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Towards a new generation of non-cytotoxic shape memory thermoplastic polyurethanes for biomedical applications

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ABSTRACT

In recent decades, the technology of polymeric materials used in biomedical applications has been greatly improved, replacing the metals that had been used until now. This change has not only meant an improvement in the cost of the raw material and in its processing, but it is also due to the fact that there are applications, such as stents, where the material is required to have a certain flexibility, both during the surgical intervention and during the healing or conditioning the tissue in which the intervention is performed. In this type of application, the so-called shape memory polymers (SMPs) are very interesting, but for this, they must meet the condition of being biocompatible. In this work, new polyurethane materials have been designed in which, in addition to shape memory prevailing, adequate cell proliferation values are obtained for possible use in biomedical applications. Furthermore, during the synthesis, in order to avoid undesired and toxics subproducts, instead of the typical aromatic diisocyanates, an aliphatic 1,6-hexamethylene diisocyanate (HDI) has been selected. Moreover, neither solvents nor catalysts were used, which makes eco-friendly synthesis suitable for scaling at an industrial level. Finally, castor oil (CO) has been used as one of the main synthesis reagents, which is an abundant compound obtained from biological sources. For all this, it can be concluded that the polymers described here have a wide range of application possibilities (biomedicine, food packaging…), and are highly interesting to preserve our Planet.

1. Introduction

The development of new materials with biomedical applications is a key research sector for the improvement of life and quality of it. Thus, during the last decades the development of materials in this sector has increased significantly [1,2]. Likewise, it is important to recognize the biocompatibility and non-toxic materials. Biocompatibility is understood as a possible coexistence between a compound (biomaterial) and a biological environment, where their interaction is not harmful to the living organism. Toxicity is the ability of a chemical to produce harmful effects on a living being, upon contact with it. Many polymers have been used for biomedical purposes where these characteristics are indispensable to make them applicable. Nowadays, polyurethanes (PUs) are materials widely used in the biomedical field for the preparation of catheters, blood oxygenators, heart valves, internal lining of artificial hearts, membranes for dressings and drug delivery systems or for tissue engineering among others [3–7].

The application of PUs in the biomedical field began in the 1950 s with the work of Pangman [8]. This author studied the application of a polyester-urethane foam as a covering for a breast prosthesis. However, this material rapidly degraded in vivo due to its susceptibility to hydrolysis. Despite its failure, many researchers continued along this line and it was in 1958 when PU materials were first introduced in biomedical applications, with the development of a rigid PU foam

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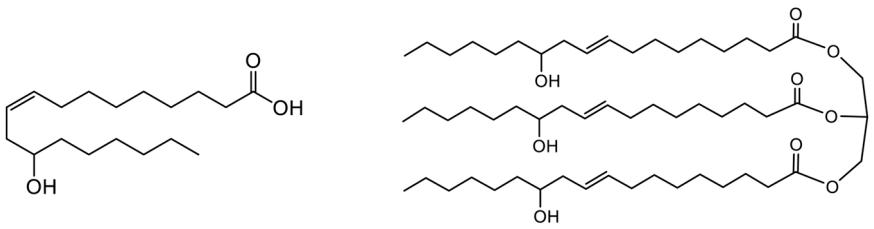


Fig. 1. Structures of ricinoleic acid (left) and castor oil (CO) triglyceride (right).

(Ostamer™) for bone fixation [9–11]. Starting from this moment, the search for biocompatible PUs has not stopped, and numerous studies have been carried out [12–16].

It should be noted at this point that it is important to find a correlation between the mechanical and biological properties of the PU so that, in addition to presenting the biocompatibility required by the device for medical use, it maintains the mechanical requirements necessary for its application. Thus, in the formulation of biocompatible PUs, it is necessary to avoid the use of aromatic reagents because during degradation processes can decompose generating highly toxic by-products [17,18]. Thus, for example, it has been found that some of the most used aromatic diisocyanates in the synthesis of PUs (2, 4-toluene diisocyanate (TDI), 4,4′-methylene bis(phenylisocyanate) (MDI), etc.) give off carcinogenic and mutagenic diamines during their degradation processes [17,18]. It will be preferred to work, therefore, with aliphatic diisocyanates instead of aromatics. In addition, aliphatic diisocyanates have greater chemical compatibility with the macrodiol chains, and the mixture of phases between them will be favored. So, in these cases, the role that the chain extender will take is more decisive in the phase separation process. In the case of chain extenders something very similar happens, aliphatic and short chain extenders are the most appropriate for biomedical uses, where the biocompatibility of PU is essential [19]. In this task, the use of vegetable oils as chain extenders has been of great help. These oils stem from bio-renewable sources, so they are very important raw materials for biopolymers production. Among their characteristics low toxicity, good biodegradability, and high purity are included [20].

Vegetable oils can be obtained from a multitude of seeds and fruits such as olives, soybeans, sunflower, palm, coconut, corn, castor, etc. In this work, castor oil (CO) has been selected as raw material for chain extender, which is obtained from the *Ricinus communis* plant and contains 40–50% of its weight in oil. Unlike other oil-producing plants, the castor plant produces triglycerides containing less than 90% of a simple fatty acid, ricinoleic acid (Fig. 1) [21–23]. This high percentage of ricinoleic acid in triglyceride makes the presence of hydroxyl groups lend it grade 3 functionality.

CO is a product used in the production of plastics, paints, lubricants, etc. It has been used for decades in the formulation of PUs as chain extender, but the nature of its structure also allows it to act as a polyol in the manufacture of PUs. PUs obtained from CO are typically flexible, due to the long chains of fatty acids in their structure. The thermostable structure of these elastomers is due to the hydroxylated nature of the vegetable oil [20]. Furthermore, the use of castor oil with hydroxylated groups means that it is not necessary to carry out the prior hydroxylation process that other vegetable oils usually require.

