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3D printing of new biobased unsaturated polyesters by microstereo-thermal-lithography

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Abstract

New micro three-dimensional (3D) scaffolds using biobased unsaturated polyesters (UPs) were prepared by microstereo-thermal-lithography (μSTLG). This advanced processing technique offers indubitable advantages over traditional printing methods. The accuracy and roughness of the 3D structures were evaluated by scanning electron microscopy and infinite focus microscopy, revealing a suitable roughness for cell attachment. UPs were synthesized by bulk polycondensation between biobased aliphatic diacids (succinic, adipic and sebacic acid) and two different glycols (propylene glycol and diethylene glycol) using fumaric acid as the source of double bonds. The chemical structures of the new oligomers were confirmed by proton nuclear magnetic resonance spectra, attenuated total reflectance Fourier transform infrared spectroscopy and matrix assisted laser desorption/ionization-time of flight mass spectrometry. The thermal and mechanical properties of the UPs were evaluated to determine the influence of the diacid/glycol ratio and the type of diacid in the polyester's properties. In addition an extensive thermal characterization of the polyesters is reported. The data presented in this work opens the possibility for the use of biobased polyesters in additive manufacturing technologies as a route to prepare biodegradable tailor made scaffolds that have potential applications in a tissue engineering area.

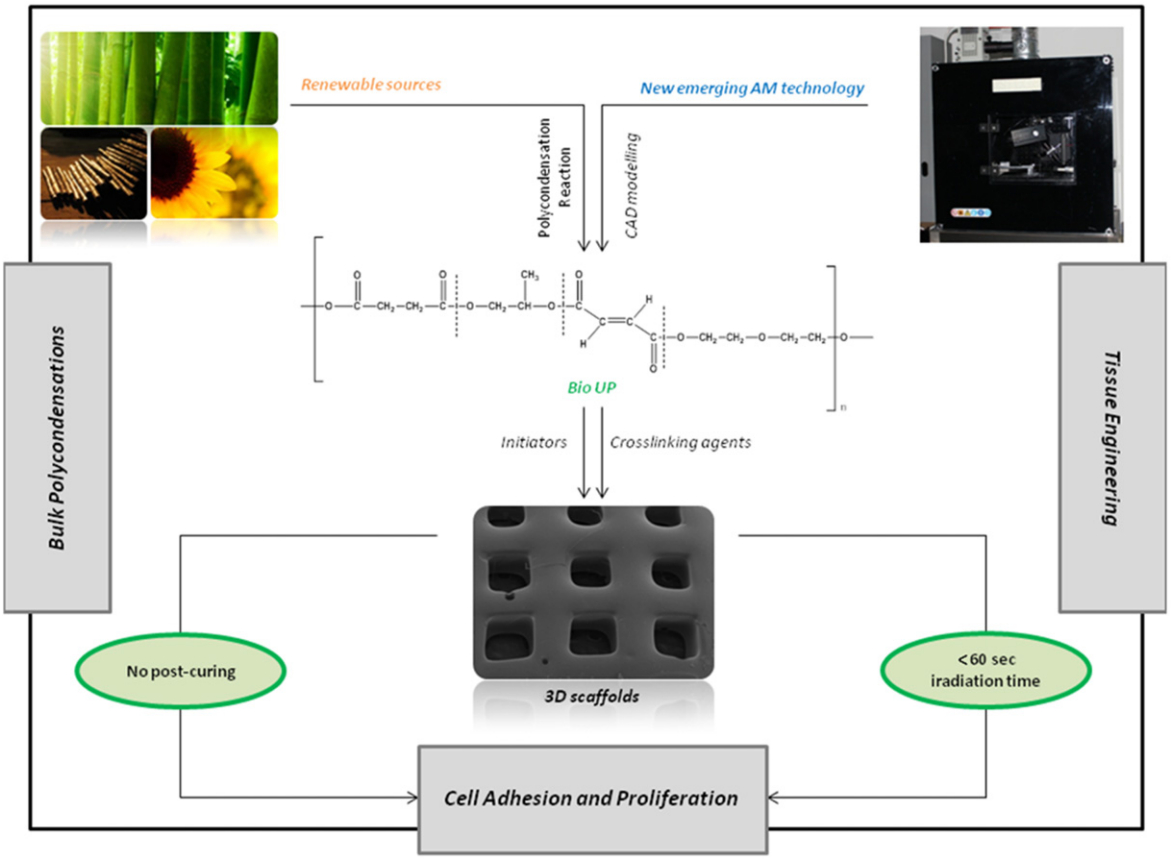
S Online supplementary data available from stacks.iop.org/BF/6/035024/mmedia

Keywords: unsaturated polyesters, biocompatibility, stereolithography, 3D scaffolds

(Some figures may appear in colour only in the online journal)

1. Introduction

Stereolithography (SL) is an additive manufacturing (AM) technique (Kruth *et al* 1998), which allows the fabrication of three-dimensional models from a computer-aided design (CAD) (Melchels *et al* 2012). This technology produces multi-layered materials through a selective photo-initiated cure reaction of specific polymers, providing very accurate and precise scaffold architectures (Yeong *et al* 2004). Microstereo-thermal-lithography (μSTLG) is an improved SL technology that was developed by Paulo Bártolo and co-workers, where thermal and optical parameters are simultaneously used to produce three-dimensional (3D) structures (Bartolo and Mitchell, 2003). The μSTLG presents several advantages when compared with the conventional stereolithography: i) the generation of radicals is more efficient; ii) small concentrations of two types of initiator (thermal and photo) can be used; iii) the combination of ultraviolet (UV) radiation and heat increases the reaction rate and hence the fractional conversion values; iv) the curing reaction is more



Scheme 1 Schematic illustration of the relationship between polycondensation bioproducts and the AM technologies in tissue engineering.

localized, resulting in more accurate models and v) the system has more tunability (Bártolo, 2011). One important issue associated with this advanced technique is related to the lack of proper biocompatible and photocurable polymers (Melchels *et al* 2010). To overcome such an issue, this work was focused on the development of suitable unsaturated polyester resins (UPRs) to be used in μSTLG. The materials need to fulfil some critical requirements: i) fast curing process, ii) low viscosity and iii) biocompatibility if the final scaffolds are intended to be used in biomedical applications. The UPs are versatile materials with a wide use in industry such as: glass-fiber-reinforcement composite materials or paint constituents for different applications such as the automotive, construction and marine industries (John Scheirs, 2003). These oligomeric structures are easily prepared and involve the use of non-expensive monomers. Therefore, these materials represent an effective and convenient technical solution (Lu *et al* 1998). Currently used UPs are characterized by having low molecular weights, and generally are obtained by polycondensation of 1,2-diols with saturated and unsaturated anhydrides or diacids (Martin, Rogers (2003)). Using UPs, it is possible to obtain a 3D network structure by crosslinking the unsaturated bonds of the polyester structure with a vinyl monomer, typically styrene (St), originating crosslinked polyester resins (UPR) with important industrial applications (John Scheirs, 2003). Other chemical modifications (e.g. oxidation of UPs, leading to unsaturated epoxy polyesters) are also possible to improve their mechanical properties (Worzakowska, 2009, Alkskas *et al* 2010, Alemdar *et al* 2010).

