Valorisation of glycerol and CO_2 to produce biodegradable polymer nanoparticles with a high percentage of bio-based components

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Abstract: The biodiesel industry has grown rapidly in the last years, leading to the production of 88 000 tons of waste glycerol in 2018. Glycerol is a bio-derived molecule already exploited in pharmaceutical and cosmetic industries for its beneficial emollient and antimicrobial properties, safety and low cost. However, since its production is increasing year by year, many efforts are being done to find new ways to exploit it and to produce new valuable bio-based molecules. In particular, glycerol carbonate is a versatile molecule that can be produced from glycerol and another waste product, CO_2. Despite its potentialities in further functionalization, harsh reaction conditions are often required for the synthesis of glycerol carbonate and its exploitation is often challenging. Therefore, an industrially scalable reaction to convert glycerol into high added-value compounds is urgently needed. Here, the aim is to demonstrate a feasible conversion of glycerol to glycerol carbonate-based vinyl monomers that can be conveniently incorporated in amphiphilic block copolymers by reversible addition-fragmentation chain transfer (RAFT) polymerization. These block copolymers can be self-assembled in water to obtain nanoparticles with a bio-based content as high as 70% w/w. Since both glycerol and glycerol carbonate are approved by the Food and Drug Administration (FDA) and considered as safe, the possibility of exploiting these bio-based nanoparticles for the controlled drug release was explored.

Keywords: Glycerol; CO_2; RAFT; Polymers; Bio-based; Biodegradable; Drug Delivery

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1. Introduction

Nowadays, a huge research effort is spent in replacing the raw materials obtained from fossil fuels with renewable alternatives, such as those extracted from biomass[1]. In those cases where biomass is used as renewable source of raw materials, these are referred to as bio-derived. An important class of bio-derived materials are polymers. Usually, bio-derived polymers are classified into four main groups depending on whether they are obtained from: CO_2, Terpenes, Vegetable oils and Carbohydrates.[2]

Glycerol is a derivative of vegetable oils that has recently attracted the industry interest because it is the main co-product of the biodiesel industry.

The market of biodiesel has grown rapidly in the last years, driven by the environmental concerns about the use of depleting fuels. In 2018, the annual production of biofuel reached 152 billion litres.[3] Considering that about 10% of the weight of biodiesel is generated in glycerol[4], a huge amount of waste glycerol was produced[3],[4]. Therefore, there is an urgent need for the glycerol reuse and valorisation.

Indeed, glycerol is a natural metabolite that serves many roles in the human body and is "generally regarded as safe" by the U.S. Food and Drug Administration (FDA)[5]. For this reason and for its physical properties, it is already extensively used as an additive in pharmaceutical and cosmetic products (*e.g.* as a plasticizer[6], thickener, emollient[7], demulcent, humectant, bodying agent, lubricant[8]). Despite these well-established applications, the size of those markets in which glycerol is nowadays resorbed is not sufficiently wide to convert all of the glycerol produced every year. Therefore, intensive researches have been conducted to find new applications for crude glycerol, such as its use as a low-cost organic solvent and as a functional building block for the synthesis of bio-based materials.[9]

A possible way of exploiting glycerol that has been recently proposed implies its conversion to glycerol carbonate[10]. This is a glycerol-derived product that possesses interesting properties, such as high boiling point, high flash point, low volatility and safety, which make it suitable as a solvent or for applications as coating and in personal care products.[10]

In addition, glycerol carbonate is a versatile molecule for further functionalization, since it possesses both a hydroxyl and a carbonate group that can be exploited in the polymer industry to obtain green polyurethanes[11] or to make it react with other groups via esterification or as initiator for the ring opening polymerization (ROP) of other cyclic monomers.

Alternatively, it can be conveniently provided with a vinyl group to obtain glycerol carbonate acrylate (GCA) and make it amenable for radical polymerization, which leads to polymers with good thermal stability and ion conductivity.[12] As previously stated, GCA can also be exploited to synthetize isocyanate-free polyhydroxyurethanes through Aza-Michael addition with diamines.[13]

Glycerol carbonate acrylate is an interesting molecule also because the ring opening of the cyclic carbonate moiety in alkaline conditions leads to a 1,2-diol increasing the hydrophilicity of the corresponding polymer. This feature has been exploited to create particles able to disassemble in alkaline environment releasing CO_2.[14]

Despite these interesting properties and versatility, the exploitation of glycerol carbonate is nowadays limited by the harsh conditions required for its synthesis as well as by the necessity of employing oil-based co-reactants together with glycerol. In fact, the main path followed to obtain glycerol carbonate still consists in its reaction with urea[15] or dimethyl carbonate[16].