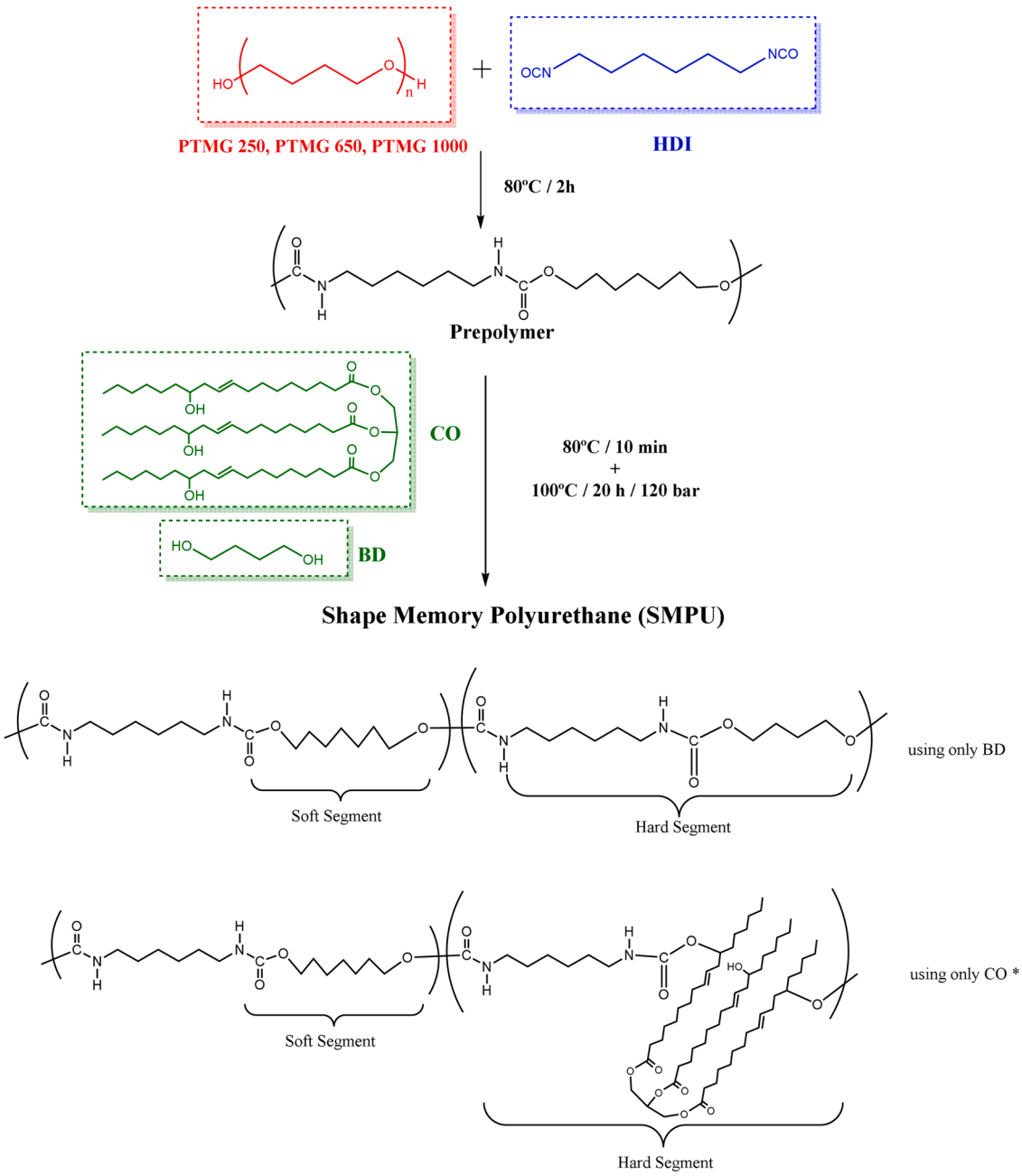
Motivated by our previous works [24–27], the present study is a first approach to achieve a new generation of shape memory thermoplastic polyurethanes (SMTPUs) more biocompatible than those previously synthesized using two long-chain polytetramethylene glycols (PTMG650 and PTMG1000) as polyols and aromatic isocyanates such as 2,4-toluene diisocyanate (TDI), 4,4′-methylene bis(phenylisocyanate) (MDI) or the TDI/MDI mixture (Scheme 1). For this purpose, a lower mass PTMG has been used (PTMG250) and the mentioned aromatic diisocyanates have been replaced for an aliphatic one, 1,6-hexamethylene diisocyanate (HDI). It must be borne in mind that although several researchers [28–32] have reported that polyurethanes based on aromatic diisocyanates are more biostable than their aliphatic counterparts, the degradation products of these aromatic diisocyanates include toxic and carcinogenic compounds such as aromatic diamines, thereby making them undesirable for use in biomedicine. Moreover, also the chain extender 1,4-butanediol (BD) has been replaced, in some cases, for one obtained from bio-renewable resources, as it is the castor oil (CO). However, thermal, mechanical and shape memory properties must be at least so good than the previously reported SMPUs [33,34]. To achieve this objective, the three components (polyol, diisocyanate and chain extender) were mixed with different molar proportions (polyol/diisocyanate/chain extender molar proportion of 1: N + 1: N with different N, but the same NCO/OH ratio). In this way, it is possible to tailor the PU properties by controlling the soft (SS) and hard segments (HS) ratio.

Previous works described and synthesized PUs derived from bio-renewable sources with good mechanical properties and shape memory behavior [35,36]. Nevertheless, to our knowledge, no works have been described these type of polymers with non-cytotoxic or biocompatible property. For this reason, the aim of this work is to achieve a new generation of biocompatible SMPUs from polytetramethylene glycol (PTMG), 1,6-hexamethylene diisocyanate (HDI) and 1,4 butanediol (BD) and/or castor oil (CO). To this end, the prepolymer method has been used to control the final architecture of the PU and produce linear PU chains [37]. Catalyst has not been used in the synthesis of these PUs to avoid problems of final biocompatibility of the material, and no solvent system has used during the synthesis. This new generation of biocompatible polyurethanes with shape memory capabilities has potential to be used in food packaging and biomedicine. Moreover, all the mentioned reagents and processes make some of these polymers ideal to perform the synthesis at industrial scale.

