIERAKO LANA

"Waterborne degradable polyester nanoparticles: Synthesis of ε-caprolactone and L-lactide- based PEGylated nanoparticles through free radical polymerization"

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Donostia, 2021ko iraila.

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ABSTRACT

Nanoparticle (NP) based drug delivery systems offer a more efficient and controlled way of administrating drugs compared to the traditional methods, and polymeric NPs seem to be a promising platform due to its numerous advantages and properties. Conventional techniques such as nanoprecipitation require extensive use of the solvents and numerous steps to produce NPs. Therefore, in this work, the production of PEGylated polyester based NPs in a two-step process, reducing the amount of organic solvents as much as possible was studied. To do so, biocompatible ε–caprolactone and L-lactic acid based macromonomers of different chain lengths were produced by ring opening polymerization (ROP), and then polymerized with poly(ethylene glycol) methyl ether methacrylate (PEGMA) via free radical polymerization (FRP) in aqueous and water-ethanol mediums. Latexes were analysed regarding conversion of the reaction, coagulum content and particle size. To ensure the degradability of the produced NPs, a hydrolytic degradation study was performed for polymers produced by solution polymerization. Molar mass evolution, thermal properties and water absorption and remaining weight were monitored during the study.

LABURPENA

Nanopartikuletan (NP) oinarritutako sendagaiak emateko sistemek sendagaiak administratzeko modu eraginkorrago eta kontrolatuago bat eskaintzen dute metodo tradizionalekin alderatuta. NP polimerikoak aukera ona dira eskaintzen dituzten propietate eta abantailengatik. Nanoprezipitazioa bezalako teknika konbentzionalek disolbatzaileen erabilera zabala eta NPak sortzeko urrats ugari behar dituzte. Hori dela eta, lan honetan, PEGilatutako poliesterretan oinarritutako NPen ekoizpena bi urratseko prozesuan aztertu zen, disolbatzaile organikoen erabilera ahal den neurrian murriztuz. Horretarako, kate luzera desberdineko ε–caprolactona eta L-azido laktikoan oinarritutako makromonomero biobateragarriak eraztun irekitze (ROP) bidez ekoiztu ziren. Ondoren polietilenglikolarekin (PEG) polimerizatu ziren erradikal askeen polimerizazio (FRP) bidez ur eta ur-etanol ingurunean. Latexak erreakzioaren

konbertsioa, koagulo edukia eta partikulen tamaina bidez karakterizatu ziren. Ekoitzitako NPen degradagarritasuna bermatzeko, degradazio hidrolitikoaren azterketa egin zen antzeko polimeroentzako. Azterketan zehar pisu molekularraren eboluzioa, propietate termikoak eta uraren xurgapena eta pisu galera behatu ziren.

1. Introduction

Among all the numerous applications that polymers have, medicine is one of the most significant fields. Some polymers, due to their biodegradable and biocompatible properties, are used for multiple medical applications, from re-absorbable sutures to orthopaedic implants. Their carbon based chemistry make polymers closer to biological tissue than inorganic materials, and they have further the advantage to be tuneable in physical, chemical and biological properties in a wide range to meet the requirements of specific applications.[1,2,3] With the flowering of nanotechnology, revolutionary steps have been taken forward in medicine: differently functionalized nanoparticles find advanced utilizations in medical diagnostics and drug delivery. Novel drug delivery systems came into existence to overcome the shortcomings of conventional dosage, and polymeric nanoparticles seem to be a promising platform to tackle them.[4,5]

1.1. Nanoparticles as drug delivery system

NPs are particles that can be described as small nanostructured materials which are characterized for their nano-metric size, commonly not passing the 100 nm. Therefore, NPs can be used as drug delivery systems that can transport active ingredients to a targeted tissue or organ, with the specified concentration and controlled release.[6]

Since conventional dosage forms, such as pills and solutions, encounter many drawbacks like low bioavailability and efficiency, rapid clearance, and in the case of chemotherapeutic agents, high toxicity and poor specificity, different systems for administrating drugs have been developed. NPs can enable an effective delivery of the drug into the desired tissue, and concentration, and prolong circulating half-life and reduce dosage frequency. This way efficacy and bioavailability is increased, and toxicity and side effects are reduced.[7,8]

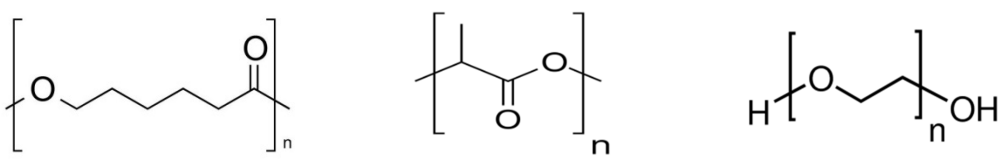
Many different types of nanocarriers exist depending on their conformation and composition. They can be made of inorganic, metallic, viral, lipid and polymer materials. However, polymer based nanoparticles exhibit many advantages over other materials like simple elaboration and design, good

biocompatibility, variety of possible morphologies and easy control over size distribution.[9]

1.2. Polymers used for the production of nanoparticles.

The selection of one polymer or another for the drug delivery system depends on the nature of the drug, the interaction and stability of the drug with the polymer, and the end use that want to be achieved. Both synthetic and natural polymers can be used. Among the natural polymers cellulose, natural latex, starches and proteins such as albumin are the most employed ones. Regarding the synthetic polymers, a large list of them can be used safely, guarantying their biocompatibility. Some examples are polylactides (PLA), poly (lactide coglycolides) (PLGA), polyglycolides (PGA), polyanhydrides, polyorthoesters (POE), polycyanoacrylates, polycaprolactone (PCL), poly (malic acid) (PMLA), polyglutamic acid (PGA), poly (methyl methacrylate) (PMMA), poly (N-vinyl pyrrolidone) (PVP), poly (vinyl alcohol) (PVA), polyacrylamide (PAM), polyethylene glycol (PEG), polyacrylic acid (PAA) and poly (methacrylic acid) (PMAA).[7]

Some of the most common ones and hence, used in this work to produce nanoparticles due to their well-reported properties were PCL, PLA, and PEG. Figure 1 shows the chemical structure of the employed polymers. PLA is a synthetic polymer that can be obtained from renewable sources and shows many attractive characteristics for the production of NPs including excellent safety, good biocompatibility, low levels of immunogenicity and toxicity, and tuneable rate of biodegradation.[7]



PCL PLA PEG

Figure 1. Chemical structure of the polymers used in the work: polycaprolactone, polylactic acid and polyethylene glycol.

Regarding PCL, this is a fossil fuel-based aliphatic polyester and the six carbon chain in the repeating unit makes it intrinsically hydrophobic. It shows poor surface wetting and interaction with biological fluids, so it avoids cell adhesion and proliferation. It displays a slower degradation time than that of polylactide. Therefore, it can be used for longer-term devices.[11]

For PEG, there is a lot to be said. This is a hydrophilic polyester that can be found in the formulation of multiple nanoparticles due to its properties. Once NPs are injected intravenously, one of the main problems to be faced is NPs not being identified as foreign bodies. This is accelerated by proteins adhering to the surface of the NPs, provoking immune responses. It has been studied that a hydrophilic and neutral outer layer with hydrogen-bond acceptors instead of donors can reduce protein absorption. Consequently, PEG has been selected as one of the most effective biopolymers against protein absorption.[12] Something to be considered is that PEG is not degradable and can be excreted in the kidney. But thanks to its hydrophilic character it does not accumulate in the tissues.[6] Therefore, it is used to elongate circulation half-lives of the polymeric nanoparticles and also provide water solubility to hydrophobic drugs. There are different methods to attach PEG layer to the surface of biomaterials: physical adsorption of PEG-containing surfactants, self-assembling of PEG diblock copolymers, covalent bonding, complexation and electrostatic interaction. Although all of these strategies have been used in practice, they are still subjected to some limitations.[13] The most efficient way of PEGylating nanoparticles is the copolymerization of PEG with the polymer or monomer that will be used for the production of NPs.

1.3. Methods for the production of polymeric nanoparticles.

In the same way that the selection of the polymer plays an important role in acquiring the properties of interest, so does the preparation method, where morphology, size distribution and particle properties are strongly influenced.[14] Numerous strategies are found in literature for the production of polymeric NPs. These strategies can be divided into two: the ones in which particle formation

takes place from a pre-existing polymer, and the ones in which it occurs starting from monomer.

In the former, nanoprecipitation, solvent evaporation, salting out, supercritical fluid and dialysis can be found. In these techniques NPs are obtained by dissolving the preformed polymer in a suitable organic solvent and mixing it with an aqueous phase, in various different ways. One of the main problems of these techniques is the difficult and incomplete removal of the solvent.[15] Other of the limitations they face are the large particle size distribution, and the impossibility of achieving nanoparticles smaller than 200 nm, required for specific targeting.[16]

For monomer polymerization techniques, emulsion, mini-emulsion, micro-emulsion and interfacial polymerization are carried out. Among them, emulsion polymerization is the most employed process. [7]

1.3.1. Emulsion polymerization

Emulsion polymerization is a heterogeneous polymerization technique in which colloidal particles are obtained in a dispersed media, usually water. The product obtained is known as latex. In a direct process, that is, oil-in-water emulsion, hydrophobic monomers are emulsified in water using a surfactant and polymerized using a water soluble initiator. Particles ranging from 50 nm to 1000 nm are obtained through this process, but more commonly from 80 nm to 300 nm. As it is known, the dispersed system is thermodynamically unstable, and colloidal stability is provided by different type of stabilizers.[17] Water dispersed particles can be stabilized by electrostatic, steric or depletion effects.

Electrostatic stability is the main mode of colloidal stability in emulsion polymerization. It is achieved by using adsorbed ionic surfactant molecules, which carry their head group charge to the particle surface. So when the particles are close to each other, the counter ions will overlap, thereby generating repulsive force. Regarding steric stability, this is provided by the complete surface coverage of strongly adsorbed and/or chemically bonded (i.e. grafted) water-soluble polymer (WSP) chains, which spreads from the particle surface into the water

phase. In the absence of electrostatic effects, the repulsive force will not be generated until the particles approach at a distance close to twice the thickness of the WSP chain surface layer. Finally, depletion effects are caused by WSP chains that are free in the aqueous phase. At the high concentrations of WSP, the probability of particles approaching without a WSP chain between them is small, so when the particles approach the chain, this creates a force of repulsion between the particles.[18,19] Figure 2 is an illustration of the mentioned stabilization forms.