However, a more environmentally friendly route to glycerol carbonate consisting in the fixation of CO_2 has been recently proposed in the literature[17]. CO_2 is notoriously a readily available raw material, which is bringing about the urgent need for capture and storage to compensate the industrial emissions in the atmosphere. In this case, the main issue is the reaction condition: high temperatures (>80 °C) and high CO_2 pressures are needed, together with a catalyst and, often, a toxic solvent. This of course compromises the carbon neutrality of the whole process. For instance, Michele Aresta et al. obtained only a 7% yield of glycerol carbonate reacting glycerol and CO_2 at 180 °C and 5 MPa for 15 hours with $Bu_2Sn(OMe)_2$ as catalyst.[18] With similar demanding reaction conditions, a slight improvement in the yield (*i.e.* 35%) could be only obtained using MeOH as solvent and then at expenses of augmented danger for the process conduction.[19] Therefore, the best results are obtained so far through a longer two-step procedure.[20] A more sustainable chemistry for the production of cyclic carbonates from diols has been recently proposed by McGuire et al. [21], who reached the efficient fixation of CO_2 at atmospheric pressure and ambient temperature using 4-toluensulfonyl chloride as coupling agent.

In this work, we explored this attractive discovery and proposed a feasible pathway for the valorisation of glycerol and the fixation of CO_2 to produce polymer nanoparticles (NPs) with a high percentage of bio-derived components. In particular, we were able to obtain biodegradable NPs from the self-assembly of amphiphilic block copolymers synthesized *via* reversible addition-fragmentation chain transfer (RAFT) polymerization and mainly constituted by glycerol, as shown in **Figure 1**. In fact, glycerol methacrylate (GM) is used to produce the hydrophilic part of the polymer and to provide

steric stabilization to the NPs (see (2) and (3) in **Figure 1**). On the other hand, the realization of the hydrophobic portion is obtained exploiting the chemistry of glycerol carbonate. For this purpose, on one side the direct RAFT polymerization of GCA was explored ((7) in **Figure 1**). On the other, a biodegradable macromonomer was synthesized by using the glycerol carbonate as the initiator in the ROP of lactide ((8) in **Figure 1**) followed by acylation of the oligo(lactic acid) with acryloyl chloride ((9) in **Figure 1**, hereinafter GCPLAn-A, where n is the average number of lactic acid units).

To the best of our knowledge, this is the first report on the valorisation of glycerol and CO_2 in the production of modular amphiphilic block copolymers through RAFT polymerization.