2. Materials and methods

2.1. Reagents

All polyurethanes synthesized in this work have been obtained combining the three components (polyol, diisocyanate and chain extender) in different molar stoichiometric proportions polyol/diisocyanate/chain extender = 1 / N + 1 / N, where N = 1–4 in 1 intervals (NCO/OH ratio equal to 1 in all cases). As polyol three polytetramethylene glycols with molecular weights of 250 (PTMG250), 650 (PTMG650) or 1000 g·mol^{-1} (PTMG1000) were used. 1,6-hexamethylene diisocyanate (HDI), an aliphatic diisocyanate, was used due to its lower toxicity with respect to aromatic ones. Finally, two chain extenders were used: 1,4-butanediol (BD) and castor oil (CO). All reagents, purchased from Sigma Aldrich, were used as received except the chain extenders (BD and CO), which were dried under vacuum 24 h at 80 °C due to their strong hydrophilicity. All the samples have HDI, so they are named based on the PTMG, chain extender system and N number (Table 1S). For example, PTMG650-BD-4 is synthesized using PTMG650, HDI and BD and the N number is equal to 4.



* this is one of the possible SMPU synthetized using only CO as chain extender

** when a mixture of CO and BD are used as chain extender, the number of possible synthesized SMPUs increases, being able to produce cross-linking reactions between the different structures

Scheme 1. Shape memory thermoplastic polyurethanes (SMTPUs) synthesis from polytetramethylene glycol (PTMG), 1,6-hexamethylene diisocyanate (HDI) and 1,4 butanediol (BD) and/or castor oil (CO) using the pre-polymer method.

2.2. Synthesis

The synthesis took place as previously reported [27]. The three components react using the polyol/diisocyanate/chain extender molar proportion of 1: N + 1: N (N = 1–4), with equal molar ratio of isocyanate groups to hydroxyl groups (NCO/OH = 1). In a 250 mL five-neck round bottom flask equipped with a mechanical stirrer and a nitrogen inlet was added the polyol (PTMG250, PTMG650 or PTMG1000) and stirred (250 rpm) for 30 min at 80 °C. The inert atmosphere of nitrogen during the reaction was used to avoid humidity that could have a plasticizing effect on the final polyurethane that would modify its properties [38]. Then, the diisocyanate (HDI) was added dropwise. The reaction continued at 80 °C for 2 h obtaining an intermediate polymer called pre-polymer. Finally, the chain extender (BD, CO, or a mixture of both, where the OH diol/ OH triol ratio used was 1/1) was added dropwise to the reaction medium to react for 8–10 min until a significant increase in viscosity was detected and allowing the polyurethane chains to grow (Scheme 1). For instance, for the PTMG250-CO-1 sample, 100 g of polyurethane were synthesized from 20.7 g of PTMG250, 27.8 g of HDI and 51.5 g of CO. At that moment, the obtained low molecular

weight polyurethane was poured into a preheated at 100 °C stainless steel mold (dimensions: 50 mm × 50 mm x 1.5 mm). This mold was immediately placed into a hydraulic press (100 °C, 120 bar) for about 18–20 h. Two Teflon sheets were placed on both sides of the metallic mold to reduce the surface roughness of the PU sheets obtained. The final linear PUs were obtained cooling at room temperature under constant pressure. After that time, it was considered that the reaction has been completed. Nevertheless, in order to be sure that no isocyanate groups are free in the medium, FTIR analysis was performed, confirming the complete polyurethane polymerization. FTIR was used to follow and control the degree of the reaction. Fig. 1S shows an example of FTIR spectra corresponding to the PTMG650-HDI-BD N3 sample. These polyurethane specimens were used for all the characterization described below.

3. Characterization

The synthesized PUs were characterized by different techniques in order to know the thermal and mechanical properties, as well as, evaluate the shape memory capacity. So, thermogravimetric analysis (TGA) was used to check the thermal stability (the initial degradation temperature, T_i); differential scanning calorimetry (DSC) to calculate the fusion and transition temperatures (the glass transition temperature measured in the second heating scan, T_g); transmission electron microscopy (TEM) to determine the cristallinity; and dynamic-mechanical thermal analysis (DMTA) to estimate the mechanical properties of the synthesized PUs. Moreover, to evaluate the shape memory behavior thermo-mechanical analysis (TMA) was used. Finally, cytotoxicity assays were performed to the most interesting samples in order to check the biocompatibility using Crystal Violet assay for determining adhesion of cultured cells.

3.1. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

Infrared spectra were collected on a Nicolet Nexus FTIR spectrophotometer (Thermo Electron Corporation). The FTIR spectra were obtained using an ATR accessory with Zinc Selenide crystal (ZnSe) in the range 400–4000 cm^{-1} at a resolution of 4 cm^{-1} and 64 scans per spectrum. This technique was used to assess the extent of reaction between the isocyanate and hydroxyl groups, identifying the functional groups present in the final SMPU to evaluate the degree of reaction between the isocyanate group (-N═C═O) and the hydroxyl groups.

3.2. Thermogravimetric analysis (TGA)

A Mettler Toledo TGA/SDTA851e thermobalance was employed to evaluate the thermal stability of the synthesized PUs. TGA test takes place, under a nitrogen atmosphere, heating the sample (10–15 mg) from 25° to 800°C at a heating rate of 10 °C·min^{-1}. The initial temperature of degradation (T_i) was estimated when the loss of mass was 2 wt%, whereas the maximum temperature of degradation (T_d) of each degradation step was calculated from the minimum in the first derivative curves. TGA experiments were done in duplicate.

3.3. Differential scanning calorimetry (DSC)

A Mettler Toledo model DSC 822e calorimeter was used to determined the thermal properties of each PU sample. Samples around 10 mg, sealed in aluminum pans, were subjected to a heating/cooling/heating cycle from − 100–250 °C at a scanning rate of 10 °C·min^{-1} under constant nitrogen flow (20 mL·min^{-1}). In the first heating scan, the thermal history of the polyurethane was erased. Thus, the glass transition temperatures ($T_{g\text{-onset}}$, $T_{g\text{-endset}}$) were obtained from the second heating scan. These tests were also done in duplicate.