Nowadays, growing attention is being paid to environmental and sustainability issues involving the preferential use of biobased monomers in order to reduce the CO_2 footprint. This concern is particularly relevant in polymeric materials that use monomers derived from fossil resources. In addition, UP based materials are receiving particular attention in the biomedical areas considering their potential biodegradability (Guo *et al* 2007). On this matter, the selection of monomers is crucial since UP properties can be fine-tuned by changing the structure of the diacid or the dihydroxy compounds. For instance, it is known that an increase in the propylene glycol content in UP formulation increases the UP hardness (Irfan, 1998). Also, the presence of aromatic rings in the polyester structure enhances the rigidity of the resin. The opposite effect occurs by increasing the content of aliphatic acids, leading to more flexible polyesters. Other studies related to UP synthesis are focused on the influence of the glycol's nature or the styrene concentration in the final properties of the crosslinked polyesters (Worzakowska, 2009, Matynia *et al* 2006, Sanchez *et al* 2000). In industrial applications isophthalic acid and propylene glycol are the most commonly used reagents (Martin, Rogers (2003)). Several authors have already reported the synthesis of UPs using the succinic (Jasinska and Koning, 2010a, Tawfik, 2001), adipic (Barrett *et al* 2010) (Nebioglu and Soucek, 2007) and sebacic

(Guo *et al* 2007, Najafi and Sarbolouki, 2003) acids or other biobased materials (Jasinska and Koning, 2010b) but, to the best of our knowledge, the formulations here described have never been reported and characterized. In this work, biobased UPRs were synthesized via bulk polycondensation. Biobased monomers such as succinic acid (SuCA), adipic acid (AA) and sebacic acid (SeBA) were used with the purpose of replacing the isophthalic acid (IA), while fumaric acid (FA) was used as the source of double bonds for further cross-linking reactions, instead of maleic anhydride (MA), which is widely reported in the literature for that purpose due to its high reactivity and low cost (Tawfik, 2001, Takenouchi *et al* 2001, Cherian and Thachil, 2007). However, the use of MA is responsible for the occurrence of *cis-trans* isomerism in the final materials (Grobelny, 1995, Grobelny and Kotas, 1995). Propylene glycol (PG) and diethylene glycol (DG) were the selected glycols. The biobased UPs were used to prepared 3D scaffolds by μSTLG, using at this stage only the UV irradiation.

2. Materials and methods

2.1. Materials

Isophthalic acid (99%) (IA), succinic acid (99%) (SuCA), adipic acid (99.6%) (AA), sebacic acid (94.5%) (SeBA), diethylene glycol (99%) (DG), propylene glycol (99%) (PG) and potassium hydroxide (90%) were purchased from Sigma/Aldrich Chemical Company and used as received. Hydroquinone (99%) was purchased from Analar, ethanol (96%) from Panreac, tetrahydrofuran-D (99.5%) from Euriso-top and phenolphthalein from Niedel-deHaën Allied Signal. Styrene, 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol were also purchased from Sigma.

2.2. Methods

2.2.1. Synthesis of unsaturated Polyesters by bulk polycondensation. The UPs were prepared by bulk polycondensation. The diacid, the source of double bonds, glycols and hydroquinone (0.02% of the total weight) were charged into a four-necked glass reactor, equipped with a mechanical stirrer, a nitrogen inlet and a condenser connected to a receiver flask. The reactor was first heated at 190–200 °C, and then the temperature during reaction was raised to 220 °C. The polycondensation (Scheme 1) was carried out for at least 14 h. The end of the reaction was determined by monitoring the acid value (AV) (according to ASTM 109-01).

2.2.2. Physicochemical characterization. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopic analysis was carried out with a JASCO FT-IR 4100. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained at room temperature on a Varian Unity 600 MHz spectrometer using a 3 mm broadband NMR probe, in deuterated tetrahydrofuran (THF-d_8). Tetramethylsilane (TMS) was used as the internal reference.

The thermal stability of UPs was evaluated in the range of *ca*. 25–600 °C, in a TA Instruments Q500 thermogravimetric analyzer (thermobalance sensitivity: 0.1 μg) at a heating rate of 10 °C min^{-1} and under a dry nitrogen purge flow of 100 mL min^{-1}. The modulated differential scanning calorimetry (MDSC) studies were performed within a temperature interval ranging from 90 to 50 °C, in a TA Q100 instrument at a heating rate of 2 °C min^{-1} in the temperature modulated mode, and under a nitrogen flow of 50 mL min^{-1}. Dynamic mechanical thermal analysis (DMTA) was performed in Tritec 2000 DMA equipment at a heating rate of 5 °C min^{-1}, in the temperature range −150 to 300 °C, with a multifrequency mode (1 and 10 Hz). The polyesters were placed into stainless steel pockets. The pocket was clamped directly into the DMTA using a single cantilever configuration.

The chromatographic parameters of the samples were determined using high-performance gel permeation chromatography (HPSEC; Viscotek TDAmax) with a differential viscometer (DV), right-angle laser-light scattering (RALLS, Viscotek), low-angle laser-light scattering (LALLS, Viscotek), and refractive-index (RI) detectors. The column set consisted of a PL 10 mm guard column (50 × 7.5 mm^2) followed by one Viscotek T200 column (6 μm), one MIXED-E PL gel column (3 μm) and one MIXED-C PLgel column (5 μm). A high-performance liquid chromatography (HPLC) dual piston pump was set with a flow rate of 1 mL min^{-1}. The eluent (THF) was previously filtered through a 0.2 μm filter. The system was also equipped with an on-line degasser. The tests were done at 30 °C using an Elder CH-150 heater. Before the injection (100 μL), the samples were filtered through a polytetrafluoroethylene (PTFE) membrane with 0.2 μm pore. The system was calibrated with narrow PS standards. Number average (M_n), weight average (M_w) and polydispersity (PDI) of the synthesized polymers were determined by conventional calibration (OmniSEC software version 4.6.1.354).

The biobased UPs were analyzed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). The dried-droplet sample preparation technique was used, applying 2 μL of 2,5-dihydroxybenzoic acid (DHB) matrix solution (20 mg mL^{-1} in THF) directly on a MTP AnchorChip™ 800/384 TF MALDI target (Bruker Daltonik, Bremen Germany), and, before drying the matrix solution, 2 μL of sample (20 mg mL^{-1} in THF) was added and allowed to dry at room temperature. External mass calibration was performed with a calibration standard (Bruker Daltonik, Bremen Germany) for the range 700–3000 m z^{-1} (9 mass calibrant points): 0.5 mL of calibrant solution and DHB matrix previously mixed in an eppendorf tube (1:2, v/v) were applied directly on the target and allowed to dry at room temperature. Mass spectra were recorded using an Autoflex III smart beam MALDI-TOF mass spectrometer Bruker Daltonik (Bremen, Germany), operating in a linear positive ion mode. Ions formed upon irradiation by a smart beam nitrogen laser (337 nm) using an accelerating potential of 20 kV and a frequency of 200 Hz. Each mass spectrum was produced by averaging 2000 laser shots collected across the

whole sample spot surface by rastering in the range 400–3000 m z^{-1}. The laser irradiance was set to 45–50% arbitrary units according to the corresponding threshold required for the applied matrix system. Low molecular ion gating was set to 400 Da to remove the ions below this value arising from the matrix and their clusters or other unknown contaminants. All spectra were acquired and treated using the flexControl 3.0 and flexAnalysis 3.0 softwares (Bruker Daltonik), respectively.

A controlled stress rheometer, Haake, model RS1, was used to measure the viscosity. The geometry used was a plate/plate system PP20 (titanium for the rotating part and stainless steel for the stationary part). The UPs were dissolved in 37% (w/w) of styrene and measured under certain conditions. The biobased polyesters were also studied at 25 °C. All measurements were made in triplicate.

2.2.3. Cell viability assays. For cell viability tests the UPs were crosslinked with styrene[4]: once the UP synthesis was finished the temperature was decreased to 80 °C–100 °C and the reaction mixture was discharged to a vessel containing styrene. The mixture was continuously stirred and warmed until total dissolution of the UP occured. After the dissolution to a concentration of 37% (w/w), UPs were photo polymerized in a UV chamber, in the presence of 2 to 5% (w/w) of the photoinitiator Irgacure 651, and using a UV light (253.7 nm) for a period time not exceeding 15 min, at room temperature. The cytotoxicity of the different polymers was evaluated in the 3T3-L1 cell line by an extraction test according to ISO 10993-5 Standard (Organization, 2009). 3T3-L1 cells, seeded in 48-well culture plates, were incubated with extraction fluid for 24 h and the cell viability was assessed by a modified *Alamar Blue* assay (Faneca *et al* 2008). This assay measures the redox capacity of the cells due to the production of metabolites as a result of cell growth. Briefly, following cell incubation during 24 h with the extraction medium, the medium of each well was replaced with 0.3 ml of DMEM-HG containing 10% (v/v) of *Alamar Blue* (0.1 mg ml^{-1} in PBS) and, after 1 h of incubation at 37 °C, 170 μL of supernatant was collected from each well and transferred to 96-well plates. Then, absorbance at 570 and 600 nm was immediately measured in a SPECTRAmax PLUS 384 spectrophotometer (Molecular Devices, Union City, CA).