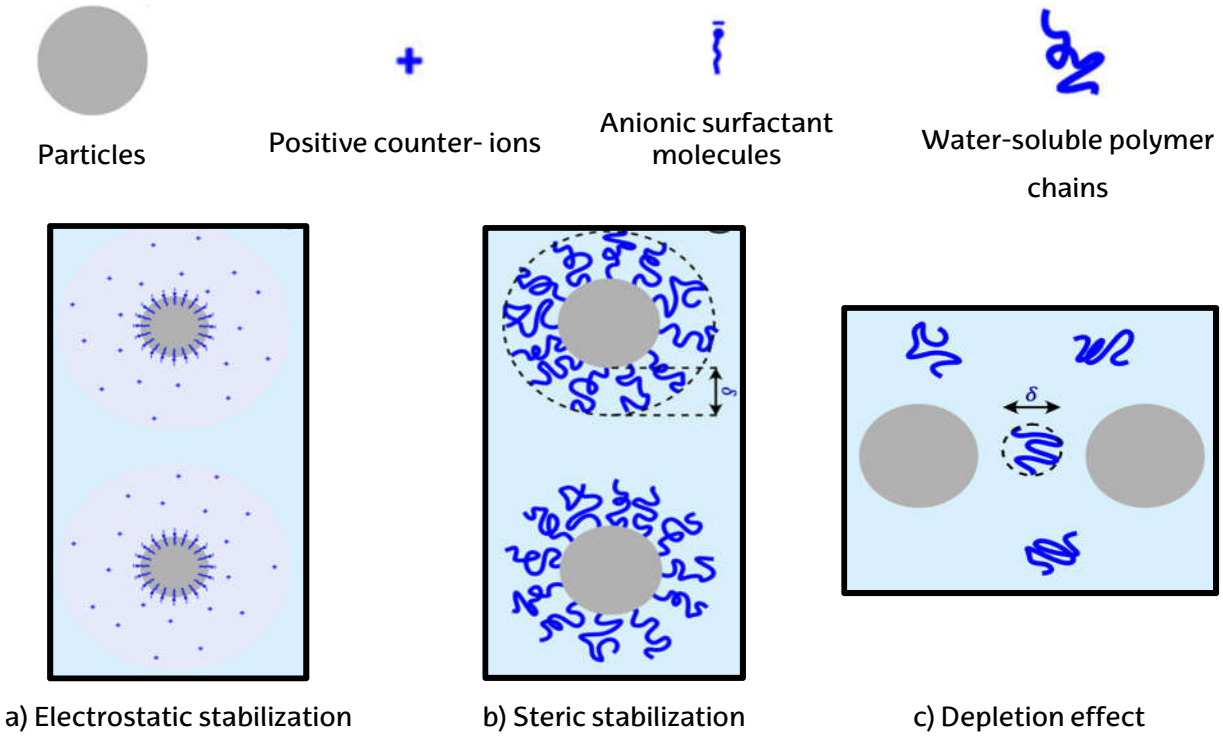


Figure 2. Representation of the different stabilization forms reproduced from reference.[19]

It is, therefore, the water as continuous reaction medium and the achievement of submicron nanoparticles what makes emulsion polymerization a suitable technique for the production of polymeric nanoparticles for drug delivery systems. However, the use of emulsifiers is a problem as they have to be removed after the polymerization process due to biocompatibility and toxicity issues. It is commonly removed by using ion exchange resins, but its effectiveness is difficult to be proved in many cases.[20]

1.3.2. Dispersion polymerization for producing nanoparticles.

Although dispersion and suspension polymerization are not normally used as route to obtain nanoparticles for drug delivery applications, colloidal polymeric particles can be obtained as well through these techniques. In this work, dispersion polymerization was studied.

Unlike emulsion polymerization, in dispersion polymerization the monomer is soluble in the continuous phase but not the polymer formed. Polymerization starts from a homogenous phase where the initiator, the stabilizer and the monomer are all dissolved in a suitable solvent or mixture. The continuous phase usually consists of water and alcohol (such as methanol or ethanol). Polymerization begins in the continuous phase, and the formed oligomers precipitate and aggregate to form particles, which are usually stabilized by nonionic surfactants such as polyvinyl pyrrolidone. Due to the short nucleation time, monodisperse particles with a size range of 1-5 μm are usually formed.[21]

1.4. Motivation and objectives of the work.

Many examples on the production of polyester nanoparticles can be found in literature. However, in most of those examples, techniques requiring large amounts of organic solvents and several synthesis and purification steps are used.[22,23] Consequently, the synthesis of polyester nanoparticles in a straightforward way reducing the use of surfactants and organic solvents is the need of the hour. Free radical polymerization techniques are a good alternative, and some authors have already reported examples.[7,14,15,24] However, problems when using long polyester macromonomer chains have to be still faced.

The main objective of the project is to produce polymeric nanoparticles which can be used in biomedical applications such as drug delivery. Therefore, biocompatible and degradable polymers are needed. With this aim, PEGylated polyesters (PCL and PLA) as biocompatible polymers are proposed to be produced by emulsion and dispersion polymerization, and their ability to form nanoparticles and to hydrolytically degrade is assessed in this project.

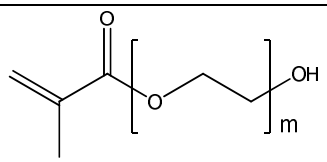
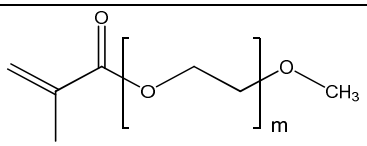
2. Experimental part

2.1. Materials and reagents

L-Lactide (Alfa Aeser), ε-caprolactone (Sigma Aldrich), 2-hydroxyhethyl methacrylate (HEMA, Sigma Aldrich) and tin(II) 2-ethylhexanoate ($SnOct_2$, Sigma Aldrich) were used as received for the synthesis of the MMs.

For the polymerization of the synthesised MMs potassium peroxodisulfate (KPS, Sigma Aldrich) was used as initiator. Ethanol was used as solvent (EtOH, Scharlab) in the polymerization of larger macromonomers. Regarding the stabilizers, different polyethylene oxide macromonomers were used as received: poly(ethylene glycol) methacrylate (PEGMA, Sigma Aldrich) of Mn = 300 g/mol, Mn=950 g/mol and Mn=2000 g/mol. Deionized water (Milipore Mili-Q purification system) was used during the whole process, and deutered chloroform ($CDCl_3$, Sigma Aldrich) was used as solvent for ^{1}H-NMR experiments. In Table 1 the chemical structure, ethylene oxide units and molar mass of the stabilizers employed is reported.

Table 1. Chemical structure of the different stabilizers employed.

	
Poly(ethylene glycol) methyl ether methacrylate **(PEGMA)**	***Methoxy Polyethyleneglycol Methacrylate*** **(Bisomer S20W)**
m = 5 Ethylene oxide units (EO) Mn = 300 g/mol	m = 45 EO Mn = 2080 g/mol Water content % (mass): 48 - 52
m = 19 EO Mn = 950 g/mol	
m= 43 EO Mn=2000 g/mol Water content %(mass):5O	

For the degradation study, the synthesised MMs were polymerized in solution using tert-butylbenzene (Aldrich) as reaction medium, azobisisobutironitril as initiator (AIBN, Sigma Aldrich) and as steric stabilizer methoxi polyethylenglycol methacrylatets (Bisomer S20W, GEO Speciality Chemicals). Phosphate buffer with

a pH=7.4 was prepared (NaCl 8,0 g/L, KCl 0,2 g/L, Na_2HPO_4 1,42 g/L and KH_2PO_4 0,24 g/L) and employed as degradation medium. GPC grade tetrahydrofuran (THF, Scharlab) was used as received.

2.2. Synthesis of the Macromonomers

ε-Caprolactone (CL) and L-Lactide (LA) based macromonomers (MM) and comacromonomers (co-MM) were synthesised using ring opening polymerization (ROP) in bulk, as described in various papers.[25–27] This is a well-controlled polymerization process in which tin(II) bis-(2-ethylhexanoate) ($Sn(Oct)_2$) was used as a catalyst and 2-hydroxyethyl methacrylate (HEMA) as initiator to obtain HEMA functionalized macromonomers. The repeating units of the macro- and co-macromonomers are controlled by the molar ratio of the monomer (L-lactide and -caprolactone) and HEMA. The products obtained from the ROP process were suitable for further free radical polymerization, since the vinyl group from HEMA is preserved during ROP. MMs and co-MMs of different chain length were synthesised. For the case of caprolactone, MMs with 3, 6 and 12 repeating units were synthesised, and named as MCL3, MCL6 and MCL12, respectively. For lactide based MMs, repeating units of 6, 12 and 20 were chosen namely MLA6, MLA12 and MLA20. Finally, a co-MM was synthesised with 4 repeating units of CL and 4 of LA, named as MCL4-co-LA4.

The reactants and the catalyst (0.1% wbm) were placed in a 50 mL round-bottom flask (RBF) at 130 °C, and stirred continuously under N_2 flux (flow rate 15 ml/min). The reaction was left for 6 hours. The same reaction conditions were employed for the synthesis of each MM and the final amount was 20 g in total. In order to control the final MM average chain length, the desired molar ratio of the reagents was used and the formulations employed are reported in Table 2. The reaction route for the synthesis of each MM is shown in Figure 3.

Table 2. Formulation used for the synthesis of each type of macromonomer.

Type of MM	Theoretical repeating units (n)	Mn of the theoretical MM (g/mol)	Monomer (g)	HEMA (g)	$SnOCt_2$ (g)
MCLn	3	472	14.52	5.51	0.057
	6	814	16.85	3.20	0.063
	12	1498	18.29	1.74	0.067
MLAn	6	562	15.37	4.63	0.048
	12	994	17.38	2.62	0.051
	20	1570	18.34	1.66	0.053
MCL4-co-LA4	4CL+4LA	874	10.45CL+6.59LA	2.98	0.059

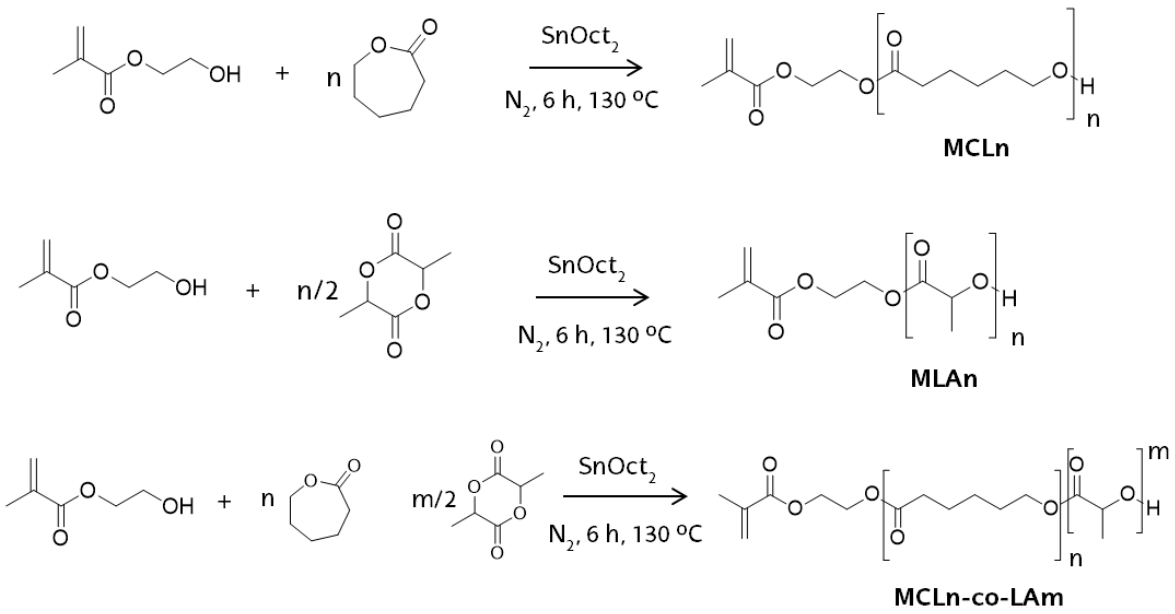


Figure 3. Scheme of the reaction route for the synthesis of the macromonomers.