3.4. Transmission electron microscopy (TEM)

TEM work was done on a TECNAI G2 20 TWIN operated at 120 kV and equipped with LaB6 filament. The ultrathin films were obtained at − 40 °C using a cryoultramicrotome device (Leica EMFC6) equipped with a diamond knife. The ultrathin sections of about 80 nm of thickness were placed on 300 mesh copper grids.

3.5. Dynamic-mechanical thermal analysis (DMTA)

DMA-1 Mettler Toledo equipment was used to estimate the mechanical properties of the PU samples. DMTA tests were perfomed twice in tensile mode. For that, it is necessary PU specimens with dimensions 1.5 mm thick, 5 mm wide and 10 mm long. These samples were measured from − 100–150 °C at a heating rate of 3 °C·min^{-1}. The deformation frequencies (1, 3 and 10 Hz) and the displazament (20 μm) used are within the linear viscoelastic region (LVR) of the synthesized PUs. Therefore, the storage module E' (which is a measure of the elastic response), the loss modulus E'' (which measures the viscous response), and the loss factor (tan δ = E''/E') can be evaluated. Moreover, the glass transition temperature ($T_{g\text{-peak}}$) can also be determined at the maximum in tanδ.

3.6. Thermo-mechanical analysis (TMA)

DMA-1 Mettler Toledo equipment in tensile mode was also employed to evaluate the shape memory capacity of the synthesized PUs. In the thermo-mechanical analysis, the variation in the sample dimensions can be measured as a function of the temperature while it is subjected to a force (which can be zero). As it has been reported previously [25,26], the temperature range chosen to perform the TMA experiment must be below and above the temperature that active the shape memory effect, which for the polyurethanes synthesized in this work is the glass transition temperature (T_g), specifically that measured by DMTA, $T_{g\text{-peak}}$.

Briefly, a rectangular sample (10 mm × 5 mm x 1.5 mm) was heated above $T_{g\text{-peak}}$ of the PU tested, and then was elongated by the action of a 2 N force. So, after 5 min, the maximum strain can be measured (ε_m). After, keeping the force constant, the sample was cooled quickly below its $T_{g\text{-peak}}$, in order to fix the temporary shape (ε_u) once this force was removed. In the recovery step, the PU was reheated above its $T_{g\text{-peak}}$ to recover its permanent shape (ε_p). Therefore, the deformation (R_d), fixation (R_f) and recovery ratios (R_r) were calculated. There are different equations to calculate these parameters [39]. In this work Eqs. 1–3 are used, where L is the initial dimensions of the sample (L = 10 mm).

$$R_d(\%) = \frac{\varepsilon_m}{L}\cdot 100 \quad (1)$$

$$R_f(\%) = \frac{\varepsilon_u}{\varepsilon_m}\cdot 100 \quad (2)$$

$$R_r(\%) = \frac{\varepsilon_u - \varepsilon_p}{\varepsilon_m}\cdot 100 \quad (3)$$

3.7. Crystal violet assay for determining adhesion of cultured cells

HEK293 cells (20 ×10^3 or 40 ×10^3) in DMEM supplemented with 10% FBS (v/v), 100 μg/mL streptomycin, 100 U/mL penicillin, L-glutamine were seeded by adding a 50 μL drop in the different PUs (2 mm diameter) and allowed to attach during 1 h at 37 °C and at 5% CO_2. Then, PUs were transferred to a 96-well culture plate and cells were maintained for 72 h in complete DMEM 150 μL/well. The cells were washed with phosphate-buffered saline (PBS) and fixed with 4.5% paraformaldehyde in PBS for 30 min at room temperature. After fixation, cells were washed with PBS again and stained with 0.5% solution of crystal violet for 20 min at room temperature. Cells were then washed thoroughly in water. After washing, the PUs were allowed to air-drying,

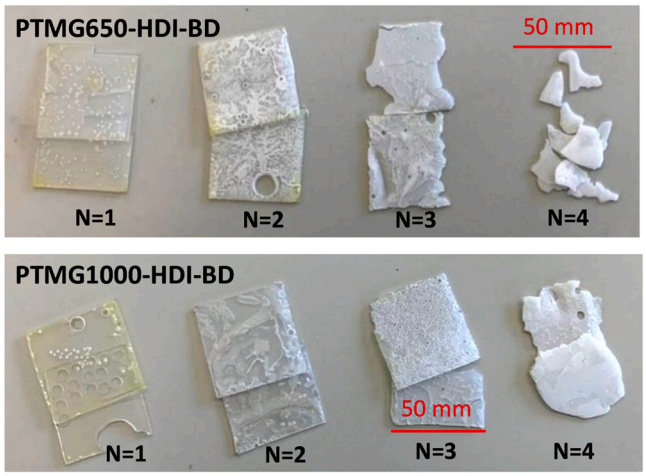


Fig. 2. Samples resulting from the use of BD.

then transferred again into a 96-well culture plate and 200 μL of acetic acid (15%) was added to each well. PUs were incubated for 20 min at room temperature on a bench rocker with a frequency of 20 oscillations per minute. Finally, acetic acid was transferred to a new 96-well culture plate and optical density (OD) of each well was measured at 570 nm (OD570) with a plate reader. The percentage of viable cells (attached) is calculated by comparing the average OD570 values of cells seeded directly in the 96-well culture plate with the OD570 values of the cells seeded in the PUs. Image acquisition was performed before acetic acid addition in an OLYMPUS CX43 microscope using a x10 objective.

3.8. Contact angle measurement

In order to corroborate that the added droplet during Crystal Violet assay was the same in all the PU surfaces in all the samples, static contact angle was measured in the OCA 15EC goniometer (Dataphysics OCA 15 EC Neurtek Instruments). Serum was used as a testing liquid and sessile drop method (2 μL per drop) was carried out at room temperature to do the measurements. Reported data are the average of 6 measurements. In order to measure the angle between the surface and the drop OCA 15EC camera was set at 0.7x zoom and images recorded the drop right after is placed in the surface. The contact angles were analyzed and determined with SCA20-U software.