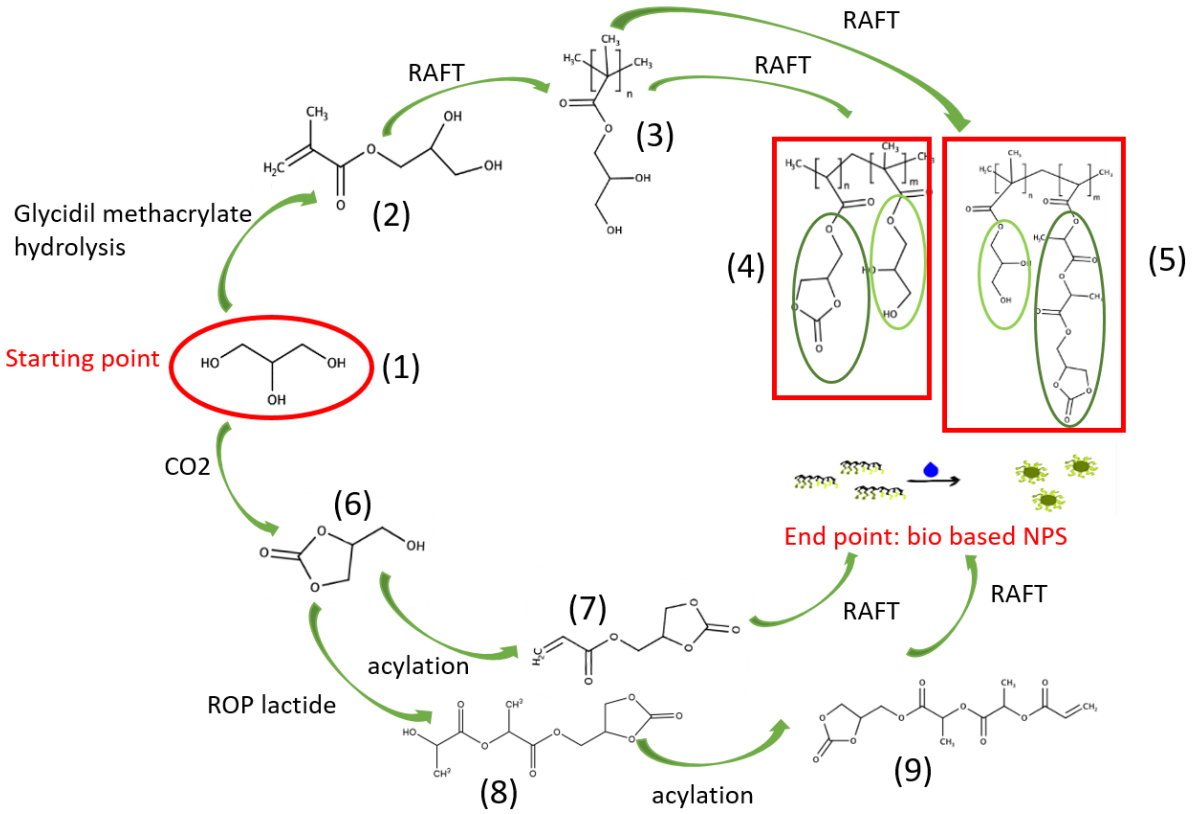

Figure 1. Synthesis of glycerol methacrylate–glycerol carbonate acrylate particles (GM-GCA) and glycerol methacrylate–glycerol carbonate-poly lactic acid-acrylate particles (GM-GCPLA-A). Starting from glycerol and following the numbers are represented respectively: (1)glycerol (2)glycerol methacrylate (3)poly(glycerol methacrylate) (4)poly(glycerol methacrylate-glycerol carbonate acrylate) (5)poly(glycerol methacrylate-glycerol carbonate-poly lactic acid acrylate) (6)glycerol carbonate (7)glycerol carbonate acrylate (8)glycerol carbonate-poly lactic acid (9)glycerol carbonate-poly lactic acid acrylate.

For both the kind of NPs, poly(GM-GCA) and poly(GM-GCPLA$_2$-A), we studied the influence of the polymer structure and composition on the size and the degradation in alkaline and acidic pH.

Since glycerol carbonate, PLA and glycerol are regarded as safe by the FDA, the use of our NPs for the controlled drug release was envisioned. To demonstrate this possibility, the NPs were loaded with pyrene, a hydrophobic drug mimic molecule, and the possibility of achieving a sustained release at different pH was studied.

2. Materials and Methods

2.1 Materials

4-(Hydroxymethyl)-1,3-dioxolan-2-one (GC, >99%, MW=118.09), Tin(II) 2-ethylhexanoate (Sn(Oct)$_2$, MW=405.12), 3,6-Dimethyl-1,4-dioxane-2,5-dione (lactide, LA, 99%, MW=144.13), acryloyl chloride (AC, 97%, MW=90.51), methacryloyl chloride (MAC, 97%, MW=104.53), 2,2′-Azobis(2-methylpropionitrile) (AIBN, 98%, MW=164.21), Glycidyl methacrylate (GlyMA, 97%, MW=142.15), 4-cyano-4-(phenylcarbonothioylthio)-pentanoic acid (CPA, 97%, MW = 279.38), 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (alpha- alpha, 97%, MW = 271.19), 4-4'azobis (cyanovaleric acid) (ACVA, 98%, MW=280.2), refined glycerine (100%, MW=92.09, Dutch glycerin refinery), *p*-Toluenesulfonyl chloride (tosyl chloride, TsCl, 98%, MW=190.65), chloroform (99,8%, MW=120.38), trimethylamine (TEA, >99%, MW=101.19), methanol (MeOH, MW=32,04), dimethyl sulfoxide (DMSO, >99%, MW=78.13), ethanol (EtOH, 99.8%, MW=46,07), acetonitrile (ACN, MW=41.05), hydrochloric acid (HCl, MW=36.48) tetrahydrofuran (THF, MW=72.11), dimethyl sulfoxide-d (DMSO-d6, MW=84.17), chloroform-d ($CDCl_3$, MW=120.38). All the solvents and the chemicals used were of analytical-grade purity and were purchased from Sigma-Aldrich if not explicitly stated.