2.3. Polymerization of the macromonomers in dispersed media

Batch emulsion polymerization was proposed in this project to synthesize nanoparticles. The procedures carried out are described below, and a scheme of the reaction is shown in Figure 4.

a) Polymerization in aqueous medium (PA):

MCL3 and MLA6 were used for this technique since they were the shortest MMs synthesised that can be employed without using any solvent and therefore, can be polymerized in water medium. 1 g of the MM was copolymerized with PEGMA 300, PEGMA 950, and PEGMA 2000 at different concentrations, with 18 g of deionized water in a 50 mL two-neck RBF, producing 20 g reaction. While stirring at 75 °C under N_2 flux (flow rate 15ml/min), KPS 1% weight based on the macromonomer and the stabilizer (wbtm) dissolved in 1 g deionized water, was injected as a shot. The reaction was carried out for 3 h. A copolymerization was carried out between the two synthetic MMs by employing 0.5 g of MCL3, 0.5 g of MLA6, and 0.5 g of PEGMA 950 in 18 g of deionized water in a 50 mL RBF in the same conditions as in the previous reaction.

b) Polymerization in water-ethanol medium (PWE):

In this technique the largest MMs were tested (MCL6, MCL12, MLA12, MLA20, and MCL4-co-LA4) with stabilizers of different chain lengths (PEGMA 950 and 2000) and concentrations (25%-50% wbm). Additionally, different solvent:water proportions were tested. The solvent and the deionized water were added to the reactor, together with the MM and the stabilizer, and stirred under a N_2 atmosphere at 75 °C. The initiator was then added to the reactor in a shot (KPS, 1 wbtm% dissolved in 1 g deionized water) and the reaction was stopped after 3 h.

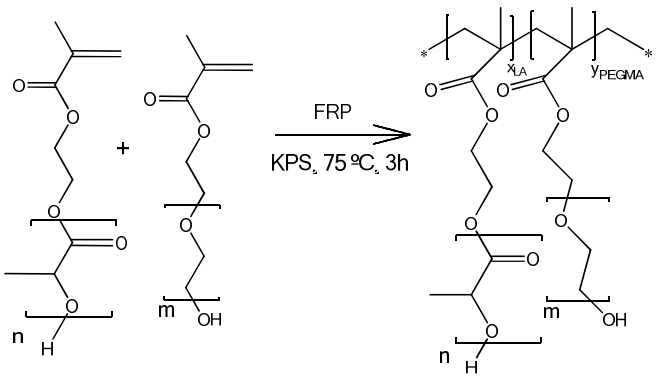


Figure 4. Reaction scheme of the polymerization of MLA type macromonomers.

2.4. Polymerization of the macromonomers in solution

Considering the stability and reproducibility issues faced in the polymerization of the MMs in emulsion and dispersion polymerization, same polymer systems were reproduced via solution polymerization. Subsequently, the degradability of the polymers obtained from solution polymerization with similar composition as those of the nanoparticles produced were studied. Three macromonomers (MLA6, MCL6 and MCL4-co-LA4) were chosen to be polymerized with Bisomer S20W and the produced polymers were named as PLA6, PCL6 and PCL4-co-LA4, respectively.

5 g of the synthesised MM, 1.11 g of Bisomer S20W (22.2 wbm%) and 14.2 g tert-butylbenzene were placed in a 100 mL round bottomed flask at 80 ºC, with a reflux condenser under a N_2 atmosphere. The initiator (1 wbtm%) was dissolved in 1 g of tert-butlybenzene and added in a shot to the reaction medium. The reaction was left for 6 h and in total 20 g were produced for each polymer. When the reaction was finished, the polymers were placed in a silicon mold and dried in the oven at 65 ºC for one day to remove the solvent and obtain a film. Afterwards, 5 mg from each polymer were taken to be characterized via ^{1}H-NMR. Figure 5 shows a scheme of the reaction carried out.

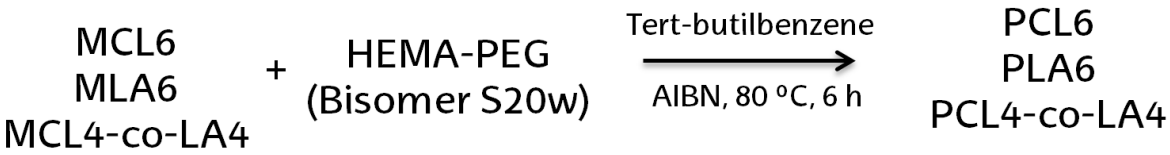


Figure 5. Scheme of the solution polymerization reaction.

2.5. Characterization methods and techniques

2.5.1. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance spectroscopy, known as NMR, is a non-invasive and non-destructive analytical method for materials characterisation. It is a spectroscopy technique that is based on the absorption of electromagnetic

radiation by nuclei of the atoms (the most common stable isotopes are ^{1}H and ^{13}C). The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. When an external magnetic field is applied, the nuclei can be excited and an energy transfer is possible between the base energy to a higher energy level. When the spin returns to its base level, energy is emitted. The energy absorbed during this transition is a function of nucleus type and its chemical environment in the molecule. As a consequence, variations in electron density around each nucleus will cause each nucleus to experience a different magnetic field, and that differences are measured by the chemical shift, which gives us significant information to characterize a molecule.[28,29]

In this project ^{1}H-NMR has been employed for the characterization of the synthesised MMs and to follow the conversion of the polymerization reactions. All measurements were carried out in a Bruker AVANCE 400 MHz equipment.

Characterisation of the synthesised macromonomers:

The experimental repeating units of each MM were characterize as reported in literature.[27,25,26] 5 mg of each sample were dissolved in 1 mL of deuterium chloride ($CDCL_3$) and transferred into the NMR tube. A proton automatic measurement was carried out.

Figure 6 shows a typical spectrum for CL-based macromonomers. The two first peaks present (around δ of 6.1 and 5.6 ppm) correspond to the vinyl group of HEMA. All the area integrations were made by setting the area of one of the vinyl hydrogen atoms of the HEMA group (peak X and X'). For the characterization of the ε-caprolactone based MMs, peak *H* (δ 4 ppm) is representative of the total number of repeating ε–caprolactone units added, while *I* is of the total number of terminal groups. Therefore, ratio between the two terms to which the last unit is added, not considered in peak *H*, represent the number of experimental units. Equation 1 was used to obtain the experimental units of MCLn type macromonomers.

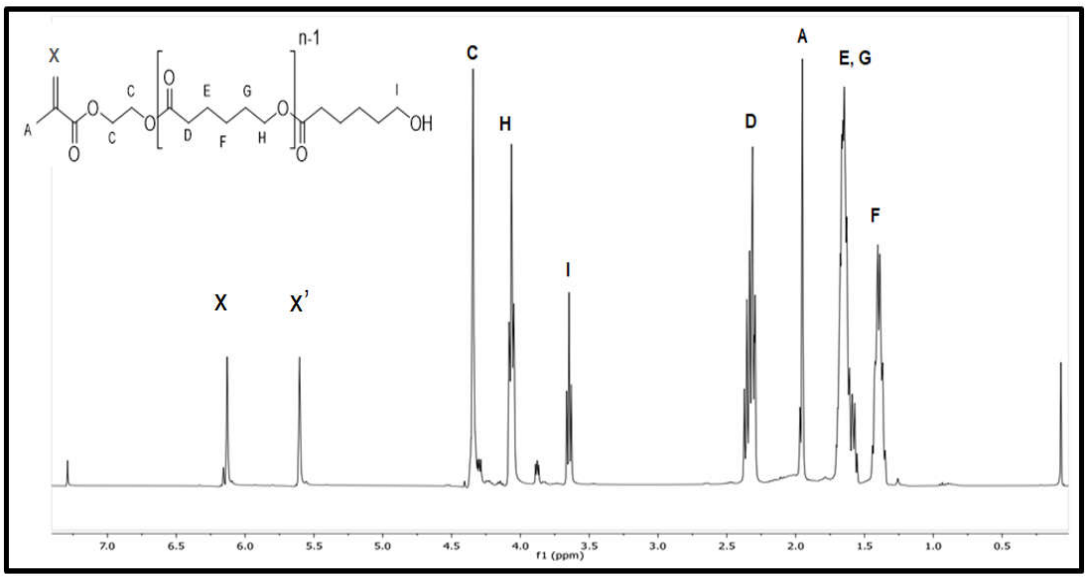


Figure 6. Typical spectrum for CL- based macromonomers.

$$n_{CL} = \frac{Integral\ of\ peak\ H\ (methylene\ proton\ signal)}{Integral\ of\ peak\ I\ (\alpha - methylene\ proton\ signal)}$$

Equation 1. Experimental units of CL-based macromonomers.

Figure 7 depicts typical spectrum for LA-based MMs. Using the same approach as in CL-based MMs, the average number of LA units were obtained. Peak *B* corresponds to the total number of LA units, and peak *D* refers to the number terminal units which is equivalent to signal X, the number of vinyl groups. Signal *D* overlaps with *E*, so X was used in Equation 2 to calculate the ratio between the two terms to which 1 is added to count for the last unit not considered in *B*.

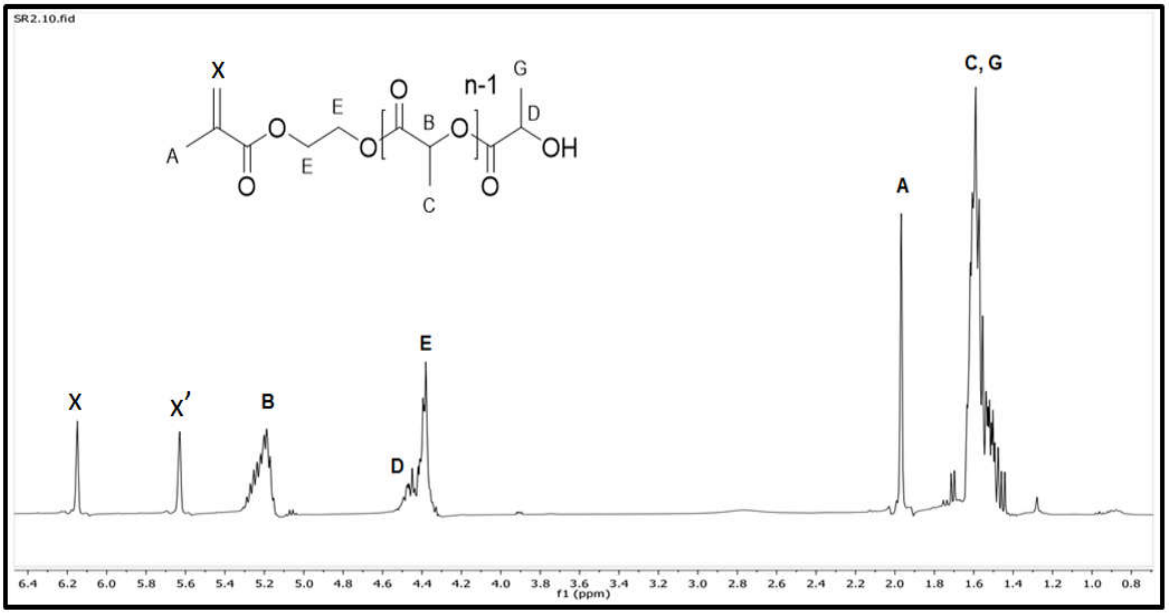


Figure 7. Typical spectrum for LA- based macromonomers.

$$n_{LA} = \frac{Integral\ of\ peak\ B\ (methine\ proton\ signal)}{Integral\ of\ peak\ F\ (vinylic\ proton\ signal)} + 1$$

Equation 2. Experimental units of LA-based macromonomers.

Figure 8 shows the spectrum for CL- and LA-based MM. To obtain the experimental units of LA and CL in the comacromonomers, two different equations were used. Equation 3 refers to the average number of CL units, where *D* is representative of the total CL units and *B* to the number of vinyl groups. Equation 4 refers to the number of LA units, where *H* represents the number of LA units and *X* the number of vinyl groups which is equivalent to *P*, the terminal units of LA. 1 is added to the equation to take account of the terminal units not considered in *H* signal.