4. Results and discussion

As mentioned previously, three polyols with different molecular weights (PTMG 250, 650 and 1000 g mol^{-1}), diisocyanate (HDI), and three different chain extenders (BD, CO and a mixture of both) have been used in this work. The study started using BD as a chain extender (PTMG650 and PTMG1000). In view of the first results, it was decided to replace BD for a mixture of BD and CO (maintaining NCO/OH = 1 relationship) and, finally, evaluate the results obtained when only CO was used (in this case, and in view of the previously obtained results with the other chain extenders, PTMG250 it was also used).

4.1. BD as chain extender

Work started using PTMG650 and PTMG1000 as polyols and BD as chain extender. These samples resulting from the use of BD did not turn out to be as expected (Fig. 2). As the N number is increased, that is, as the hard phase of the PU increases, a separation of zones occurs in the specimen that makes the sample inhomogeneous. In addition to this fact, at higher N, the PUs are more fragile and in some cases were not suitable to be subsequently characterized by some selected techniques (DMTA, TMA) since they do not support the mechanical stress exerted by the measuring instrument. Therefore, for these PUs, only the minor N samples (N = 1 and 2) could be analyzed by DMTA and TMA. Moreover, even having good flexibility properties, sometimes their mechanical properties are not as expected. Anyway, all the synthesized polyurethanes using BD as chain extender were characterized by DSC and TGA. DMA and TMA techniques were only used, as mentioned above, for samples with N = 1 and 2.

Table 1
T_g peak (°C) obtained from DMTA data in different samples.

N	PTMG-250	PTMG-650			PTMG-1000		
	CO	BD	BD+CO	CO	BD	BD+CO	CO
1	-26.2	-46.5	-39.6	-41.2	-59.8	-49.9	-49.6
2	-19.9	–	-35.4	-33.0	-59.0	-43.9	-41.9
3	-19.7	–	-35.0	-31.5	–	-37.6	-36.0
4	-16.4	–	-27.5	-28.6	–	-36.1	-34.7

Table 2
Shape memory data.

Sample Code	R_d (%)	R_f (%)	R_r (%)
PTMG650-BD-1	0.4	-371.3	-27.3
PTMG650-BD-2	–	–	–
PTMG650-BD-3	–	–	–
PTMG650-BD-4	–	–	–
PTMG1000-BD-1	9.7	99.6	18.5
PTMG1000-BD-2	3.0	50.0	48.7
PTMG1000-BD-3	–	–	–
PTMG1000-BD-4	–	–	–
PTMG650-BD/CO-1	5.8	87.6	87.5
PTMG650-BD/CO-2	5.5	71.7	85.7
PTMG650-BD/CO-3	4.4	39.3	88.4
PTMG650-BD/CO-4	3.3	94.5	93.1
PTMG1000-BD/CO-1	3.2	37.0	90.7
PTMG1000-BD/CO-2	3.5	52.3	88.5
PTMG1000-BD/CO-3	2.4	26.2	95.9
PTMG1000-BD/CO-4	2.8	46.1	82.3
PTMG250-CO-1	7.1	84.4	95.7
PTMG250-CO-2	7.7	81.7	93.7
PTMG250-CO-3	7.0	82.5	88.0
PTMG250-CO-4	7.0	88.0	90.5
PTMG650-CO-1	2.9	38.6	98.6
PTMG650-CO-2	7.7	85.6	100.0
PTMG650-CO-3	8.4	92.5	98.8
PTMG650-CO-4	8.7	94.1	99.9
PTMG1000-CO-1	8.2	99.7	90.5
PTMG1000-CO-2	6.6	81.4	89.0
PTMG1000-CO-3	7.8	96.8	90.2
PTMG1000-CO-4	7.8	97.9	87.6

In TGA analysis, it has been seen that all these PU have good thermal stability, with T_i temperatures above 250 °C. Moreover, the TGA curves (not shown) show a thermal degradation in two stages. The first stage of degradation occurs between 350 and 380 °C and corresponds to the hard phase of PU. Next, the second degradation stage occurs between 422 and 430 °C for PUs derived from PTMG650 and between 406 and 412 °C for those derived from PTMG1000 (Table 2S), and corresponds to the soft phase degradation (Table 2S).

From DSC measurements, it is possible to determine the thermal transitions of the samples, such as the glass transition (T_g) or the melting temperature (T_m). Table 3S shows these transition temperature values ($T_{g\text{-onset}}$, $T_{g\text{-endset}}$ and T_m), as well as the melting enthalpy (ΔH_m) for those semicrystalline samples that present a melting point. In the case of the PUs with BD as chain extender, this occurs. The fusion of HS occurs as a consequence of the breakdown of the hydrogen bonds formed between the urethane groups. Because of this, these samples can contain a slight degree of crystallinity, which in general increase as N increases. Moreover, two glass transitions were detected by DSC for these PUs, corroborating, as had already been observed with the naked eye, that there is a separation of zones and the sample is not homogeneous. It is

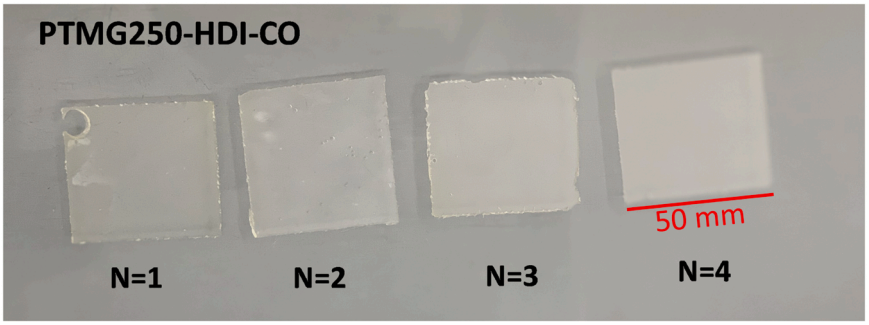


Fig. 3. Samples resulting from the use of PTMG250, HDI and CO N = 1 (left), N = 2–3 (middle), and N = 4 (right).

also observed how, as the molecular weight of the PTMG increases, the separation of the microdomains rises and the glass transition temperature decrease. This happens because the longer polyol chains increase the flexibility of the material. Another noteworthy aspect of the results obtained in the PTMG1000-BD samples is that as the hard phase content is increased (higher N) an increase in T_m is observed, this may be related to the increase in the polarity of the hydrogen bonds that are created between groups in the hard region of PU.