2.2.4. Scaffold fabrication. 3D scaffolds were prepared by means of μSTLG. The μSTLG can create scaffolds with specific geometries, varying in shape and thickness. The UPs and the corresponding unsaturated monomer was loaded into the reservoir and exposed to UV light, in a period of time between 25 and 60 s. μSTLG system uses a mercury lamp of 350 W as a light source. Optical fibers, projection and focal lenses irradiate a UV-DMD and an IR-DMD (Bartolo and Mitchell, 2003) (Pereira, 2014). A dichroic mirror captures the images projected on DMD (1024 × 768 pixels, 14 mm in size), combining them into a single image that is transferred to the reactive resin (monomer or oligomers). The equipment also includes a multi-vat system. The vertical displacement of the platform is secured by an uniaxial MYCOSIS Translation Stage VT-80. This positioning system allows vertical increments of 1 μm, at a speed ranging between 0.001 and 20 mm s^{-1}.

2.2.5. Scaffold morphology. The morphologies of the processed scaffolds were observed by means of scanning electron microscopy (SEM) (MEV)/EDS, JEOL, model JSM-5310, at an accelerating voltage of 10 kV. The topography of the scaffolds surface was observed by an Optical Microscopy 3D micro, Alicona, IFM G4 3.5 EN.

3. Results and discussion

3.1. Polycondensation of unsaturated polyesters with biobased dicarboxylic acids

UPs were prepared through a bulk polycondensation reaction between biobased dicarboxylic acids, two glycols and an unsaturated acid monomer in different relative ratios. The selection of the diacid monomers was based on their linear chain structure. Succinic acid plays an important role in the Krebs cycle, that occurs naturally in plant and animal tissues, while sebacic acid is a derivative of vegetable oils (e.g. castor oil) (Bechthold *et al* 2008). Adipic acid is one of the most used acids at the industrial scale in different applications, such as medicine and food industries.

These three monomers are among the most common diacids used in polyester synthesis (Edlund and Albertsson, 2003) and can be considered as a potential candidate to substitute phthalic acids (Ma *et al* 2012). The formulations were developed to fulfil two important requirements: the incorporation of biobased monomers and preparation of low molecular weight structures with internal double bonds for further crosslinking (figure 1).

The results of polycondensation reaction formulations are presented in table 1. For comparative purposes, UPs based on isophthalic acid were prepared with different ratios between the isophthalic acid and the glycols, in order to evaluate the results of increasing the number of aromatic rings, as well as the influence of decreasing the percentage of glycols in the final properties of the UPs (UP1, UP2 and UP3).

In the formulations UP4, UP5 and UP6 the main objective was to determine the effect of the replacement of the isophthalic acid by aliphatic biobased diacids with different chain lengths. In this case, a polymer structure with a higher flexibility is expected compared to the counterpart using isophthalic acid (Stevens, 1999). As expected, the molecular weights of the oligomers are small (between 604 and 1221) and the PDIs are higher than 2 (Martin, Rogers (2003)).

The viscosities of the polyesters dissolved in styrene (UP/St) were determined and are presented in table 2 and figure 2(a). The viscosity values of UPs based on alkylic carboxylic acids were substantially low (between 0.24 and 0.58 Pa s), when compared with those based in isophthalic acid. This can be ascribed to the higher mobility of the polymeric chains which incorporate a long chain alkylic

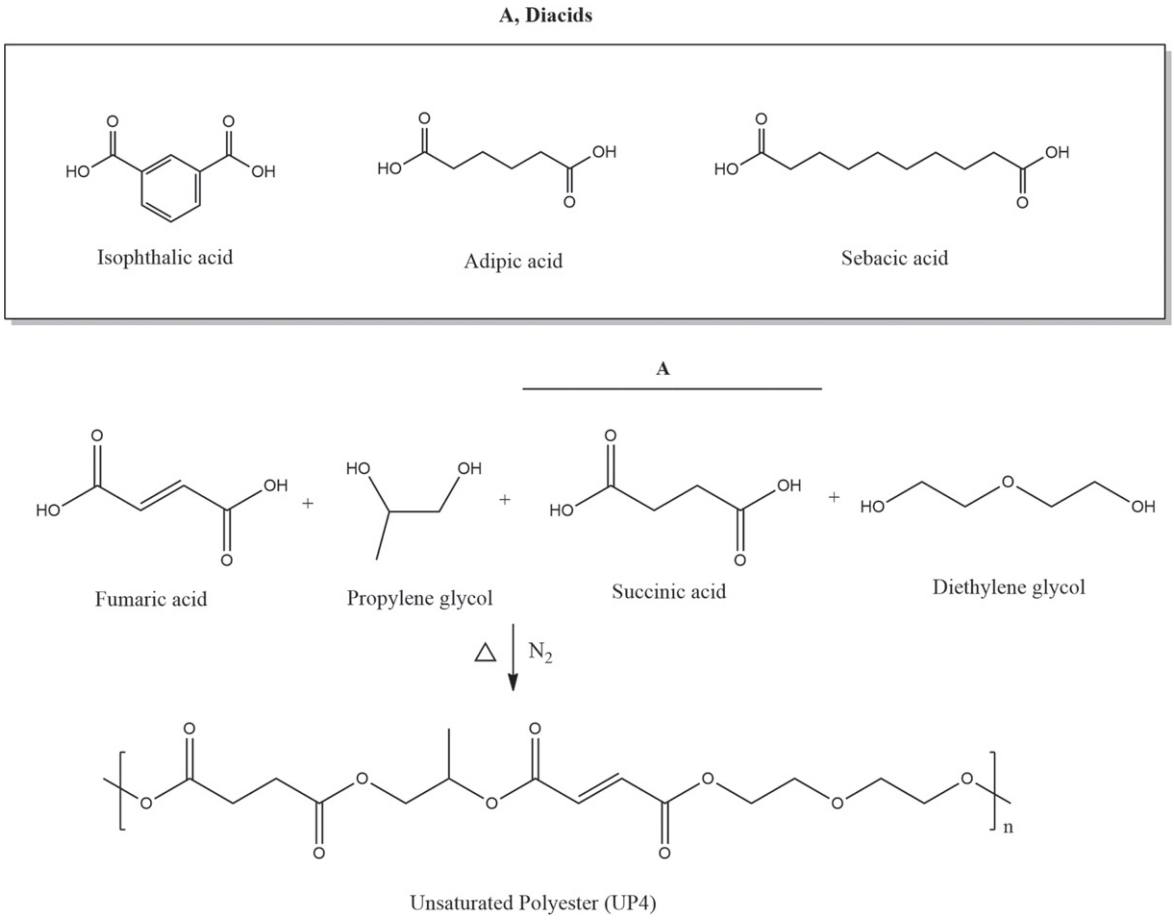


Figure 1. Monomer structure used in the synthesis of unsaturated polyesters.

backbone. Determination of the viscosity for UP3 was unabled by the extremely high hardness of the polyester, which may be related to the high concentration of carbon-carbon bonds in the polymer backbone and the possibility of the occurrence of crosslinking reactions during the polymerization reaction (table 1).

Regarding only the UPs stability, UP viscosities were monitored during 6 months. The biobased UPs showed an increase of the viscosity over time. This characteristic suggested that UPs present some rheological instability, probably due to the occurrence of some gelation processes. Such behaviour has already been reported by others (Takenouchi *et al* 2001), being characterized by a change of state of the material, i.e. a liquid-to-rubber transition, and is associated to non-reversible events (Bártolo, 2011). The rheological curves of the polyesters determined six months after the synthesis are presented in figure 2(b).

It was possible to observe that UPs showed a constant value of viscosity, with the exception of UP4—in this case the observed viscosity was unexpectedly high. Additionally, the viscosity of UP4 decreased sharply with the increase of shear rate. Although this result can be expected for polymers due to a pseudo plastic behaviour (Shaw, 2012), the abrupt decrease in UP4 was not expected.