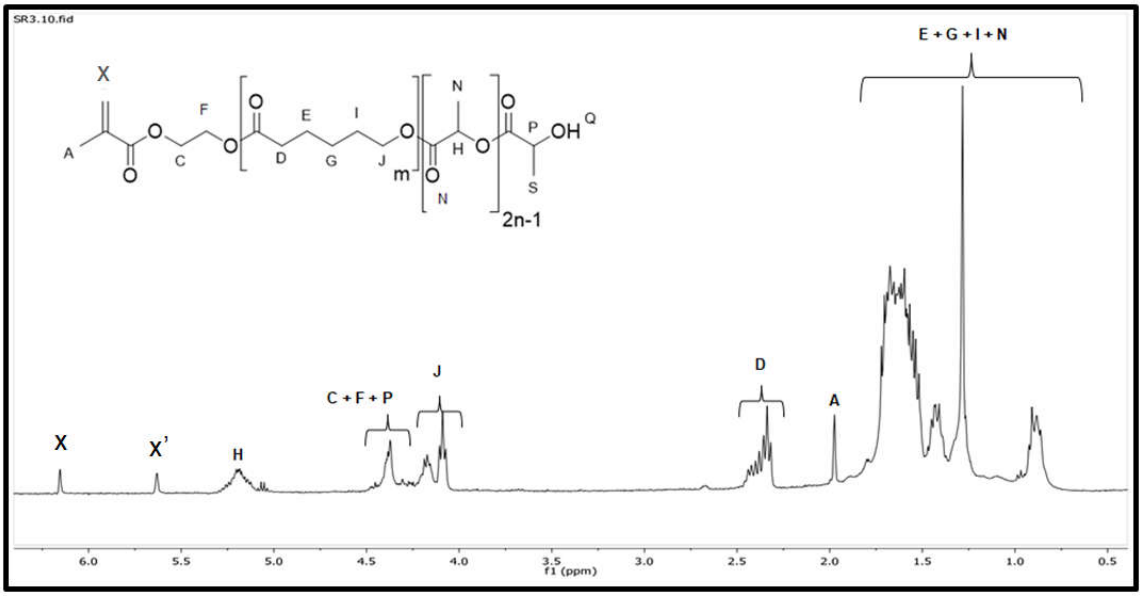


Figure 8. Typical spectrum for CL-and LA- based comacromonomers.

$$n_{CL} = \frac{Integral\ of\ peak\ D\ (methylene\ proton\ signal)}{Integral\ of\ peak\ X\ (vinylic\ proton\ signal)x2}$$

Equation 3. CL experimental units of the co-macromonomers.

$$n_{LA} = \frac{Integral\ of\ peak\ H\ (methine\ proton\ signal)}{Integral\ of\ peak\ X\ (vinylic\ proton\ signal)} + 1$$

Equation 4. LA experimental units of the co-macromonomers.

Additionally, the molar mass of the produced macromonomers was calculated via ^{1}H-NMR as well. Therefore, Equation 5 was used to obtain the experimental molar mass of the MMs, where *Mn* is the experimental molar mass, M_{HEMA} the molar mass of HEMA, $M_{monomer}$ the molar mass of the repeating unit (for LA 72 g/mol and for CL 114 g/mol), and *n* the experimental average repeating units of the MM calculated previously.

$$Mn = M_{HEMA} + M_{monomer} \cdot n$$

Equation 5. Molar mass for the produce macromonomers.

Conversion of the polymerization reactions:

Due to the low volatility of the synthetic macromonomers, gravimetric measurements could not be employed as a technique to obtain conversion. Instead, ^{1}H-NMR was employed. Figure 9 is a spectra of the polymerization of MLA6 type macromonomers and PEGMA 950, showing uncomplete conversion of the reaction. Equation 6 was followed for the conversion of the reactions. The vinyl protons from HEMA that correspond to the MM and the stabilizer, that is peaks X and X' (around δ 6.1 and 5.6 ppm), change to single bond as a result of polymerization, which means that at complete conversion no X peak is observed. Monitoring the changes in the vinyl protons we can calculate the conversion, using peak A (Figure 9) as internal standard, since it remains unchanged during polymerization.

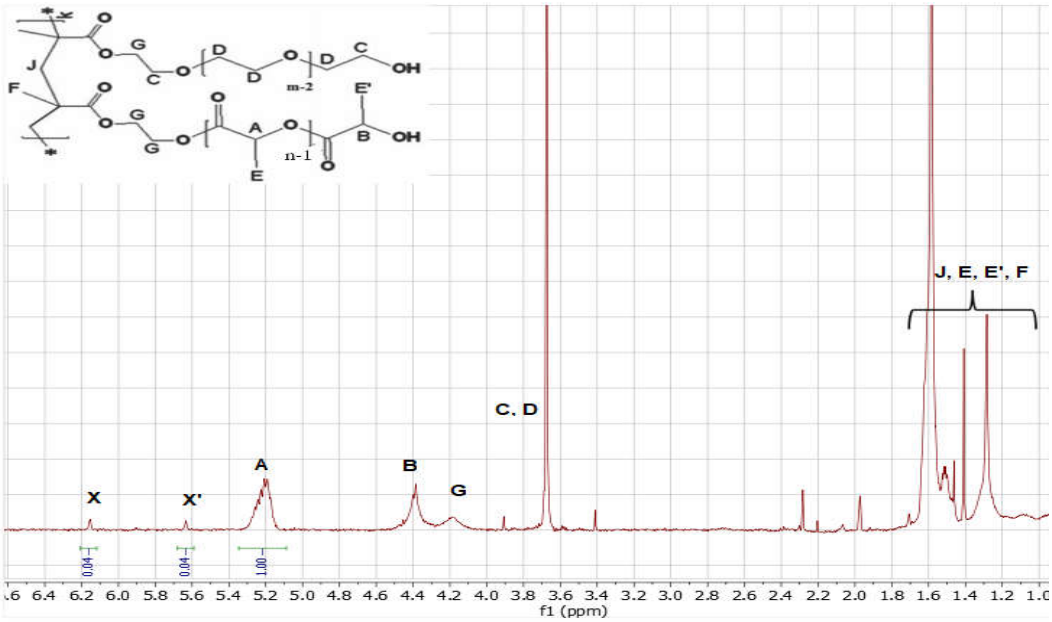


Figure 9. Spectra of polymerization reaction of MLA type and PEGMA macromonomers

$$\text{Conversion } (\%) = \left(1 - \frac{Intgral\ of\ peak\ X\ in\ polymerization}{Integral\ of\ peak\ X\ before\ polymerization}\right) \cdot 100$$

Equation 6. Conversion of the polymerization reaction.

To prepare the ^{1}H-NMR samples, a few drops of the latex was placed in an aluminium capsule and dried overnight at 65 ºC to make a film. 5 mg of the dried film were taken and dissolved in 1 mL of $CDCL_3$. The samples from solution polymerization were prepared in the same way.

2.5.2. Dynamic light scattering

Particle size of the obtained latexes was determined by Dynamic Light Scattering (DLS). DLS is a non-invasive, well-established technique for characterizing the size and size distribution of proteins, nanoparticles, polymers and colloidal dispersions, typically in the submicron region. The principle behind this technique is that a laser beam is applied to the sample, and a photon detector detects the fluctuations of the scattered light at a known scattering angle (173°), as illustrated in Figure 10. From a microscopic point of view, particles scatter the light and therefore, they provide information about their motion. So data about particles is obtained from the scattered light fluctuations. Intensity fluctuations will provide information about particles diffusion. Therefore diffusion coefficient of the particles can be obtained this way. Diffusion coefficient (*D*) is related with the radius (*R*) of the particles by Stokes-Einstein equation, shown in Equation 7, where K_B is the Boltzman constant, *T* temperature and η viscosity. The smaller the particles, the faster they move and thus, the larger the diffusion coefficient will be.[30]

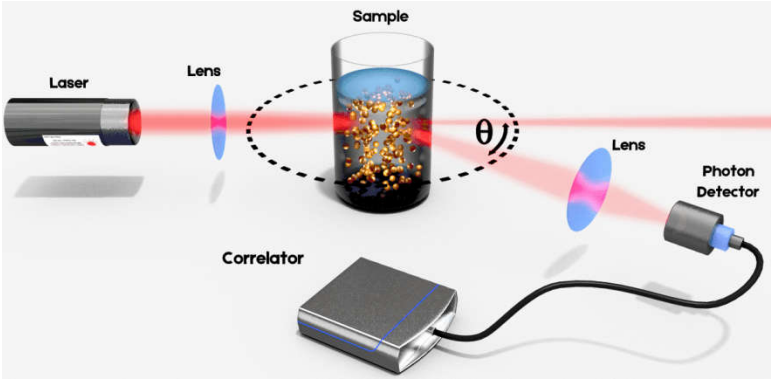


Figure 10. General scheme of the DLS equipment reproduced from reference.[30]

$$D = \frac{k_B T}{6\pi\eta R}$$

Equation 7. Expression for diffusion coefficient.

A Zetasizer Nano ZS from Malvern Instrument was used. Samples were

prepared by diluting a fraction of each latex in deionized water. The analyses were carried out at 25 ºC and a run consisted of three size measurements of 1 minute each. An average value of three measurements is reported as a result.

2.5.3. Coagulum content

A dispersion of colloidal particles in water would quickly coagulate in the absence of forces to counteract the van de Waals attraction between particles. Therefore, the presence of coagulum or aggregates during the polymerization reactions in emulsion and dispersion are a signal of the poor stability of the system.[19] After finishing the reactions, latexes were filtered using a filtering fabric to no aggregates were present. In the cases where aggregates were present, this one was transferred to a capsule and dried overnight at 65 ºC.

2.5.4. Degradation study

The polymers that were produced in solution polymerization, ~20 mg polymer were placed in a previously weighted 5 mL vial. Then, 3-4 mL of the PBS was added until the entire sample was covered by the solution. The vial was closed with the tap, covered with parafilm, and introduced in a 37 ºC water bath. Samples were withdrawn at different time intervals (Day 1, 7, 14, 21, 28, 42, 56, 71) and each sample was done by triplicate in order to do a subsequent statistical study. When the sample was taken out from the bath, the PBS was removed with a 5 mL syringe and a needle, and the sample was weighted to get the wet weight of the sample (W_w). After that, the sample was left to dry one night at room temperature, and one more night under vacuum at room temperature. When the drying process was finished, the sample was weighted to report the dry weight of the polymer (W_d). With the initial weight, wet weight and dry weight reported, the water absorption and remaining weight of each polymer were studied using Equation 8 and Equation 9. Additionally, 10 mg from each vial were taken to prepare samples for both GPC and DSC to study the molar mass and thermal transitions evolution, respectively.

$$\%WA = \frac{W_w - W_d}{W_d} 100.$$

Equation 8. Water absorption.

$$\%RW = \frac{W_d}{W_0} 100.$$

Equation 9. Remaining weight.