DMTA study allows us to verify the results obtained previously by DSC. All dynamic thermo-mechanical measurements were carried out at different frequency values (1, 3, 10 Hz) in order to distinguish between the different transitions, but the 3 Hz frequency was chosen to represent the results obtained in all cases. As said before, these PUs samples synthesized with BD are too fragile for this technique, especially for high N. Therefore, measurements were only made at low N ($T_{g\text{-peak}}$ values shown in Table 1).

From the thermo-mechanical cycles measured by TMA, it has been possible to collect the strain values necessary to calculate the quantitative parameters to evaluate the shape memory behavior (Eqs. 1–3): the deformation (R_d), fixation (R_f) and recovery ratios (R_r) (Table 2). The tests were carried out, like the DMAs, only with the samples with low N values (PTMG650-BD N = 1, PTMG1000-BD N = 1 and PTMG1000-BD N = 2). As can be seen in Table 2, the values obtained for these three samples are not good and in some cases (PTMG650-BD N = 1). The two samples with PTMG1000 show poor recovery values ($R_r < 50\%$ in both cases). Worse are the measured values for the sample PTMG650-BD N = 1. For this sample, the deformation ratio is already abnormally small. Moreover, when trying to fix the temporal shape of the sample, a negative value (−371.3%) is observed because the equipment is not capable of counteracting with the applied force (2 N) the thermal contraction exempted by the sample. An attempt was made to avoid the problem by increasing the applied force to 10 N (the maximum of the equipment), but the problem persisted.

In conclusion, these polyurethanes synthesized with BD as a chain extender, although they present good thermal stability, have certain disadvantages. First, they are semicrystalline, with relatively low melting temperatures (slightly above 100 °C). Second, they exhibit two glass transitions at very low temperatures, which is a problem considering that the shape memory transition temperature is T_g. Finally, both its mechanical properties and shape memory capacity are too poor for a possible future application in any sector.

For this reason, it was decided to continue the study by mixing 1,4-butanediol (BD) and castor oil (CO) as chain extender in future polyurethane synthesis, also using HDI as diisocyanate and PTMG650 and PTMG1000 as polyols.

4.2. BD-CO mixture as chain extender

Polyurethanes synthesized using a mixture of BD and CO as chain extender present better appearance than those synthesized only with BD, at least with the naked eye they are more homogeneous. TGA analysis shows similar results to the PUs synthesized with BD. Thermal stability is slightly higher, with degradation initiation temperatures (T_i) close to 300 °C (Table 2S). They also present a thermal degradation in two stages: the first one, related to the hard segment, at approximately 340 °C and 360 °C for PTMG650 and PTMG1000 polyurethanes respectively; and the second one, related to the soft segment, between 420 and 425 °C in both PUs.

For the mixture of BD and CO samples, DSC measurements does not show, despite the presence of BD as chain extender, the existence of a peak indicative of crystallinity. This fact seems indicate that these samples are amorphous. However, due to the use of BD as chain extender they could present a small cristallinity not detected by DSC. Anyway, as well as the PU samples synthesized with BD, two glass transitions are observed for these samples (Table 3S).

DMTA measurements (Table 1 show the $T_{g\text{-peak}}$ values determined at 3 Hz frequency) could be carried out without problems in this case. Slightly higher T_g-peak values are observed than for BD samples. Moreover, as expected, these values increase both as the N value increases (T_g-N4 > T_g-N3 > T_g-N2 > T_g-N1) and as the molecular weight of the polyol decreases (T_g-PTMG650 > T_g-PTMG1000).

Table 2 show the shape memory parameters (R_d, R_f and R_r) measured by TMA for the polyurethanes synthesized using a BD-CO mixture as chain extender. As can be seen in Table 2, the strain values obtained for these samples are between 3% and 6% depending on the polyol and/or the N value. It is also observed that, in general, the PTMG650 samples show slightly R_f higher values, in some cases above 80%. The R_r values are similar for both polyols, between 80% and 95%. However, no clear trend is observed in any of these parameters (R_d, R_f and R_r), neither with the value of N nor with the molecular weight of the polyol. This fact could be due to several factors: the presence of two glass transitions in the polyurethane (it must be taken into account that the T_g is the transition temperature of the shape memory effect), to the presence of a small residual crystallinity due to the use of BD as a chain extender, or to inhomogeneities of the sample not appreciable to the naked eye.

In conclusion, these PUs synthesized with a mixture of BD and CO as a chain extender have improved substantially each of the measured properties: better appearance and homogeneity, better thermal stability, higher glass transition temperatures, and better shape memory capacity. Nevertheless, they still have drawbacks: still have two T_g at relatively low temperatures, and their shape memory properties do not follow a logical trend when varying the polyol or N.

For this reason, it was decided to continue the study using only castor oil (CO) as chain extender. In this case, it was also decided to introduce a new molecular weight of the polyol in order to increase as far as possible the glass transition temperature of the final polyurethane. Therefore, PUs were also synthesized with PTMG250. That is to say, PUs with HDI as diisocyanate, castor oil as chain extender and three different polyols (PTMG250, PTMG650, PTMG1000) were synthesized.