3.2. Chemical characterization of unsaturated polyesters

The structure of the UPs was analysed by ATR- FTIR and ^{1}H NMR figure 3 shows strong bands ascribed to the carbonyl stretching group characteristic of the polyesters, at *ca*. 1750–1725 cm^{-1} (Coates, 2000). The OH band at 3600–3200 cm^{-1} is absent for the different UPs. The characteristic adsorption bands of double bonds are assigned to the C = C stretching vibration present at 1680–1600 cm^{-1} (Stuart, 2002). These double bonds are associated to FA, present in the different formulations. Also, the group frequency 3095–3075 cm^{-1} from the pendant = C-H stretch was detected (see figure 1 in SI). UP1 and UP3 show additional bands in the 1615–1580 cm^{-1} and 1510–1450 cm^{-1} regions, characteristic of the aromatic ring from the isophthalic acid (Coates, 2000). A distinct peak at 725 cm^{-1} was observed for isophthalic UPs and it is assigned to the aromatic C-H out-of-plane bending vibrations. The peaks in the characteristic region 1150–1050 cm^{-1} observed in all cases can be ascribed to the C-O group.

Figure 4 shows the ^{1}H NMR spectra of the UPs based on isophthalic acid. The spectrum of UP1 exhibits three distinct peaks (***a***) at 8.63, 8.19 and 7.57 ppm, which are assigned to the aromatic ring protons. The proton characteristics of the double bonds of fumaric acid (***b***) are located between 6.50

Table 1. Synthesis conditions and properties of the UPs synthesized from dicarboxylic acids (IA, SuCA, AA and SeBA) and diethylene glycol (DG), propylene glycol (PG) and fumaric acid (FA) as a double bond provider.

UPs	Initial Molar ratio (%)	AV (mg KOH/g)[a]	M_w	M_n	(PDI)[b]	Final Molar ratio(%)[c]	Acids/Alcohols Final Molar Ratio
UP1	IA/FA/PG/DG 24/22/25/29	29.0	2768	951	2.91	33/19/20/28	52/48
UP2	IA/FA/PG/DG 16/30/25/29	40.0	1988	604	3.29	28/24/20/28	52/48
UP3	IA/FA/PG/DG 25/40/15/20	28.0	—	—	—	35/26/18/21	71/29
UP4	SuCA/FA/PG/DG 24/22/25/29	41.0	3639	1138	3.20	34/11/40/15	45/55
UP5	AA/FA/PG/DG 24/22/25/29	21.4	3081	1221	2.52	13/13/57/17	26/74
UP6	SeBA/FA/PG/DG 24/22/25/29	43.3	4700	928	5.10	18/16/46/20	34/66

[a] AV, acid value;
[b] PDI, polydispersity, was determined by SEC, using conventional calibration with polystyrene standards and THF as eluent;
[c] Monomers molar percentages from ^{1}H NMR spectra.

Table 2. Viscosity data of UPs and UPs in styrene (37% (w/w)) at 100 s^{-1} and 25 °C.

Polyesters	η (UP/St) (Pa s), 37 s^{-1}	η (UPs) (Pa s), 100 s^{-1}
UP1	2.09	—
UP2	2.08	—
UP3	—	—
UP4	0.38	125.00
UP5	0.24	6.16
UP6	0.58	29.90

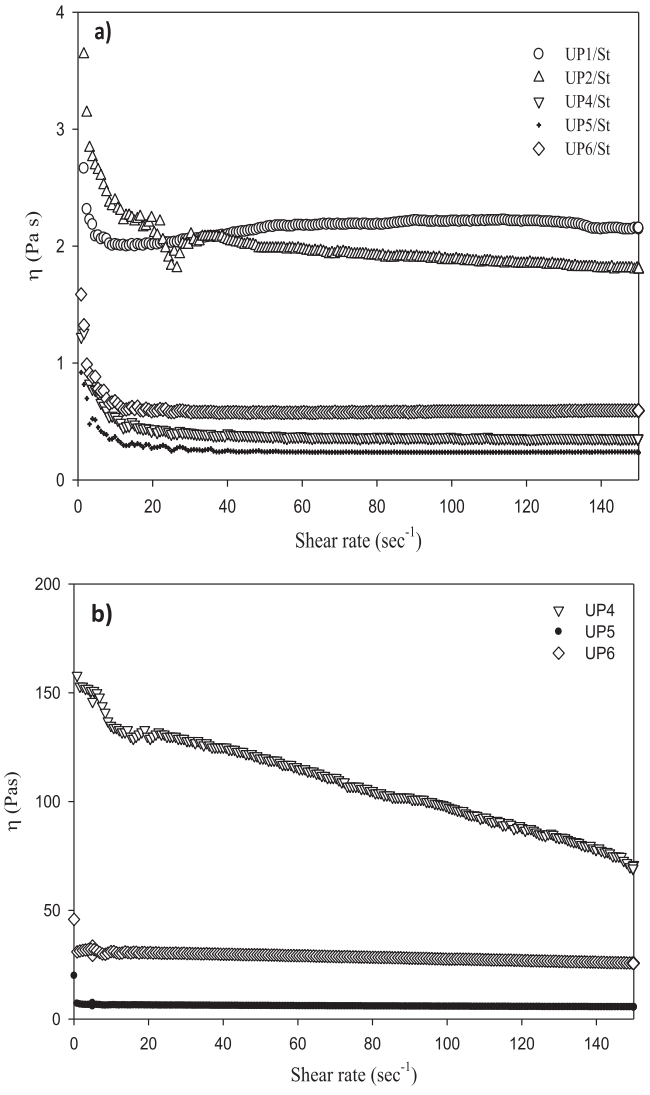


Figure 2. Rheological behavior of: (a) the UPs in styrene (37%) and (b) the biobased UPs after 6 months.

and 7.0 ppm. The CH_3 signals (***c***) of PG are located between 1.03 and 1.47 ppm, while the CH signal (***d***) of PG was found at around 5.25 and 5.54 ppm. The CH_2 signals (***e***) of both glycols are located between 3.71 and 4.45 ppm. The close proximity of the (***e***) peaks due to glycols, which appear almost overlapped, avoids a straightforward analysis. The glycol content was determined from the signal of the methyl proton of PG (***c***) and the CH_2 signals of DG and PG (***e***).

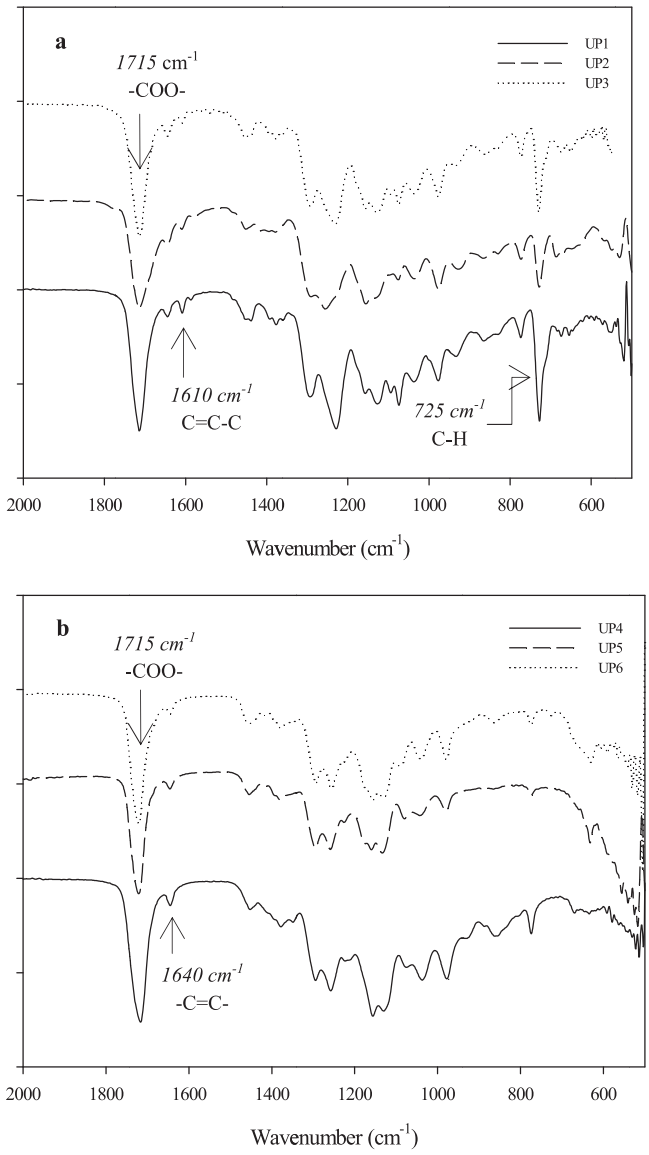


Figure 3. ATR-FTIR spectra for (a) UP1 to UP3 and (b) UP4 to UP6.