2.5.5. Gel Permeation Chromatography (GPC).

Size-exclusion chromatography (SEC) or Gel permeation chromatography (GPC) is the most widely used method to measure molar mass averages and distribution. It does not measure the molar mass directly, but the relative size of the chains when they are in solution. Therefore, it is not an absolute method and requires calibration. The technique is based on the separation of the dissolved macromolecules by size using porous columns. So the chains with higher molar mass travel less path and exit first the column while the polymer chains with smaller molar mass can penetrate all holes or pores and therefore travel further and take longer to cross the column. In Figure 11 a diagram of what would be the operation of this technique is shown. The column is the stationary phase which separates by size the different species that make up the sample and the detector detects the species. The most common detectors are those that measure RI refractive indices, in which the difference in refractive indices between the pure carrier liquid and the solution is measured.[31]

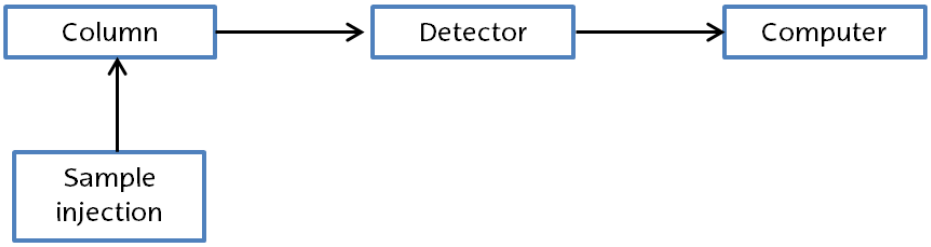


Figure 11. Scheme of the GPC system.

In this work GPC has been used to study the molar mass evolution of the degradation samples. Around 2.5 $mg_{polymer}/mL_{THF}$ concentration samples were prepared and filtered before injection. The GPC set up consists of a pump (LC-20A, Shimadzu), an autosampler (Waters 717), a differential refractometer (Waters2410) and three columns in series (Styragel HR2, HR4 and HR6) with pore

sizes ranging from 102 to 106 Å. The chromatograms were obtained at 35 C using a THF flow rate of 1ml/min. The equipment was calibrated using polystyrene standards (5th order universal calibration) and Mark Houwink constants from poly(HEMA-g-CL3) were selected (a = 0.571 and K = 2 x 10^{-4} dL/g) to read the chromatograms.

2.5.6. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is a method of thermal analysis where the mass of the sample is measured constantly as a function of temperature or time while the sample is subjected to a controlled temperature program in a controlled atmosphere. In a desired temperature range, if a species is thermally stable, there will be no observed mass change. However, when the thermal curve is descending, it indicates that mass loss occurred and so the material is not stable anymore in that temperature range.[32]

In this project TGA was carried out for the degradation samples. This analysis is important because it gives information about the thermal stability of the sample, and the maximum temperature before degradation. This information is required for further thermal analysis such as differential scanning calorimetry, so that analyses are made below degradation temperature. The measurements were conducted from 25 ^{0}C to 800 ^{o}C, with a heating rate of 10 ^{o}C/min, under nitrogen atmosphere, in a TA instruments Q500.

2.5.7. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry is a thermoanalytical technique in which the temperature of a sample and reference material is increased at a constant rate. The heat flow required to increase the temperature of the sample and the reference material at a constant rate is measured. The technique is used for

determining phase transitions, relying on the principle that, as the sample undergoes a phase transition more or less heat will need to flow to it than to the reference to maintain both at the same temperature.[33] It is a very powerful technique to evaluate material properties such as glass transition, melting and crystallization temperatures and the specific heat capacity. In Figure 12 a basic scheme of the different parts of the DSC is depicted.

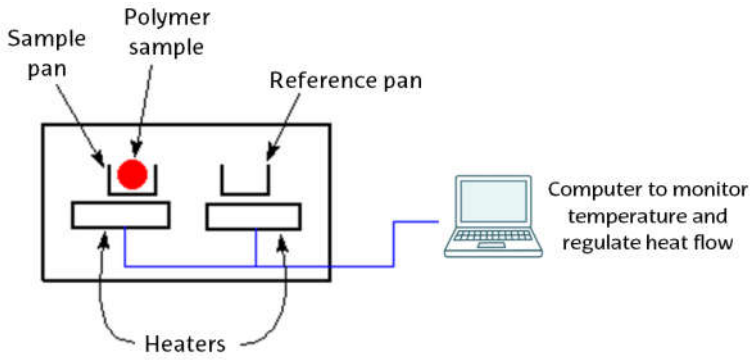


Figure 12. Scheme of the DSC.

In this work DSC was employed to study the thermal properties of the samples before and after degradation. The scans were performed in a Q1000, TA Instrument. The samples were cooled to -80 ºC and then the analysis started by heating up to 150 ºC at 10 ºC/min. In a second scan, the sample was again cooled to -80 ºC and heated again up to 150 ºC at 10 ºC/min. The first scan was done for removing the thermal background of the polymer, and the second one to obtain the real properties of the material. This way, heat flow versus temperature curves were obtained. The glass transition temperature (T_g) was taken from the inflection point in the curve, the crystallisation temperature (T_c) form the lowest point of the dip, and the melting temperature (T_m) from the highest temperature of the curve.

3. Results and discussion

3.1. Synthesis of the CL and LA based Macromonomers

CL- and LA-based macromonomers of different chain lengths were produced by ROP. In some cases the same MM type was synthesized more than once. For these, the experimental number of repeating units (n) of all the reproductions calculated based on their ^{1}H NMR spectra is reported in the table below (Table 3). Additionally, the molar mases of the mentioned MMs were calculated with Equation 5, presented in the experimental part, and the results are reported in Table 4.

Table 3. Experimental repeating unit of the synthesised MMs.

MM type	Theoretical n		Experimental n	
MCLn	3		3.9	
	6		6.2, 6.8	
	12		12.6	
MLAn	6		5.3, 5.6	
	12		11.7	
	20		19.4	
MCLn-LAn	CL	LA	CL	LA
	4	4	4.5	4.0

Table 4. Experimental molar masses of the synthesised macromonomers.

Type of MM	Theoretical Mn (g/mol)	Experimental repeating units of the MM (n)	Experimental Mn (g/mol)
MCL3	472	3.9	575
MCL6	814	6.2	837
		6.8	905
MCL12	1498	12.6	1566
MLA6	563	5.3	512
		5.6	533
MLA12	994	11.7	972
MLA20	1570	19.4	1526
MCL4-co-LA4	874	CL=4.5, LA=4.0	931

Generally good agreement with the targeted repeating unit was achieved, however higher deviation was observed in some cases. The reproducibility was also tested by producing the same MM several times, and similar values were observed. This confirms the controllable behaviour of the MM synthesis process.[25,27]

3.2. Synthesis of polymeric Nanoparticles

The characterized MMs were polymerized through different processes based on the nature of the MM. Latexes were obtained as final product and these were analysed regarding the conversion, coagulum content and particle size.

3.2.1. Polymerization and copolymerization in aqueous medium

In Table 5 the characteristics of the latexes produced by emulsion polymerization are presented. MCL3 and MLA6 were polymerized varying the concentration of the different stabilizers (PEGMA 300, 950 and 2000).

Table 5. Results obtained by emulsion polymerization and copolymerization.

Name	MMn		PEGMAm, (wbm%)[1]	Z-avg size (nm)	PDI	Conv. (%)	Coagulum content (wbtm%)[2]
PA-CL_3-1	MCL3 n = 3.9		PEGMA 950 50	175	0.152	›98	N.O.[3]
PA-CL_3-2	MCL3 n = 3.9		PEGMA 950 25	203	0.044	›98	N.O.
PA-CL_3-3	MCL3 n = 3.9		PEGMA 300 25	121	0.597	›98	30
PA-LA_6-1	MLA6 n = 5.6		PEGMA 950 50	159	0.103	›98	N.O.
PA-LA_6-2	MLA6 n = 5.3		PEGMA 950 25	240	0.141	›98	N.O.
PA-LA_6-3	MLA6 n= 5.6		PEGMA 2000 50	162	0.200	97	4.6
PA-LA_6-4	MLA6 n= 5.6		PEGMA 2000 25	220	0.151	›98	N.O.
PA-CL_3-LA_6	MCL3 n=3.9	MLA6 n= 5.6	PEGMA 950 25	197	0.104	›98	N.O.

[1]weight based on the macromonomer.

[2]weight based on total macromonomer (the synthesised macromonomer and the stabilizer).

[3]Not observed.

For all the reactions, conversions higher than 97% were achieved. Nanoparticles smaller than 250 nm were produced, except for reaction PA-CL_3-3, and a polydispersity smaller than 0.2 was achieved indicating the uniformity of the obtained dispersed particles.

Regarding the effect of MM type on particle size, no specific trend was seen. Comparing PA-CL_3-1 and PA-LA_6-1 where 50 %wbm PEGMA 950 was employed, smaller particle size was obtained for the system based on MLA6. However, when using stabilizer at 25 wbm% concentration in PA-CL_3-2 and PA-LA_6-2, the reverse trend was obtained.

Looking at the results, it is apparent that higher amount of PEGMA yields smaller particle size which is rooted in the stabilization effect of PEGMA. Furthermore, effect of the lateral chain length of PEGMA (m) on the stability of the system was studied. Using 25 wbm% of shorter PEGMA (PEGMA 300) to produce

PA-CL_3-3, considerable coagulum amount was obtained. For PA-CL_3-2, on the other hand, no coagulation was observed when using PEGMA 950. This can be attributed to the low steric stability that shorter chains provide. In the case of larger chain PEGMA (PEGMA 2000), aggregates were obtained at 50 wbm% concentration, but not at 25 wbm%. With PEGMA 950 (m=19), no aggregates were observed in the reactor even when concentration was varied. Based on the results, it seems that PEGMA 950 is the best candidate among our choices to stabilize the CL- and LA- based nanoparticles.

Copolymerization of MCL3 and MLA6 macromonomers using PEGMA 950 as stabilizer was also performed. As a result, a dispersion with a PDI of 0.104 and a particle size of 197 nm was obtained. Comparing the mentioned system with PA-CL_3-2 and PA-LA_6-2, where polymerization was carried out for MCL3 and MLA6 respectively at the same conditions, smaller particle sizes were achieved in the copolymerization. Figure 13 shows some of the latexes produced via emulsion polymerization.

A) B)

Figure 13. Example of some of the latexes obtained via emulsion polymerization: A) PA-CL_3-3, B) PA-LA_6-1

3.2.2. Polymerization in water-ethanol medium

For the macromonomers of longer chains, it was not possible to disperse them properly in water since they were solid and more hydrophobic (compared with shorter macromonomers). In order to disperse the solid macromonomers in the reaction media, they were first dissolved in ethanol. PEGMA was dissolved in water separately. Then, the two solutions were mixed, getting a turbid mixture for the 30:70 ethanol:water ratio employed and at 40:60 ratio, tested in reaction PWE-

CL_6-Et. Finally the reaction was started by adding the initiator at the reaction temperature. Although the reactions were designed to be carried out in dispersion polymerization, that is starting form an homogenous mixture, in all the cases nanoparticles of longer macromonomers were produced starting from a two phase water ethanol mixture.

Table 6 represents the results obtained for the reactions carried out in water-ethanol medium. Five MMs of different chain length were studied and polymerized with PEGMA 950, except for reaction PWE-CL_6-2 where PEGMA 2000 was employed. Additionally, the ethanol:water proportion used was 30:70 in all the cases except for reaction PWE-CL_6-Et in which it was changed to 40:60 in order to check the effect.

Table 6. Results obtained from dispersion polymerization.

Name	MM	PEGMA content (wbm%)	Z-avg size (nm)	PDI	Conv.%	Coagulum content (wbtm%)
PWE-CL_6-1	MCL6 n=6.6	PEGMA 950 50	144	0.076	89	23
PWE-CL_6-2	MCL6 n=6.6	PEGMA 2000 25	172	0.181	86	11
PWE-CL_6-Et	MCL6 n=6.2	PEGMA 950 50	337	0.481	92	25
PWE-CL_{12}-1	MCL12 n=12.6	PEGMA 950 50	97	0.079	96	23
PWE-LA_{12}-1	MLA12 n=11.7	PEGMA 950 50	203	0.199	94	40
PWE-LA_{12}-2	MLA12 n=11.7	PEGMA 950 25	242	0.249	92	33
PWE-LA_{20}-1	MLA20 n=19.4	PEGMA 950 25	146	0.048	93	35
PWE-CO-1	MCL4-co-LA4 n = 4.5, 4.0	PEGMA 950 50	214	0.197	94	23
PWE-CO-2	MCL4-co-LA4 n = 4.5, 4.0	PEGMA 950 25	255	0.230	92	18

In these reactions particle sizes in the range of 97 to 337 nm were obtained, with PDI values from 0.048 to 0.481. As shown in the table above, it was not possible to obtain conversions higher than 96% in none of the reactions and in all the cases coagulation content higher than 10 wbtm% was achieved.