2.2 Glycerol Carbonate

Glycerol carbonate (GC) ((6) in **Figure 1**) was synthesized according to a variation of the method described by McGuire et al.[21] More in details, 1 g of glycerol was mixed in a 50 mL round bottom flask with 2.07 g of tosyl chloride (1:1 mol/mol with respect to glycerol) and 30 mL of anhydrous acetonitrile (ACN, 10% w/w). The flask was soaked in a water/ice bath to cool down the reaction to 0 °C and the atmosphere was saturated with CO_2.

Under a continuous feed of gas, 2.8 g of TEA (2:1 mol/mol with respect to glycerol) were added dropwise over one hour. Then, the reaction was left stirring at room temperature for 18 hours. After 20 minutes, a bright white precipitate formed.

The final mixture was diluted with about 75 mL of ACN. The reaction products were analysed via nuclear magnetic resonance (NMR) on a Bruker 400 MHz spectrometer, high performance liquid chromatography (HPLC) and Fourier-transform infrared spectroscopy (FT-IR, Nicolet iS50).

The HPLC analyses were carried out by reverse phase chromatography on a Agilent C18 column (3.9x150 mm^2; particle size 3.5mm) using a Waters Associates, Milford, MA, USA, model 2487 instrument equipped with UV detector set at 230 nm. The mobile phase was a water acidified with phosphoric acid 0.1% v/v/acetonitrile mixture in gradient elution. The mobile phase flow rate was maintained at 1 mL/min. The gradient profile started with a 98% v/v of water. After 2 min, the ACN concentration was increased linearly to 100% in 25 min, maintained constant for 5 min and finally returned back to 2% v/v in 5 minutes.

Finally, glycerol carbonate was recovered pure by chromatography using a silica gel column. The elution was performed from an 80/20 v/v ACN/water mixture.

2.3 Glycerol Carbonate Acrylate

Glycerol carbonate acrylate ((7) in **Figure 1**) was obtained by reacting glycerol carbonate with acryloyl chloride. In particular, 8 g of GC were dissolved into a 50 mL round bottom flask in 20 mL of chloroform. 12.33 g of TEA (1.8 mol/mol with respect to glycerol carbonate) were then added to the mixture.

The mixture was stirred until dissolution of the reactants and cooled down in a water/ice bath to 0 °C. Then 9.8 g of acryloyl chloride (1.6 mol/mol with respect to glycerol carbonate) were added dropwise with a syringe pump (New Era Pump System, NE 300) over one hour.

The mixture was then left reacting at room temperature for one hour. Afterwards, the mixture was filtered with a filter paper to eliminate the salts formed from the neutralization of HCl by TEA and washed three times with 30 mL of HCl 0.1 M.

To confirm the correct synthesis of GCA, the product was analysed by NMR in deuterated chloroform.

2.4 Glycerol Carbonate-PLAn

PLA-glycerol carbonates ((8) in **Figure 1**) with different PLA average chain length were synthetized via ROP by modulating the ration between GC, used as initiator, and lactide. For instance, in order to produce 3 g of GC-PLA2 (where the number indicates the average number of lactic acid units in the oligoester), 21 mg of Na_2SO_4 and 1.06 g of lactide were melted in a 25 mL round bottom flask equipped with a magnetic stirrer immersed in an oil bath at 130 °C. Then, 0.87 g of GC (1:1 mol/mol

with respect to lactide) were added together with 0.015 g of $Sn(Oct)_2$ in a vial, mixed until reaching an homogenous mixture and injected in the flask. The mixture was left to react under stirring at 130 °C for 3 hours.