Figure 5 shows the ^1H NMR spectra of UP4 to UP6. Besides the signals corresponding to FA, PG and DG parts in UP4 to UP6, which are easily identified, on the UP4 spectrum the signal at 2.58 ppm (***f***) corresponds to the CH_2 of succinic acid. On the other hand, UP5 shows two peaks, $\delta = 2.30$ ppm (***g***) and $\delta = 1.68$ ppm (***h***) assigned to the CH_2 bonds of the adipic acid. In UP6 spectra three peaks at $\delta = 2.27$ (***i***) ppm, $\delta = 1.58$ ppm (***j***) and $\delta = 1.31$ ppm (***k***) correspond to the CH_2 of sebacic acid (Stuart, 2002). Relative integration of (***k***) protons and (***i***) + (***j***) protons confirm this assumption. From the integration of the ^1H NMR signals, it was possible to determine the relative molar amount of the monomers that are incorporated in the polymer structure (see table 1). Regarding the glycols quantification, a higher amount of PG is observed in the final UP for the formulations having biobased acids rather than for the formulation using IA. This result suggests that alkylic diacids react preferentially with this glycol rather than IA.

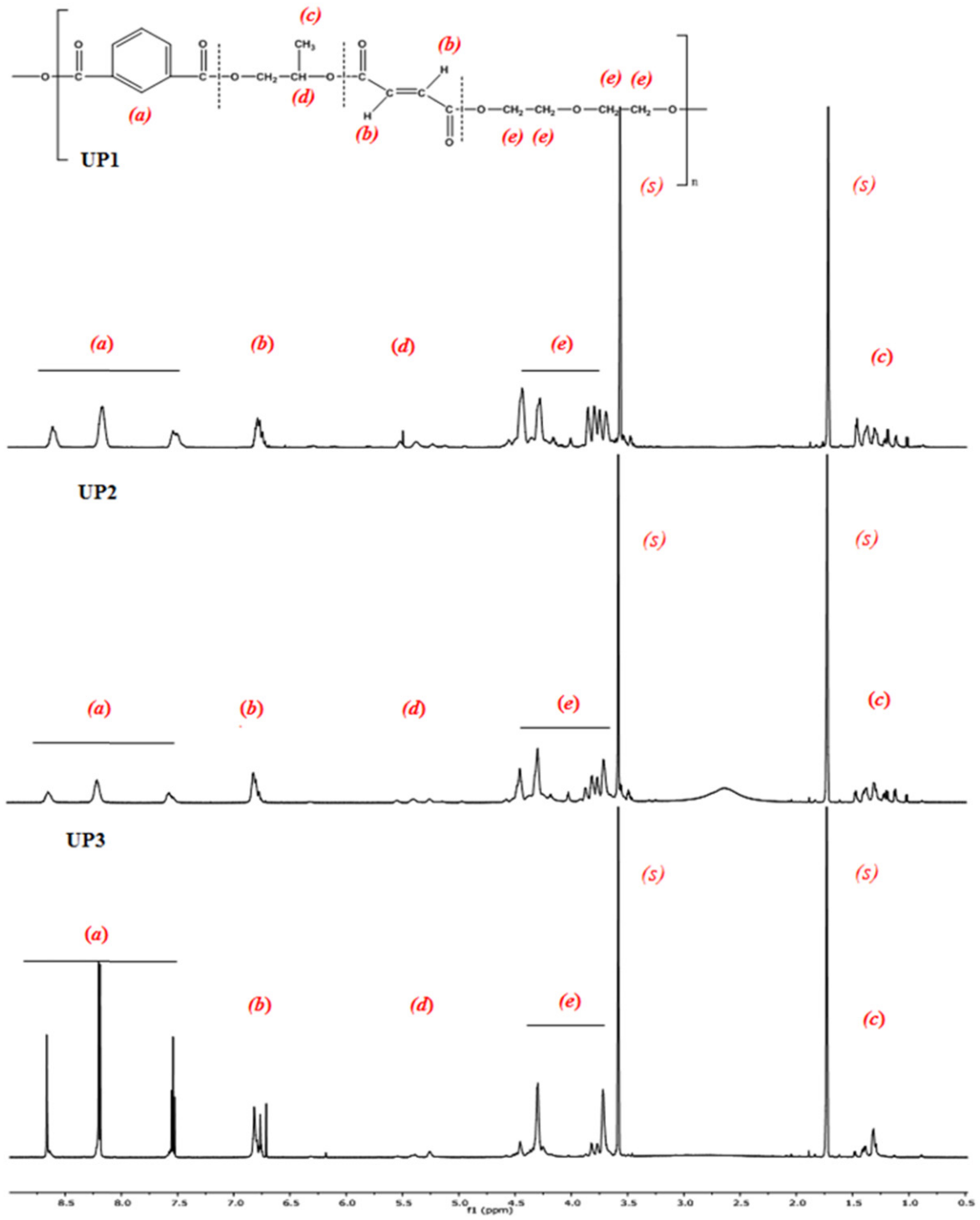


Figure 4. ^{1}H NMR spectra of unsaturated polyesters based on isophthalic acid, UP1 to UP3, where (*s*) correspond to the solvent peaks of THF- d_8.

The structures of UPs were also confirmed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). As an example, the mass spectrum obtained for UP5 from 400 to 2000 m z^{-1} is showed in figure 6. The spectrum presents five different polymer populations indicated in letters from A to E. For some MALDI signals, it is possible to assign the monomer composition of the different oligomers as showed in table 3 with two degrees of polymerization (DP = 2 and DP = 3). Some of the observed polyester m/z values were associated with the Na^+ or K^+ cations. Likewise, UP4 and UP6 revealed values in the range of 500 to 1500 m z^{-1} and 500 to 2000 m z^{-1}, respectively.

The qualitative information collected suggest that the observed mass of UP5 (table 3) was identified on the B and D groups, where the B population showed the peaks with the