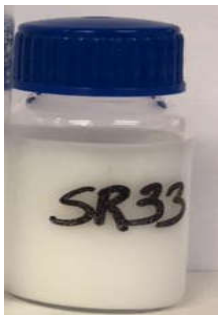
Looking at the result of the CL-based systems, PWE-CL_6-2 showed the lowest amount of coagulum, which can be attributed to the longer chain of

stabilizer (PEGMA 2000) enhancing the steric stability. Consequently, PEGMA 2000 outperformed PEGMA 950 regarding the stabilization of the CL-based systems. Additionally, in PWE-CL_6-1 and PWE-CL_{12}-1 the same coagulum content was obtained regardless of the chain length of the MM. Comparing PWE-CL_6-Et and PWE-CL_6-1, it was observed that using higher proportion of ethanol (PWE-CL_6-Et) the conversion and level of coagulation was relatively similar to that of lower ethanol amount (PWE-CL_6-1). Although higher particle size was observed for the system of higher ethanol content.

For LA-based systems, it was observed that in PWE-LA_{12}-1 and PWE-LA_{12}-2 lower coagulum content was obtained when using lower PEGMA 950 concentration. Same behaviour was seen for the reaction carried out with MCL4-co-LA4 (PWE-CO-1 and PWE-CO-2). Also, similar coagulum contents were obtained independently of the MLA chain length.

It can be concluded that, when the repeating units of the MM are increased, many stability issues are faced compared with the reactions carried in water medium where the shortest MM were used. Still dispersed nanoparticles were obtained from the larger MM by separating the coagulum. Based on the coagulum contents reported, not significant effect of the chain length in the stability was observed. But lower contents of coagulum were seen when concentrations of 25 wbm% of PEGMA 950 and a larger PEGMA was employed. In Figure 14 are shown some of the latexes obtained.

A)



B)

Figure 14. Picture of some of the dispersions obtained: A) PWE-CL_6-1, B) PWE-CO-1

3.3. Degradation study of the polymers synthesized by solution polymerization

The third part of this work consists of studying the degradability of the polymers. As mentioned in the experimental part, instead of studying the degradability of the nanoparticles, the same monomer formulation was employed to produce the polymers via solution polymerization and study their degradability. In general, the molar mass obtained from solution polymerization is lower than that from emulsion, although in this case the molar mass of the polymer backbone is not playing an important role in the degradability. Brush copolymers constituted of a HEMA backbone with PEG, PLA and/or PCL pendants were obtained.

Degradation process occurs through the hydrolysis of ester bonds of the PLA or PCL chains and subsequent release of acidic species, leaving the water-soluble poly(HEMA-co-HEMA-g-PEG45) as a secondary product. That means that degradation of these polymers is dominated by the attack of water to the ester bonds, and therefore influenced by the composition and length of the side chains and not by the molar mass of the whole polymer, as concluded in many papers.[13,14,17,21] A schematic representation of the produced polymers is shown in Figure 15.

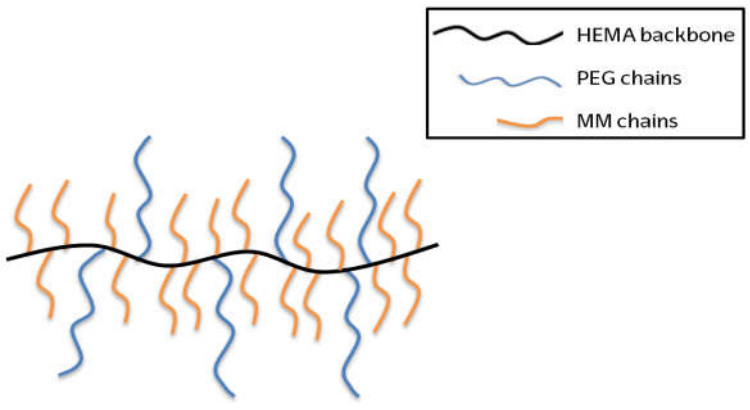


Figure 15. Schematic representation of the brush polymers produced.

In Table 7 are presented the molar mass, the polydispersity and the conversion obtained for PCL6, PLA6 and PCL4-co-LA4 polymers produced in solution polymerization.

Table 7. Characterisation of the polymers carried out in solution.

Polymer	Mw (g/mol)	Đ	Conversion%
PCL6	84390	1.59	85
PLA6	52870	1.56	94
PCL4-co-LA4	74250	1.53	95

Weight-average molar masses between 50000 and 85000 g/mol were obtained. The reactions could not achieve complete conversions, and due to time concerns, the polymers were not purified. That means that in the degradation samples unreacted macromonomer can be found.

3.3.1. Molar mass evolution.

The molar mass evolution during the degradation of the samples was monitored using GPC by reporting average molar mass in weight (Mw), and the molar mass distribution (MWD).

Figure 16 shows an example of the integration of the two different peaks obtained in the chromatograms of the polymer before degradation. The first peak corresponds to the polymer, while the second peak to the unreacted MM. The second peak could not be characterized because it was out of the calibration of the GPC. Therefore, for the evolution of the Mw only the first peak was integrated from 23 min to 28 min elution time. On the other hand, for the study of the molar mass distribution, both peaks were integrated at the same time, starting from elution time at 23 min to 31 min. By adopting these criteria the next results are presented.

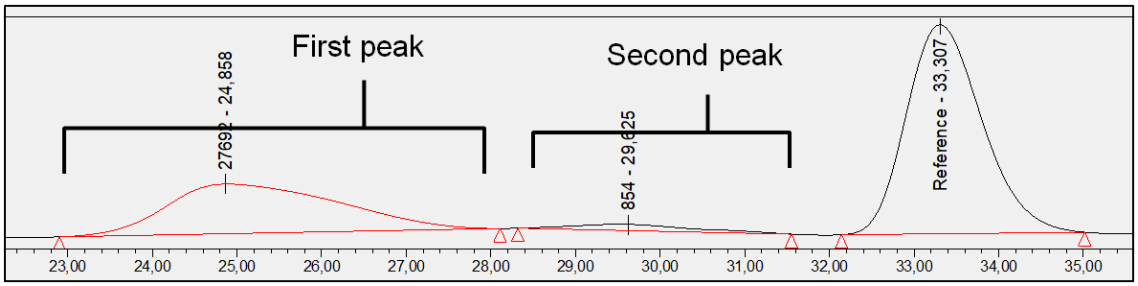


Figure 16. Example of the two peaks obtained at the chromatograms.

Figure 17 presents the molar mass distribution (MWD) of the polymers at day 0, 42 and 56 overlapped with the MWD of the macromonomer used for each polymer.

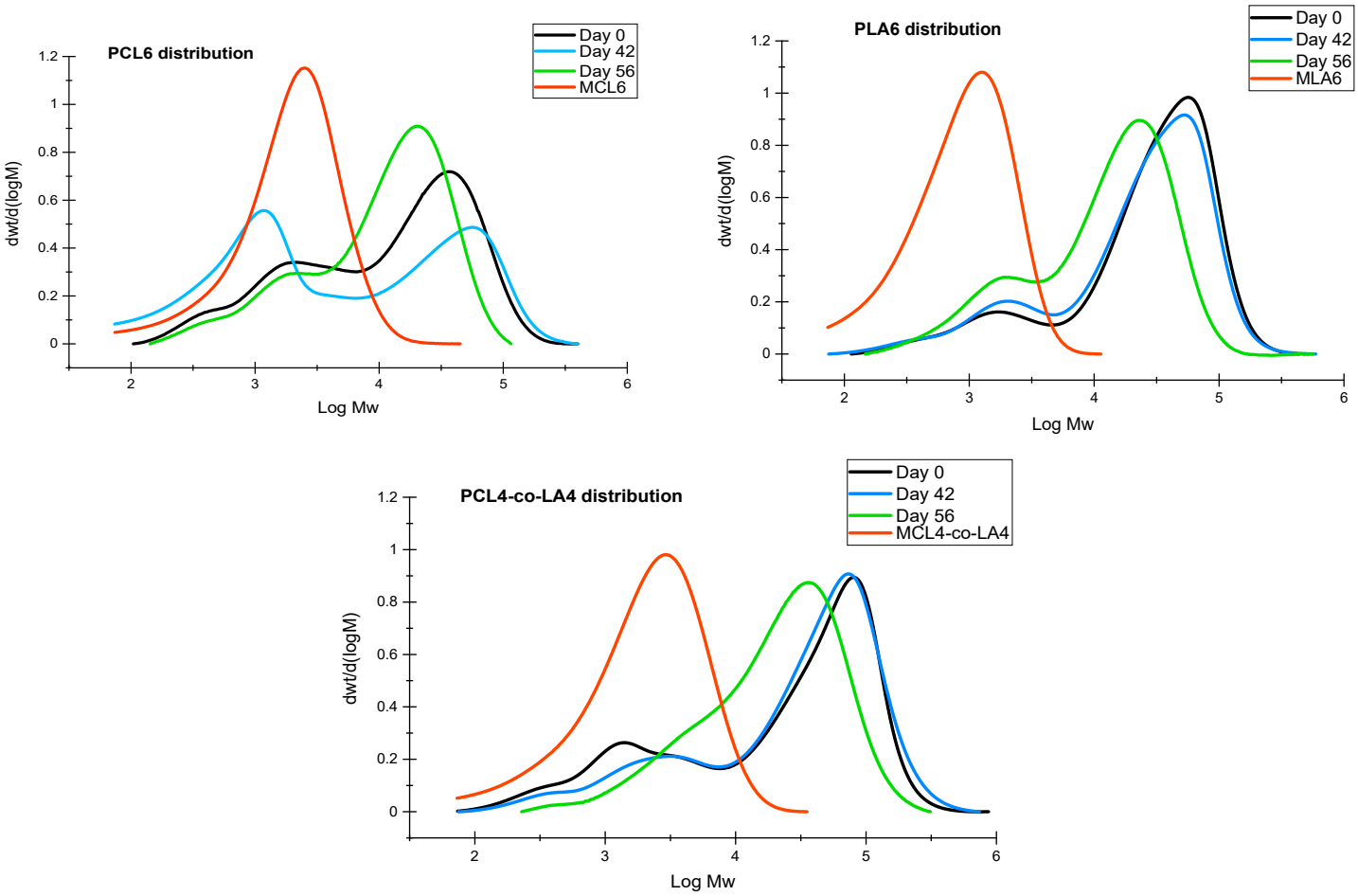


Figure 17. Molar mass distribution of PCL6, PLA6 and PCL4-co-LA4 at different degradation times.

As a consequence of the presence of unreacted MM, bimodal distributions were obtained at day 0 for the three polymers. The peak placed at higher Mw corresponds to the polymer, while the peak placed at lower Mw to the left to the unreacted MM. To confirm this, the MWD of the MM employed in the reaction was plotted, and as it can be seen, the peak of the MM overlaps the smaller peak in all the cases. The smaller peak also shows transformations during degradation time, which can be attributed to species generated from the MM chains' hydrolysis.