The same procedure was applied for the production of GC-PLAn with n equal to 2, 6, 8 and 12.

The average molecular weight (MW) and the MW distribution were characterized using size exclusion chromatography (SEC) analysis with THF as eluent and a 0.5 mL/min flow rate at a temperature of 35 °C. The instrument (Agilent, 1100 series, Germany) was equipped with differential refractive index (RI) three PL gel columns (Polymer laboratories Ltd., UK; two columns had pore sizes of the Mixed-C type and one was an oligopore; 300 mm length and 7.5 mm ID) and a precolumn. A universal calibration was applied based on polystyrene (PS) standards from 580 Da to 3,250,000 Da (Polymer Laboratories).
The results were confirmed by NMR analysis.

2.5 Glycerol Carbonate-PLAn Acrylate

To synthesize the glycerol carbonate-PLAn acrylate ((9) in **Figure 1**), a procedure similar to the one adopted for the acylation of glycerol carbonate was followed. As an example, 8 g of GC-PLA2 were dissolved into 20 mL of chloroform. 12.33 g of TEA (1.8 mol/mol with respect to the GC-PLA2) were then added to the mixture, the flask was then cooled down in a water/ice mixture to 0 °C and 9.8 g of acryloyl chloride (1.6mol/mol with respect to GC-PLA2) were added dropwise. After one hour, the reaction was filtered and purified by washing three times with 50 mL of HCl 0.1 M.

The solvent was removed under reduced pressure and the product characterized *via* NMR in deuterated chloroform.

2.6 Glycerol Methacrylate

Glycerol methacrylate ((2) in **Figure 1**) was obtained from the hydration of glycidyl methacrylate. In particular, 4.3 g of glycidyl methacrylate were dissolved in 39 g of distilled water and the solution was poured in a 50 mL round bottom flask equipped with a magnetic stirrer. The flask was then left to react at 80 °C for 9 hours. The almost complete conversion of glycidyl methacrylate was assessed via NMR. The product was then freezed at -30 °C for 6 h and freeze-dried overnight at -56 °C and 0.1 mbar using a Telstar Lyoquest freeze-dyer.

2.7 Nanoparticle Synthesis

Glycerol methacrylate was used to synthesize hydrophilic macromolecular chain transfer agents (macro CTAs) ((3) in **Figure 1**) with different degrees of polymerization (DP), namely 5, 10 and 20,

via RAFT polymerization. For instance, to obtain polyGM20 with the number accounting for the target DP, 11.25 g of GM were put in a 25 mL round bottom flask together with 98 mg of CPA (GC/CPA=20 mol/mol), 20 mg of ACVA (CPA/ACVA = 5 mol/mol) and 2 g of ethanol. The mixture was stirred until complete dissolution of the ingredients and purged with nitrogen for 15 minutes. Then, it was heated up to 70 °C in an oil bath and left reacting for 24 hours under stirring.

The reaction was quenched by cooling to room temperature and exposure to air and the product dried under reduced pressure. The viscous red liquid was dissolved in 2.5 mL of methanol and precipitated in 40 mL of chloroform to remove the unconverted monomer. The final product was recovered through atmospheric filtration and finally dried in a vacuum oven at 35 °C overnight.

The average molecular weight (MW) and the MW distribution of the hydrophilic macro CTAs were determined *via* size exclusion chromatography (Jasco 2000 Series apparatus). The samples were dissolved at 5 mg/mL in 0.05 M Na_2SO_4/acetonitrile (80/20 v/v) solution and filtered through a 0.45 μm pore-size nylon membrane. The separation was performed at a flow rate of 1 mL/min, at 35 °C with a guard and three Suprema columns (Polymer Standards Service; particle size 10 mm and pore sizes of 100, 1000, and 3000 Å), and polyethylene glycol standards were employed for the construction of the calibration curve. The monomer conversion was assessed *via* ^{1}H NMR.