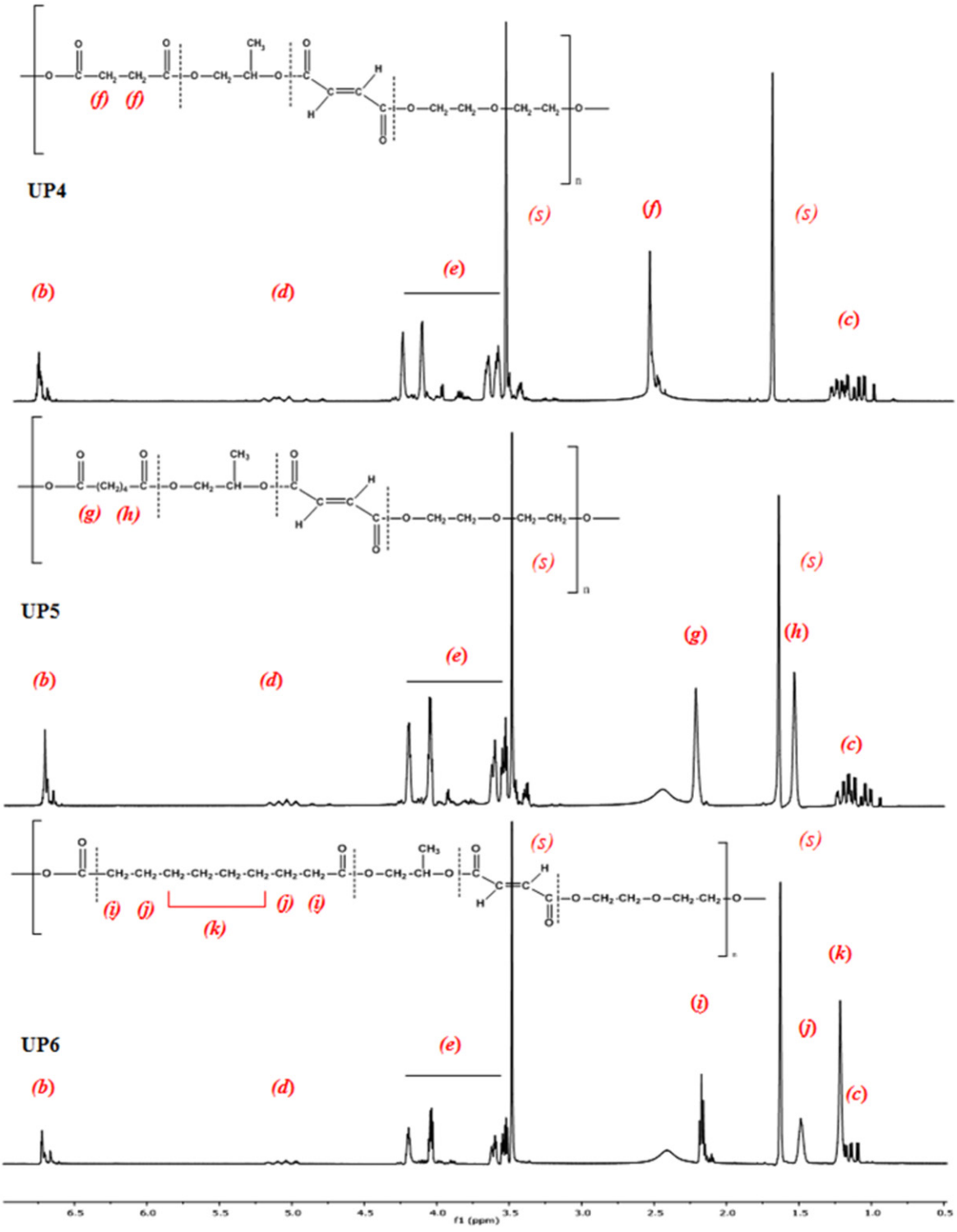


Figure 5. ^{1}H NMR spectra of UP4 to UP6, where (*s*) corresponds to the solvent peaks of THF- d_8.

highest intensities. Also, in the MALDI-TOF spectrum of UP5 a repeating unit was identified, differing from 30 Da. This value can correspond to the difference between FA (116.07 g mol^{-1}) and AA (146.14 g mol^{-1}) units or between DG (106.12 g mol^{-1}) and PG (76.09 g mol^{-1}) units. Based on the MALDI-TOF data it can be suggested that other combinations of monomers were obtained besides the structure presented in table 3.

It was also possible to confirm the existence of several combinations of monomers present in other polyester structures (see table 1 in SI). Between the five populations observed (A-E) almost all the peaks were ascribed to a specific formulation, e.g. $(AA)_n + (FA)_m + (PG)_p - (H2O)_y$ or $(AA)_n + (FA)_m + (DG)_q - (H2O)_y$. The differences between near peaks are usually due to the number of the monomer in the formulation—*n*, *m*, *p* and *q*. The formation of other

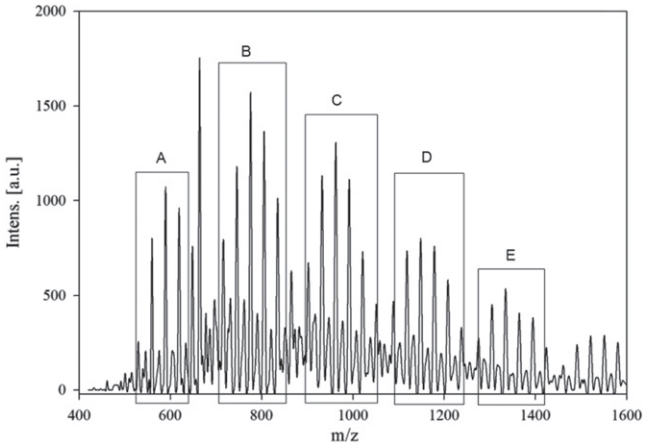


Figure 6. MALDI-TOF-MS in the linear mode (using DHB as matrix) of UP5 from m/z 400 to 1600 and the different observed populations from *A* to *E*.

oligomers is expected due to the nature of the step growth polymerization.

3.3. Thermal and Mechanical Analysis of Unsaturated Polyesters

Figure 7 and table 4 present the data obtained from the thermogravimetric analysis. Comparing UP1, UP2 and UP3 profiles, it is possible to observe that changing the percentages of the components in the formulation led to minor differences in the thermal decomposition. The differences between UP1 and UP2 thermal profiles were not relevant—only a slight decrease on the thermal stability was observed, probably due to a decrease in the aromatic content. UP3, however, presents two significant mass losses of 256 and 337 °C. This profile can indicate that the polyester has a heterogeneous composition. The onset temperature (T_{on}) of the fumaric acid is 248 °C, which is fairly close to the value obtained for the UP3 first step, 255 °C. Despite not being possible to support this hypothesis in definitive terms at the current stage of investigation, it is our belief that the first step corresponds to a possible sub product of the polycondensation reaction between FA and the other monomers. This assumption is in agreement with the ^{1}H NMR final molar %, being UP3 the polyesters with the higher percentage of FA (table 1).

Figure 7(b) comprises a low temperature region, making it clear that UP4, UP5 and UP6 formulations have similar mass loss profiles, which is explained by their comparable structures. These results also suggest that the stability of the UPs increase with the number of carbons in the main chain. For UP6, which contains SeBA in its structure, the stability is similar to the more stable UP1. However, this is not valid for temperatures below 330 °C. Strictly speaking, at lower temperatures (see figure 7(b)) UP1 shows a less stable behaviour when compared with UP6. Nevertheless, in general, UP1 appears to be the most stable formulation, with the main mass loss stage starting at 320 °C.

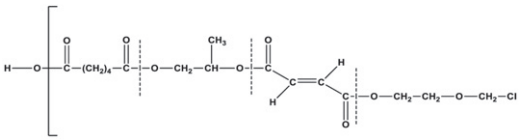
The thermal characterization of the UPs was extended by using the MDSC (the identified thermal events correspond to the glass transition temperature, T_g, of the UPs). According to table 5, the UPs revealed similar MDSC profiles, having T_g values between 8.9 and 39.94 °C. With the exception of UP1, the other UPs showed only one transition.

The dynamic mechanical results expressed as loss tangent (tan δ) as a function of temperature are shown in figure 8. The UPs were submitted to two frequencies of 1 and 10 Hz (figure 8 includes the values at 1 Hz as a representative result). DMTA, in multifrequency analysis, allows distinguishing between frequency dependent and nondependent thermal events. Molecular relaxations such as α (or glass transitions), β and γ relaxations are always frequency dependent. Figure 8 shows the tan δ traces for all synthesized UPs. The peak observed in the curves corresponds to the glass transition temperature (T_g), whose values are summarized in table 5. As indicated above, UP1, UP2 and UP3 differ only in the percentages of monomers. The T_g values of UP2 and UP3 are similar (18 and 21 °C) and higher than the obtained values for the biobased UPs. This fact is explained by the high amount of unsaturated monomer (FA and IA) incorporated into UP1 and UP2 formulations (see table 1) and also because the polymer chains of these formulations are much more rigid. Therefore, a higher energy is necessary in order to allow the mobility of the UP2 and UP3 chains. Although UP2 and UP3 have similar T_g, the UP3 shows a higher ratio of loss and storage energies (table 5). The study of the mechanical properties by DMTA of UPs is rarely reported in the literature with the exception of the UPRs, which were extensively studied (Nebioglu and Soucek, 2008, Nebioglu and Soucek, 2007, Worzakowska, 2009, Mironi-Harpaz *et al* 2010, Vilas *et al* 2001). Therefore, it is not possible to establish a comparison of our UP results with other reported values.