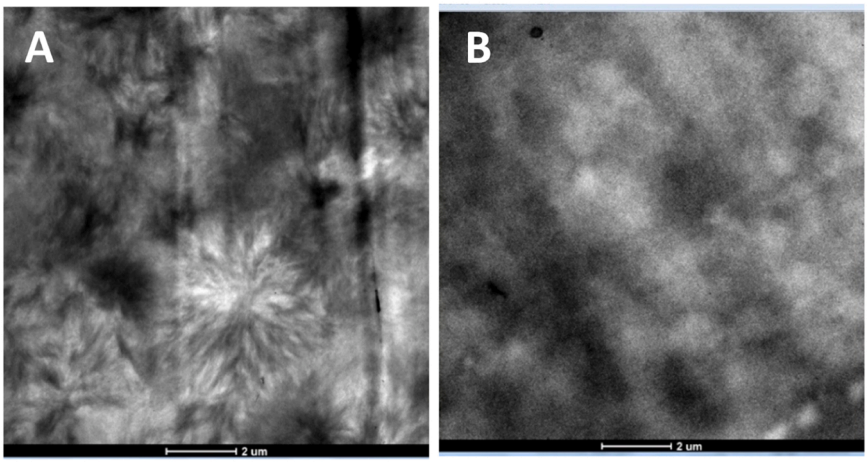


Fig. 4. TEM images. (A) PTMG 1000-HDI-BD N = 4 and (B) PTMG 1000-HDI-CO N = 4.

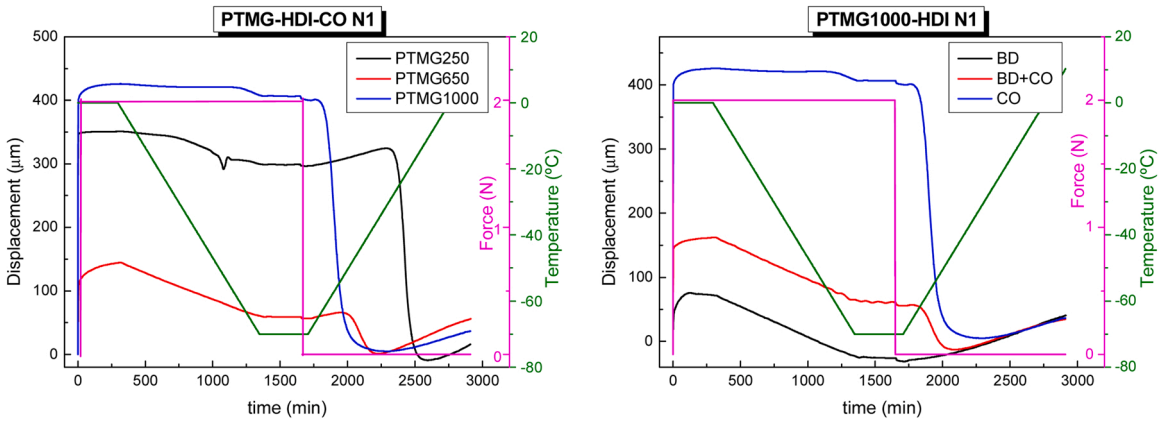


Fig. 5. Shape memory behavior of PTMGX-HDI-CO N = 1 samples in function of the polyol used (left), and PTMG1000-HDI-X N = 1 samples in function of the chain extender used (right).

4.3. CO as chain extender

Polyurethanes synthesized using CO as chain extender present the best appearance (Fig. 3). For these samples TGA analysis show high thermal stability, with T_i temperatures also close to 300 °C (Table 2S). Thermal degradation, as for the other PUs, occurs in two stages: at 350–360 °C the first one (hard segment), and at 420–450 °C the second one (soft segment). DSC measurements show that these samples are amorphous and present only a glass transition temperature (T_g), higher that the other PUs when PTMG250 polyol were used ($T_{g\text{-onset}}$ around −35 °C as can be seen on Table 3S).

In view of these results, it was decided to observe these samples microscopically to corroborate their crystalline structure using the Transmission Electron Microscopy (TEM) technique. The obtained images are shown in Fig. 4. Due to the change of chain extender it can be observed that BD containing PUs have some crystallinity, but the CO containing PUs are totally amorphous.

Table 1 shows the $T_{g\text{-peak}}$ values determined by DMTA at 3 Hz frequency. When PTMG250 were used, the measured values range from − 16 to − 26 °C depending on N, higher T_g as N is higher due to the greater presence of hard segment in the polyurethane. Furthermore, these values are considerably higher than those obtained with the polyurethanes synthesized with BD or BD+CO (T_g = −30/−60 °C), and then those synthesized with CO and PTMG650 (T_g = −30/−40 °C) or PTMG1000 (T_g = −35/−50 °C) as polyol.

Table 2 show the shape memory parameters (R_d, R_f and R_r) measured by TMA for the polyurethanes synthesized using CO as chain extender. Data obtained from the TMA measurements show, for most of the samples, strain values between 7% and 8%, fixation ratios above 80% and recovery ratios above 90%. Looking at the samples with PTMG250, those with higher $T_{g\text{-peak}}$ values, strain is 7% for all samples, and the fixation and recovery ratios are above 80% and 90% respectively. That is, it has been possible to improve the shape memory capacity (see Fig. 5) and increase the glass transition temperature (Table 1) of the polyurethane at the same time by using a lower molecular weight polyol (PTMG250), without loss of thermal stability.

In conclusion, these PUs synthesized with CO as a chain extender seem suitable for application in biomedicine. It has been greatly improved the shape memory capacity, increasing also its T_g. It only remains to check its biocompatibility.

4.4. Cytotoxicity assays

Finally, after determining which samples have the best mechanical properties, and in order to determine morphology and proliferation of the HEK293 cells grown on the PUs, we seeded the cells as described in Section 3.7. and stained the cells with crystal violet. Staining of cell grown on PUs was necessary for image acquisition because unstained cells were not visible and also allows spectrophotometric quantification of cellular growth. Microscopic observation revealed that after 72 h of seeding, HEK293 cells presented normal morphology and an adherent spindle shape of epithelial phenotype. However, proliferation rates compared to control cells (grown in 96-well culture plates) were different depending on the chain extender of the PUs and the molecular