To better monitor the changes in the molar mass during degradation time, Mw of the three different polymers against time was calculated and plotted in Figure 18, with the error bars from the deviation standard of the statistical analysis. Table 8 provides the variations in the molar mass by day. The average of the triplicated results of the samples is shown together with the deviation standard obtained.

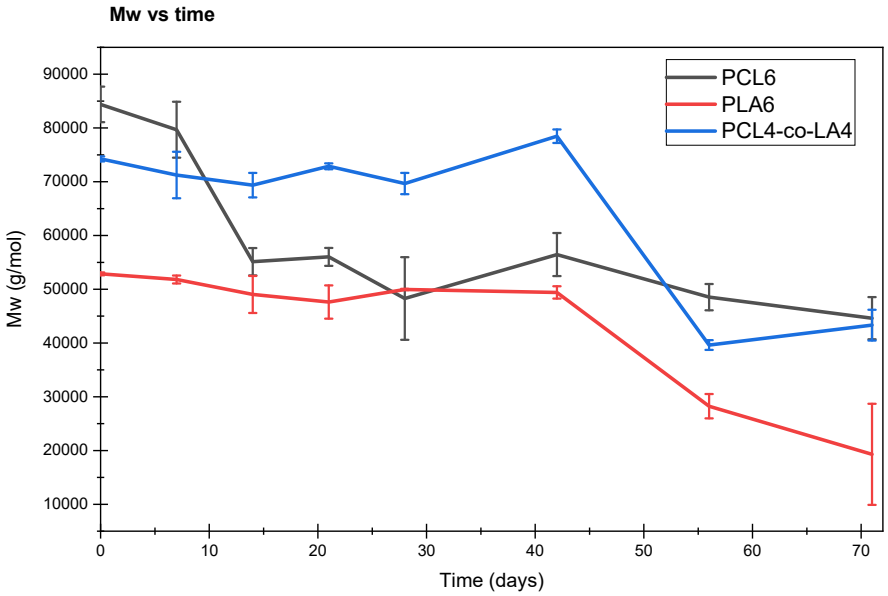


Figure 18. Evolution of the Mw against time for PCL6, PLA6 and PCL4-co-LA4 polymers.

Table 8. Molar masses obtained during degradation days.

Day	PCL6 Mw (g/mol) (x10^4)	PLA6 Mw (g/mol) (x10^4)	PCL4-co-LA4 Mw (g/mol) (x10^4)
0	8.44 ± 0.33	5.29 ± 0.03	7.42±0.05
7	7.97 ± 0.52	5.18 ± 0.07	7.13 ± 0.43

14	5.51± 0.25	4.90 ± 0.34	6.94 ± 0.23
21	5.60 ± 0.17	4.76 ± 0.31	7.29±0.06
28	4.83 ± 0.77	5.00 ± 0.01	6.97±0.20
42	5.65 ± 0.40	4.94 ± 0.12	6.78±1.84
56	4.85 ± 0.24	2.82 ± 0.23	3.96±0.09
71	4.46 ± 0.39	1.93 ± 0.94	4.33±0.29

With the information obtained from the MWD (Figure 17) and the Mw vs time plot (Figure 18), different behaviours were observed in the degradation depending on the polymer, but all of them showed a general decreasing trend in molar mass by the end of the analysis.

After the first two weeks for PCL6, a sudden decrease of the Mw occurred, obtaining a Mw of around 55000 g/mol. After that, the Mw continued decreasing steadily, achieving values of around 45000 g/mol at day 71, almost half of the initial Mw. Although changes in the Mw were observed by day 14, the MWD curve was similar to the one of the initial day until day 42. By day 56 a shift on the curve to the left was produced, as a consequence of the low Mw obtained.

Looking to the Mw evolution during time of PLA6, taking into account the deviation standard of the samples, it can be considered that during the first 42 days the Mw decreased slightly, obtaining a molar mass of around 49000 g/mol. However, during the next two weeks a sudden decrease was seen. This decline continued for the next two weeks, obtaining an average molar mass of around 19000 g/mol in the final day, more than half of the initial value. This change in the Mw was confirmed by the shift of the MWD curve to lower molar masss at day 56.

Similar behaviour was seen for the copolymer. The Mw obtained at day 42 showed a little reduction in the Mw compared with the initial value. After day 42 a dramatic decrease was observed, obtaining a final value of 43000 g/mol by the end of the study. In the MWD this was also observed. Distribution curves obtained from day 0 to day 42 were all positioned in the same Mw ranges, observing few differences between them. But at day 56 again, the curves were displaced to the left, indicating the changes in the molar mass as a consequence of the degradation taking place.

3.3.2. Thermal properties

As mentioned in the experimental part, the thermal properties of the degradation samples were also analysed. Prior to the DSC analysis, a TGA was carried out. In Figure 19 weight against temperature was plotted, and the curve obtained for PLA6 is shown.

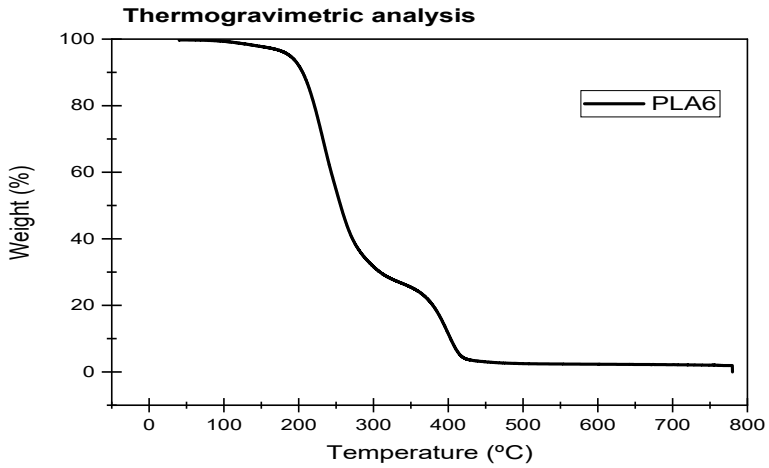


Figure 19. Thermogravimetric analysis of PLA6.

TGA analysis for the three different polymers were carried out, and similar graphs were obtained in the three cases: the curve started to descend at around 150 ºC, indicating mass loss, and complete decomposition of the samples were achieved around 400 ºC. Taking into account this information, DSC scans were carried from -80 ºC to 150 ºC. Once stablished the analysis conditions, the next results were obtained in the DSC.

In Table 9 the results obtained for the thermal transitions of the polymers before and after the degradation study are presented. Additionally, Figure 20 provides the curves obtained from DSC analyses.

Table 9. DSC results of the studied polymer before and after degradation.

Days	PCL6			PLA6	PCL4-co-LA4
	Tg (ºC)	Tc (ºC)	Tm (ºC)	Tg (ºC)	Tg (ºC)
0	-64.0 ± 1.7	-28.2 ± 1.0	22.0 ± 1.2	8.6 ± 0.1	-37.3 ± 0.9

71	-	-	26.9 ±3.92	5.2 ± 0.5	-40.3 ± 0.3

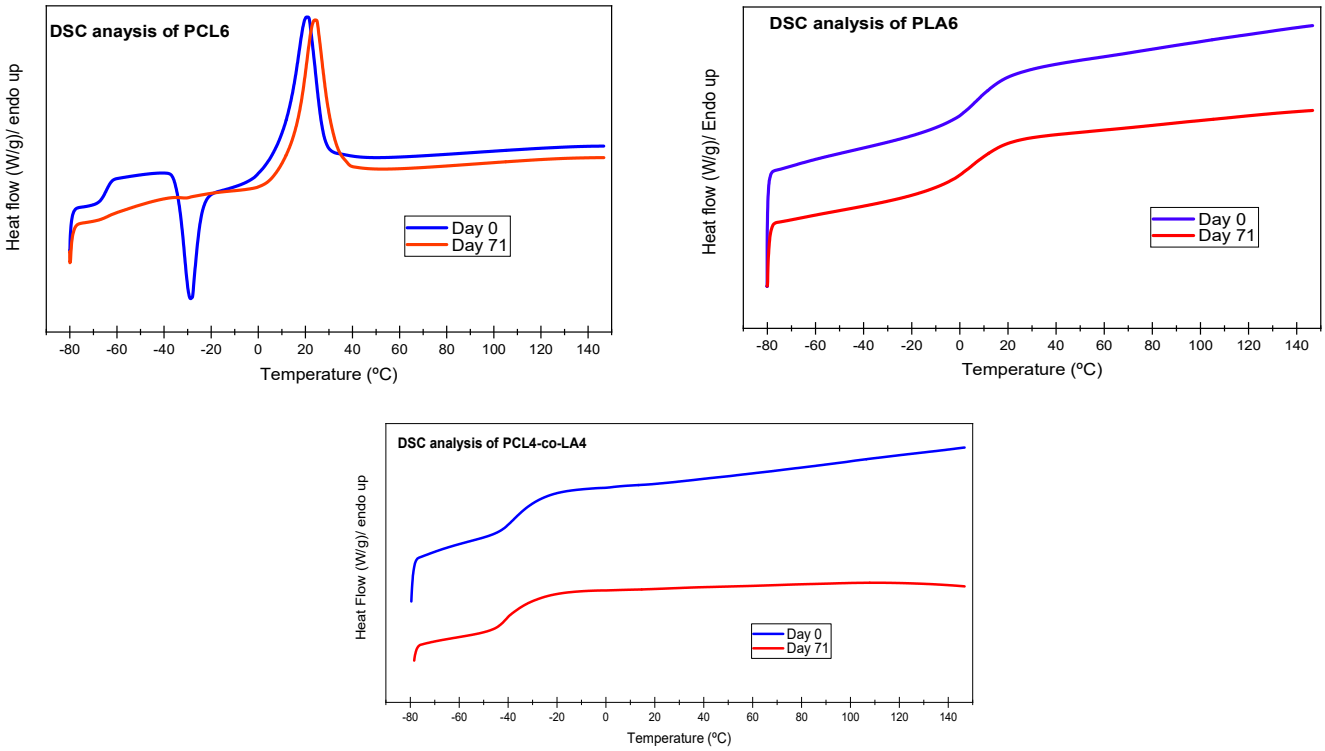


Figure 20. Second DSC scans of PCL6, PLA6 and PCL4-co-LA4 at day 0 and day 71 of the degradation study.

Thermal transitions indicate the nature of the polymers produced. PCL6 was semi-crystalline while PLA6 and PCL4-co-LA4 were amorphous. A Tg value of -64 ºC was reported for PCL6, with a crystallization temperature at -28 ºC and a melting temperature at 22 ºC. Linear polycaprolactone (PCL) also shows a partial crystalline behaviour, reporting Tg values of -60 ºC and Tm of 60 ºC. So it is due to the brush form and the presence of the PEG lateral chain why these values were obtained for our polymer.[34] At the end of the degradation study, as it can be seen in the graph, inflexion point of the curve was difficult to be seen and because of that no Tg was reported. Additionally, no crystallization occurred and melting point increased.

Regarding PLA6 samples, initial macromonomers were synthesised using L-type lactic acid, which is known to produce semi-crystalline structures.[35] However, neither Tc nor Tm was observed for our samples, which means that the polymer produced was amorphous. On the other hand, higher Tg values are

reported for pure PLA polymers (around 45-60 ºC) [35], which means that the polymer synthesized is more malleable due to its brush structure. After 71 days, a lower Tg value was observed. As polyester lateral chains degrade, a reduction in molar mass is achieved, leading to a decrease in molecular entanglement and an increase in chain mobility that affected directly the observed glass transition temperature.[36]

In the case of the copolymer, a Tg value between the PCL6 and the PLA6 values was found because of the copolymerization of MCL6 and MLA6. Furthermore, the same trend as in PLA6 was observed at the end of the degradation study: lower Tg were obtained as a consequence of the degradation state of the polymer.