According to table 5, the T_g values obtained by MDSC were in agreement with the values obtained in DMTA for the polymers UP4 to UP6. The T_g values for the aromatic based formulations were quite different for both the techniques. The differences observed between T_g values determined by MDSC and DMTA can be ascribed to differences in the principles used to determine the T_g (Lu and Anseth, 1999). The aliphatic polyesters showed negative T_g values in opposition to the positive values obtained for the isophthalic based formulations. Despite the differences in the biobased formulations, the T_g for UP4 to UP6 was very similar, with values ranging between −21 and −35 °C. UP4, UP5 and UP6 showed lower T_g values. This fact can be explained by the absence of a rigid aromatic ring in their structure, which results in much easier movements of the polymer backbone. Table 7 also reveals that for UP4, UP5 and UP6, an increase of the carbons in the main chain (from de diacid) results in a decrease of the T_g value, which was expected since for longer chains, higher mobility is achieved. In table 5 the maximum of the tan δ curve is also presented. Thus, the values of the maximum of this curve reveal that UP3 and UP4 are the polyesters with the highest capacity to dissipate energy. In summation, all the UPs reveal similar profiles, with the exception of UP1, that showed two distinct transitions (MDSC technique).

Table 3. Relationship between *m/z* and the chemical structure of UP5.

UP5 structure



Observed mass	Calculated mass	n=
805.54	803.830	2 + [Na^+]
1193.65	1194.251	3 + [Na^+]
820.80	819.941	2 + [K^+]
1208.31	1210.362	3 + [K^+]

Table 4. TGA of UPs ($T_{x\%}$: temperature at x% mass loss; T_{on}: extrapolated onset temperature).

Polyesters	$T_{5\%}$/°C	$T_{10\%}$/°C	T_{on}/°C
UP1	320.63	344.80	355.70
UP2	289.41	326.73	339.21
UP3	228.28	249.64	255.96; 336.85
UP4	199.45	262.92	342.06
UP5	240.42	295.57	328.49
UP6	308.86	339.67	350.04

Table 5. Glass transition temperatures obtained from DMTA and MDSC techniques.

Polyesters	T_g (°C)		Tanδ (máx)
	DMTA	*MDSC*	
UP1	−7.30	−28.11; 8.9	0.07
UP2	18.00	−2.48	0.12
UP3	21.10	−2.9	0.20
UP4	−21.13	−22.22	0.20
UP5	−34.38	−37.49	0.10
UP6	−35.05	−39.94	0.07

Table 6. Surface roughness of some of the developed 3D scaffolds.

UP	UM	Number of layers	Time of cure (sec)	Sa
Scf4	HEMA	1	60	518.43 nm
Scf5	HEMA	2	28	283.69 nm
Scf6	HEMA	1	28	1.649 μm

3.4. Cell viability

The application of UPRs for the biomedical field requires the preparation of crosslinked material from synthesized UPs (UPRs). The crosslinking process consists of the use of styrene and a photoinitiator. For the biobased UPs (UP4 to UP6), 37% (w/w) of styrene and 2 to 5% (w/w) of the photoinitiator, Irgacure 651 (Lu and Anseth, 1999), were used.

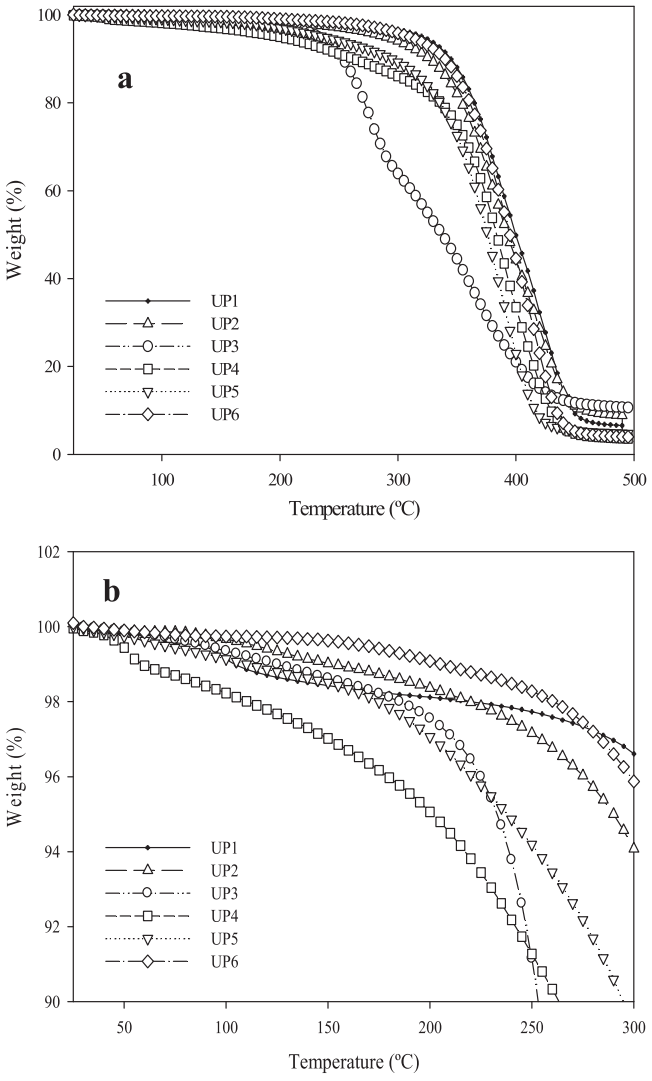


Figure 7. (a) TG curves of the synthesized UPs obtained at a heating rate of 10 °C min^{-1} and b) detailed view within the mass loss range up to 10%.

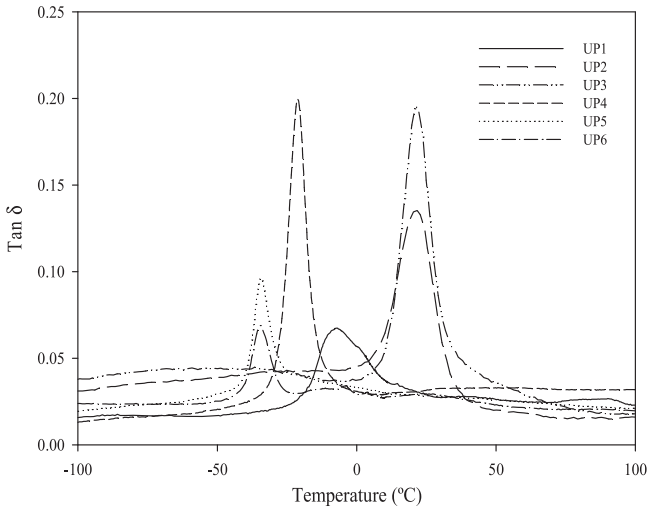


Figure 8. Loss function (tan δ) versus temperature for the synthesized UPs.

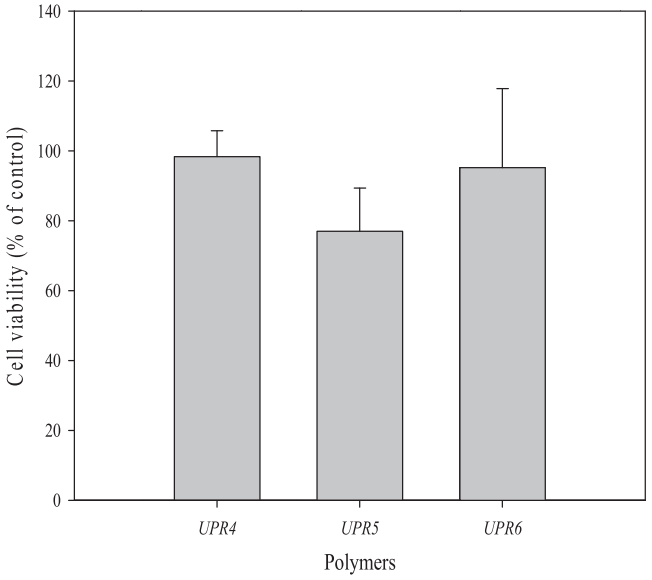


Figure 9. Effect of different polymers on cell viability. The statistical analysis (one-way ANOVA) indicates that there is no significant difference between the different conditions.