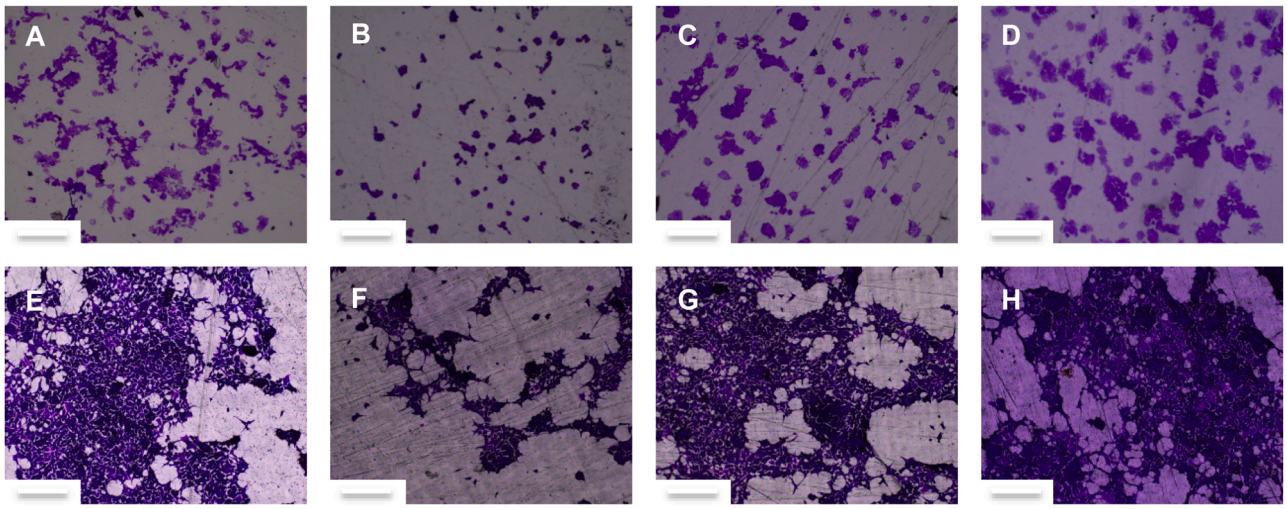


Fig. 6. Crystal violet assay in SMPU samples with same PTMG1000-HDI structure, N = 1, and different chain extender: (A) Control, (B) BD (60%), (C) BD+CO (74%), and (D) CO (90%); and samples with different polyol PTMG X–HDI-CO N = 1: (E) Control, (F) PTMG250 (74%), (G) PTMG650 (81%), and (H) PTMG1000 (94%). (A-D) 20 × 103 cell/PU and (E-H) 40 × 103 cell/PU. Scale bar = 500 μm.

Table 3
Cell viability.

Sample Code	Fig. 6	% Viability	±S. D.
Control	A and E	100	5
PTMG1000-HDI-BD N1	B	60	5
PTMG1000-HDI-BD +CO N1	C	74	6
PTMG1000-HDI + CO N1	D	90	4
PTMG250-HDI +CO N1	F	74	7
PTMG650-HDI +CO N1	G	81	3
PTMG1000-HDI +CO N1	H	94	7

mass of polyol. In order to corroborate that the added droplet morphology was the same on all the PU surfaces contact angle measurement was performed. All the samples show similar contact angles between 105° and 114° (Fig. 2S, supporting information). Moreover, in order to reduce the surface roughness of the obtained PUs, two Teflon sheets were placed on both sides of the metallic mold.

First, cytotoxicity of the chain extender was evaluated (Fig. 6, A-D). For this reason, samples with the same molecular weight of PTMG and HDI diisocyanate at the same stoichiometry and with different chain extender, were tested. As shown in Fig. 6B, when the BD was used alone, the sample was cytotoxic, attached cells did not proliferate and growth cell resulted significantly diminished compared to control cells (Table 3). When a mixture of chain-extenders was perfomed using the same proportion of BD and CO, cytotoxicity of the Pus was lower (Fig. 6C and Table 3). Finally, when only CO was used as chain-extender the sample showed a non-cytotoxic behavior.

Second, the effect of molecular weight of the PTMG was evaluated, in order to know if the PTMG affects the cytotoxicity. For this reason, the same HDI diisocyanate and the CO chain extender was used with the same N value. The results in Fig. 6 (E-H) show that higher molecular mass value of polyol has better biocompatible characteristics with cell growth similar to control cells (Table 3).

5. Conclusions

Thermoplastic polyurethanes synthesized from HDI (an aliphatic isocyanate) and BD as chain extender have been found to be unsuitable for any application where minimal mechanical properties are required. For this reason, it was decided to use a new chain extender, obtained from biological resources, such as castor oil (CO), first mixed with BD and finally alone.

Although PUs with better thermal, mechanical and shape memory properties were expected from the CO and BD mixture, the difference between these properties and those of using only CO was very small. Therefore, it can be concluded that mixing BD and CO in the same proportion during the synthesis is of no practical interest. Due to the BD molecule is much smaller and, in addition, contributes only with 2 hydroxyl groups to the reaction medium compared to the CO molecule, larger and with long chains (unsaturated triglyceride chains of castor bean) and 3 hydroxyl groups, its influence on the properties of the final polyurethane is almost negligible.

Thus, only CO was used as chain extender, also employing a new lower molecular weight polyol (PTMG250). Experimentally it has been appreciated that these polyurethanes derived from PTMG250-HDI-CO are those that show the best physical, thermal, mechanical and shape memory properties with respect to the rest of the PUs studied. Moreover, when only CO was used as chain-extender the sample show a non-cytotoxic behavior in the Crystal violet assays.

That is to say, in this work, new materials have been designed in which, in addition to shape memory prevailing, adequate cell proliferation values are obtained for possible use in biomedical applications. The combination of high molecular mass polyols, aliphatic diisocyanate (HDI) and castor oil (CO) as chain extender, without the use of solvents and catalyst, makes the obtained SMPUs good candidates to scale the synthesis at industrial level, making eco-friendly synthesis, and obtaining non-cytotoxic products. For all this, it can be concluded that the polyurethanes described here have a wide range of application possibilities (biomedicine, food packaging…). Moreover, they are highly interesting to preserve our Planet because they are synthesized in part from renewable sources.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antonio reports financial support was provided by University of the Basque Country.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mtcomm.2022.104730.

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