3.3.3. Water absorption and remaining weight

Since polyesters degrade hydrolytically, the water absorption of the samples was studied. Water absorption was measured following Equation 8. Figure 21 depicts the water absorption evolution during degradation days.

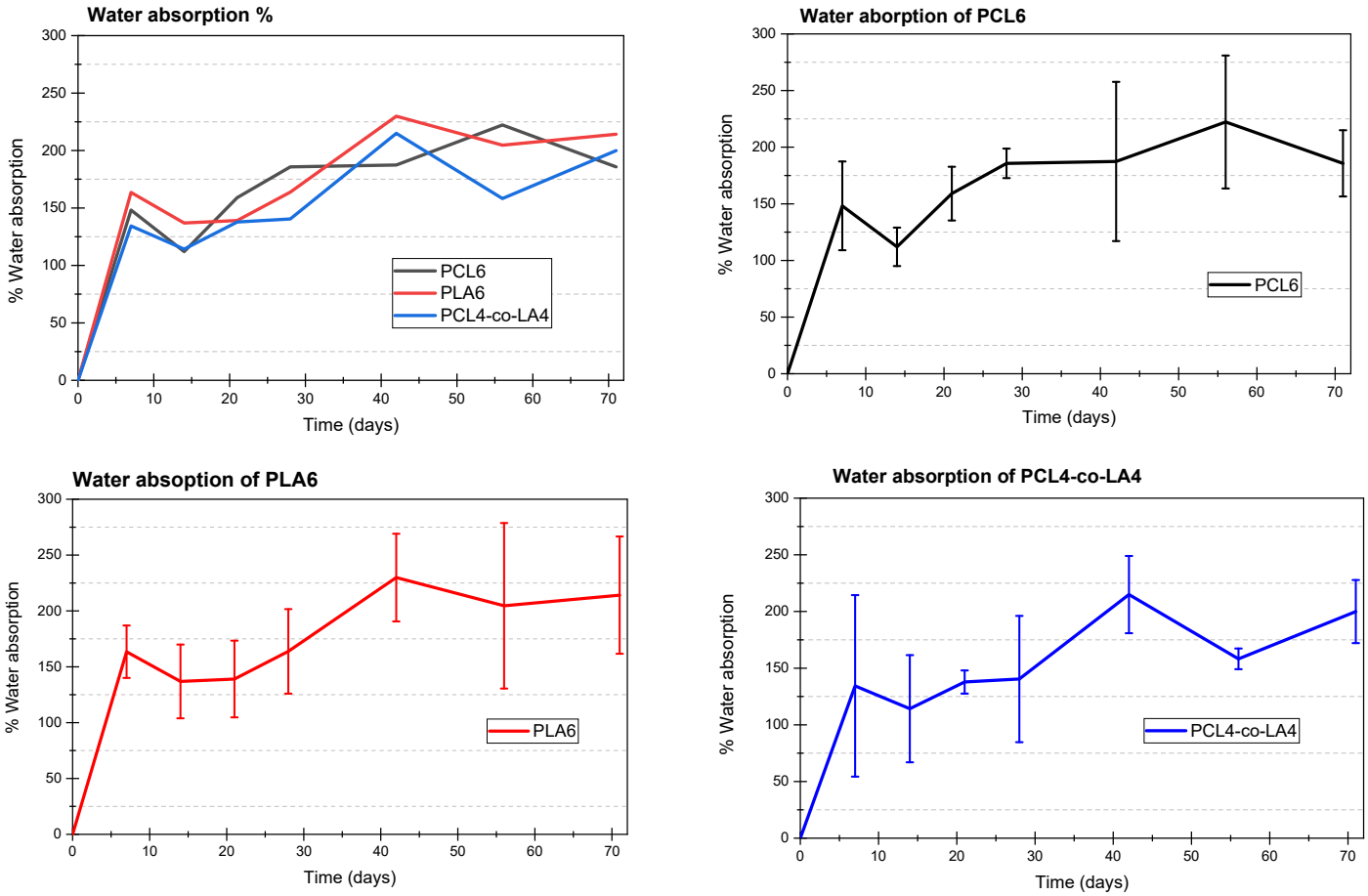


Figure 21. Evolution of the water absorption of PCL6, PLA6, PCL4-co-LA4 during in vitro degradation.

From day 7, high water absorption was reported for the three different polymers. Since all of them contain PEG side chains, which is a hydrophilic polymer, the high water absorptions can be due to the presence of this polymer.

The calculated water absorptions present high standard deviations so these data has to be interpreted prudently. What it can be inferred is that for the three polymers water absorption increases by time and then it remains steady.

These high deviation standards can be attributed to the operation method of the protocol. In the measurements of the wet weight, the surface water of the samples was not removed. It was decided to do it that way to avoid manipulation of the samples which are prone to be easily segregated.

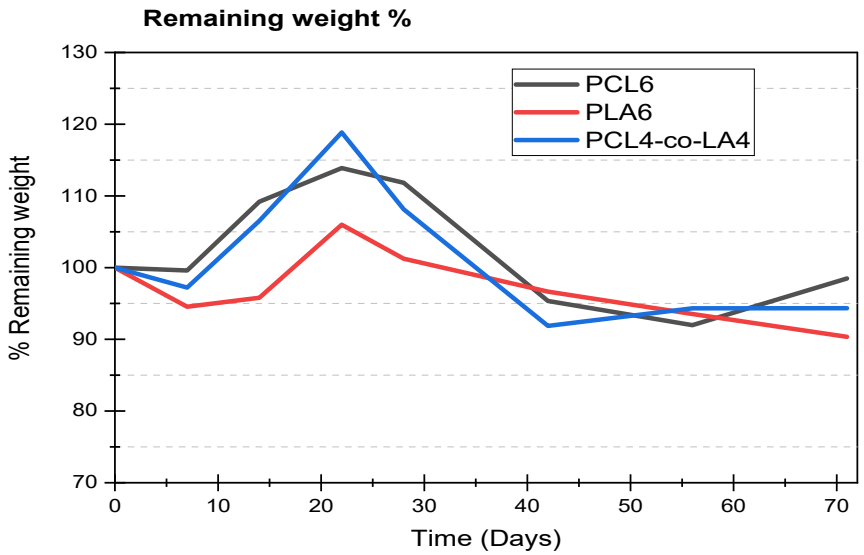


Figure 22. Remaining weight against degradation time of PCL6, PLA6 and PCL4-co-LA4.

Remaining weight (RW) of the samples was measured as well to see how much of the polymer was segregated into the water, that is, if weight loss was happening. In Figure 22 are shown the graphs of the RW of the different polymers. No error bars are illustrated because deviation standard was considerably low in the three polymers. Weight loss was observed for the three polymers at the end of the study, confirming the degradation state of the samples.

In the cases that a weight higher than 100% was reported, it indicates that the sample was not completely dried. For future degradation test, it should be considered that for theses samples two days drying is not enough due to the high water absorptions levels.

4. Conclusions

In this work biocompatible PEGylated polyester nanoparticles were produced by studying their ability to form nanoparticles and by assessing their hydrolytic degradability. For this, CL- and LA- based macromonomers (MM) of different chain length were synthesised by ROP (MCL3, MCL6, MCL12, MLA6, MLA12, MAL20, and MCL4-co-LA4).

To produce nanoparticles, the MMs of shorter chain length (MCL3 and MLA6) were polymerized with PEGMA of different molar masss in water medium. PEGMA 950 (m=19) showed the best performance regarding stability, obtaining particles the range of 159 to 240 nm, and PDI values below 0.152. On the other hand, the MMs of larger repeating units were polymerized in an ethanol-water medium due to the difficulties encountered for their dispersion in pure water. Nanoparticles were also achieved with these MMs, but many stability issues were faced. PEGMA 950 and 2000 were used at different concentrations, but in all the cases coagulum contents higher than 10 wbtm% were obtained. No relation was established between the length of the MM and the coagulum content, but it was for PEGMA concentration.

Using the same polymer formulations, polymers were produced via solution polymerization and the hydrolytic degradability of PCL6, PLA6 and PCL4-coLA4 was studied for 71 days in a PBS medium. Brushed liked polymers were produced with polyester and PEG pendants. A reduction in the Mw was observed by day 14 in the case of PCL6, while for PLA6 and PCL4-co-LA4 no considerable decrease was seen until day 56. Almost half of the initial Mw was obtained at the end of the study. The reduction in the Mw was also observed at the MWD curves. Besides, thermal properties of the polymers before and after degradation were analysed, observing a slight decrease at the Tg which can be attributed to the higher mobility of the backbone chains due to degradation. Water absorption and remaining weight contents were measured. High levels of water absorption were observed during the whole degradation process as a consequence of the PEG chins, and weight loss around 5 to 10% was seen at the end of the study for the three polymers.

Ondorioak

Lan honetan PEGilatutako poliester nanopartikula biobateragarriak produzitu dira, hauek nanopartikulak osatzeko duten gaitasuna aztertuz eta hauen degradazio hidrolitikoa ebaluatuz. Horretarako, kate luzera desberdineko CL- eta LA- oinarritutako makromonomeroak (MM) sintetizatu ziren ROP bidez (MCL3, MCL6, MCL12, MLA6, MLA12, MAL20 eta MCL4-co-LA4).

Nanopartikulak sortzeko, kate luzera txikiko MMak (MCL3 eta MLA6) ur-fasean polimerizatu ziren pisu molekular desberdineko PEGMArekin. PEGMA 950-ek (m = 19) egonkortasunari dagokionez errendimendu onena erakutsi zuen, 159 eta 240 nm bitarteko partikulak eta 0,155etik beherako PDI balioak lortuz. Bestalde, kate luzeagoko MMak etanol-ur nahastean polimerizatu ziren, hauek uretan soilik barreiatzeko zailtasunak zirela eta. MM horiekin NPak ere lortu ziren, baina egonkortasun arazo ugari agertu ziren. PEGMA 950 eta 2000 kontzentrazio desberdinetan erabili ziren, baina kasu guztietan % 10 wbtm baino gehiagoko koagulu edukiak lortu ziren. Ez da erlaziorik finkatu MM luzeraren eta koaguluaren edukiaren artean, baina bai PEGMA kontzentrazioarentzat.

Dispertsioko monomero formulazio berdinak erabiliz, polimeroak soluzio polimerizazio bidez ekoitzi ziren eta PCL6, PLA6 eta PCL4-co-LA4-ren degradagarritasun hidrolitikoa 71 egunez aztertu zen PBS-an. HEMAn oinarritutako PEG eta poliester albo kateko polimeroak lortu ziren. PCL6-ren kasuan, Mw-aren aldaketa 14. egunean ikusi zen. Aldiz, PLA6 eta PCL4-co-LA4 kasuan, ez zen beherakada nabarmena ikusi 56. egunera arte. Ikerketaren azken egunerako lortutako Mw-ak hasierakoen ia erdia ziren. Aldaketa hau Mw-ren MWD kurbetan ere ikusi zen. Gainera, degradazioa hasi aurretik eta ondoren polimeroen propietate termikoak aztertu ziren. Tg-an beherakada txikia behatu zen, eta degradazioaren ondorioz kateek lortutako mugikortasunari atxikitu zaio. Xurgatutako ura eta pisu galera edukiak neurtu ziren. PEG-kateak direla dela eta, xurgapen maila altuak ikusi ziren degradazio prozesu osoan zehar, eta %5-10 inguruko pisu galera behatu zen ikerketaren amaieran hiru polimeroetarako.

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