The obtained films were translucent and had clean, soft and flexible surfaces. Before viability tests, and in order to eliminate film acidity, they were treated with EtOH for 3-4 h, dried in vacuum at room temperature and finally placed in an oven at 50 °C for a few hours. Figure 9 shows that there are no considerable differences in cell viability of the tested biobased UPRs, with being UPR5 the less biocompatible, nevertheless with a value near 80% of the cell viability.

The *Alamar Blue* assay suggested that the cell viability was in the range 77–98% for all UPRs, showing no statistically significant differences. Therefore, the biobased polyesters synthesized in this work proved to be suitable for application in biomedical fields.

3.5. Scaffolds fabrication and characterization

At this stage, only the UV light was used to promote the scaffolds fabrication. Several biobased scaffolds (Scf4–Scf6) with different specifications were fabricated. The times of curing varied between 25 and 60 s using styrene as crosslinking agent (John Scheirs, 2003). However, HEMA was also used as UM in this work considering its intrinsic biocompatibility (Kejlová *et al* 2005). Figure 10 shows the 3D scaffolds prepared by μSTLG using HEMA as crosslinking agent. The resulting 3D structures presented distinct geometries and very homogeneous surfaces. Also, the obtained results suggest the possibility of producing biobased 3D scaffolds by just applying to the UP/HEMA mixture an UV irradiation for 45 s (2 layers and a total thickness of 0.2 mm) in the presence of the biocompatible photoinitiator Irgacure 651 (5% w/w). It was found that depending on the ratio UP/HEMA the time of cure can range from 25 to 60 s in order to afford a complete crosslinking network without the need of further post-cure.

These 3D structures are the first reported structures obtained with this new apparatus using new photo-sensitive materials. These new UPs showed very promising results for TE applications. Further studies are being performed in order to combine UV and IR radiations as well as in the development of a multi-vat system (Melchels *et al* 2012), (Bártolo, 2011), therefore the fabrication of multi-materials may be a possibility in SL.

The roughness is known to have a critical role for cell adhesion and proliferation on the surface of the 3D scaffolds. Previous analysis by SEM (figure 10) revealed several difficulties to draw any conclusion regarding this matter. Even at a higher magnification, it was impossible to detect irregularities on the surfaces. To overcome this issue, infinite focus microscopy (IFM) analysis was performed (figure 11) and the roughness results are summarized in table 6. The roughness is an important parameter in cell adhesion behaviour (Boyan *et al* 1996), (Anselme *et al* 2010) determining the cell-matrix interactions (Yeong *et al* 2004). To compare the different scaffolds using the same conditions, a similar area of the scaffold was selected to be analysed. According to IFM results, all scaffolds exhibited micro-roughness (Hsin-I Chang, 2011) showing values between 284 nm and 1.65 μm, which are consistent with literature data regarding the typical value that promotes cell adhesion (Hsin-I Chang, 2011). Further studies to evaluate the cell adhesion and proliferation in the scaffolds surface are currently in development.

4. Conclusions

New biobased unsaturated polyesters were successfully synthesized via bulk polycondensation. The success of the polymerization was confirmed through ^{1}H NMR, ATR-FTIR and MALDI-tof analyses. An extensive study of the structure properties of the obtained polymers was performed. According to thermal and mechanical results, the UPs showed a good

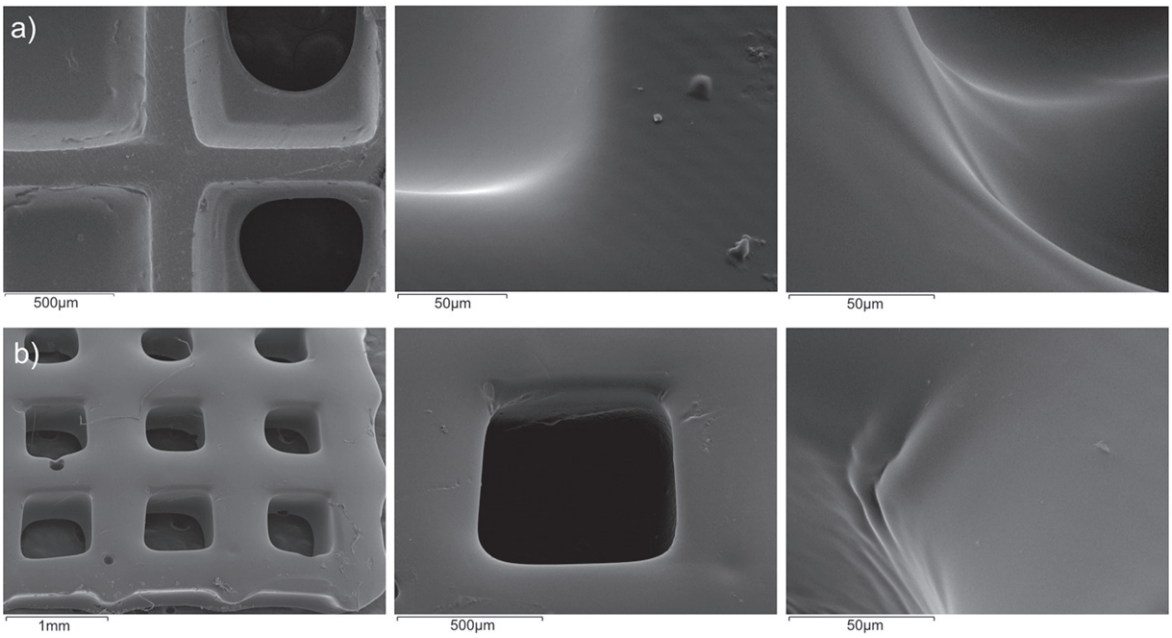


Figure 10. SEM pictures of the scaffolds obtained by μSTLG a) Scf5 based on UP5/Styrene, with different curing times and b) Scf4, based on UP4/HEMA formulation,.

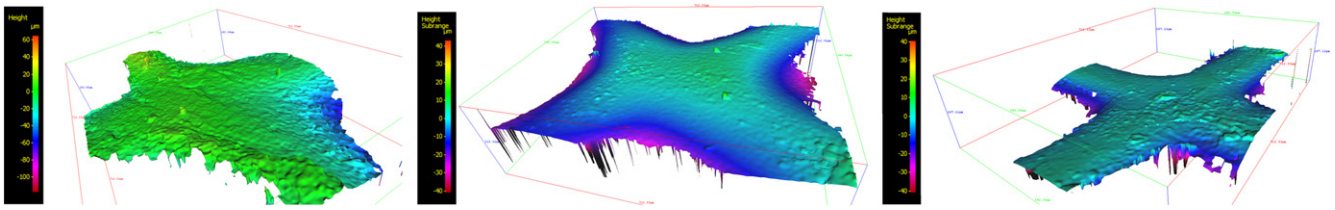


Figure 11. IFM images of 3D scaffolds: (a) Scf4/HEMA (60 s, 1 layer), (b) Scf5/HEMA (28.5 s, 2 layers) and (c) Scf6/HEMA (28.5 s, 1 layer).

thermal stability, with UP1, UP5 and UP6 being the most stable. Their thermal stability was quite similar, with onset temperature values ranging from 328.49 to 355.70 °C. The thermal properties of the UPs were analysed by DSC, MDSC and DMTA techniques, allowing the determination of the glass transition temperature. The temperature of the biobased UPs was relatively low (−21 to −35 °C). A slight decrease of T_g was found with the increase of the number of carbons in the main chain, which is explained by the enhancement of the chain mobility. The results confirm that the replacement of isophthalic acid with succinic, adipic or sebacic acid in the formulation did not reduce the thermal stability of the UPs. Also, the rheological behaviour of the biobased materials plays an important role. The biobased polyesters showed proper viscosities to be applied in the μSTLG equipment. The cell viability data suggested that synthesized UPR are good candidates for the development of new biocompatible and biobased polyesters structures. The extensive characterization reveals that the UPs properties match the μSTLG requirements. This data was confirmed by SEM and IFM results that showed very promising 3D structures, with very accurate geometries and that presented some nano-roughness, which is a very important parameter to promote cell adhesion and proliferation. The successful fabrication of the scaffolds confirms that the system can be used to fabricate 3D structures with complex micro-architectures.

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