Afterwards, the hydrophobic block of the copolymers was added by chain extending the hydrophilic macro CTAs *via* RAFT polymerization. This was obtained using either the GCA, obtaining poly(GM-GCA) ((4) in **Figure 1**) or the $GCPLA_n$-A, obtaining poly(GM-$GCPLA_n$-A) ((5) in **Figure 1**) macromonomers. Also in this case, different lengths of the hydrophobic portion were targeted by modulating the mole ratio monomer/macro CTA. For instance, considering the addition of 20 units of GCPLA2-A to the polyGM20, 0.15 g of the previously prepared polyGM20 were added in a 5 mL round bottom flask together with 0.28 g of GCPLA2-A (GCPLA2-A/polyGM20 = 20 mol/mol), 1.75 g of DMSO and 4 mg of ACVA (polyGM20/ACVA = 3 mol/mol). The flask was purged with nitrogen for 15 minutes and heated to 70 °C in an oil bath. After 24 hours of reaction, further 4 mg of initiator were added to the solution and the reaction was left to proceed for other 24 hours. The monomer conversion was assessed *via* ^{1}H NMR.

The block copolymer self-assembly leading to NPs was obtained by nanoprecipitation. In particular, the product was dissolved in DMSO at a concentration of 100 mg/mL and the solution was added drop by drop in distilled water under stirring to obtain a final latex concentration of 3% w/w. The NP suspension was dialyzed against distilled water for one week in a Spectra\Por regenerated cellulose

membrane with a molecular weight cut-off (MWCO) of 3.5 kDa to eliminate DMSO, the unconverted monomer, and eventually all the hazardous chemicals used in the synthesis steps. The dialysis medium was changed at least twice a day to preserve a large concentration gradient.

The NP size distribution was assessed *via* dynamic light scattering (DLS) performed on a Malvern Zetasizer Nano ZS instrument at a scattering angle of 173° (backscatter). The average size, polydispersity indexes (PDI) and scattering intensity were reported as a function of time. The reported data are an average of three independent measurements.
NP degradation at different pH was measured using DLS as well, recording the average size and scattering intensity every 10 minutes for about 12 hours and then every 3 days. The pH was modulated with a 0.1 M NaOH solution and checked with a FisherScientific Accumet AB150 pH meter.

2.8 Pyrene Encapsulation and Release Tests

To test the ability of the produced bio-based NPs in controlling the release of a lipophilic compound, pyrene was physically loaded in the NPs and its release tracked over time. In particular, 37 mg of pyrene were dissolved in a DMSO solution containing 740 mg of poly(GM10-GCPLA$_2$-A10) to achieve a final pyrene/polymer ratio of 5% w/w. The final product was precipitated in water as reported in Section 2.7. 3 mL of the final NP suspension were put inside a Slide-A-Lyzer® Dialysis Cassette G2 (MWCO = 3.5 kDa) and dialyzed for 48 hours against 0.5 L of solutions at different pH (*i.e.* 3, 14 and 7.4).

Aliquots of 0.1 mL of the NP suspension in the dialysis cassettes were taken at predetermined time (0, 1.5, 3, 4.5, 6, 7.5, 24 and 48 hours). The samples were dried under air and 2 mL of ACN were added to each vial. Then, they were stirred with a vortex for 30 seconds and put in a centrifuge at 5000 rpm for 5 minutes. The supernatant was analysed *via* UV-Vis spectroscopy (Jasco V-630 UV Spectrophotometer) measuring the absorbance at a fixed wavelength equal to 335 nm. The intensities of the signals were related to the pyrene concentration in ACN through a calibration curve, obtained from pyrene standard solutions in a concentration range 11.8– 0.591 mg/L.

3. Results and Discussion

3.1 From Glycerol to Glycerol Carbonate

Glycerol carbonate, a versatile molecule enabling further functionalization, was synthesized starting from two readily available molecules that are urgently pushing the researchers to find strategies for their valorisation, glycerol and CO_2.[21] Glycerol is a triol, which leads to the formation of two possible products depending on the –OH to which tosyl chloride binds. In particular, the 6-members ring 5-hydroxy-1-3-dioxane-2-one is expected to be the most abundant product due to the higher reactivity of the primary hydroxyl groups, compared to the 5-members ring 4-hydroxymethyl-1,3-dioxolan-2-one (glycerol carbonate) (**Figure 2(a)**).

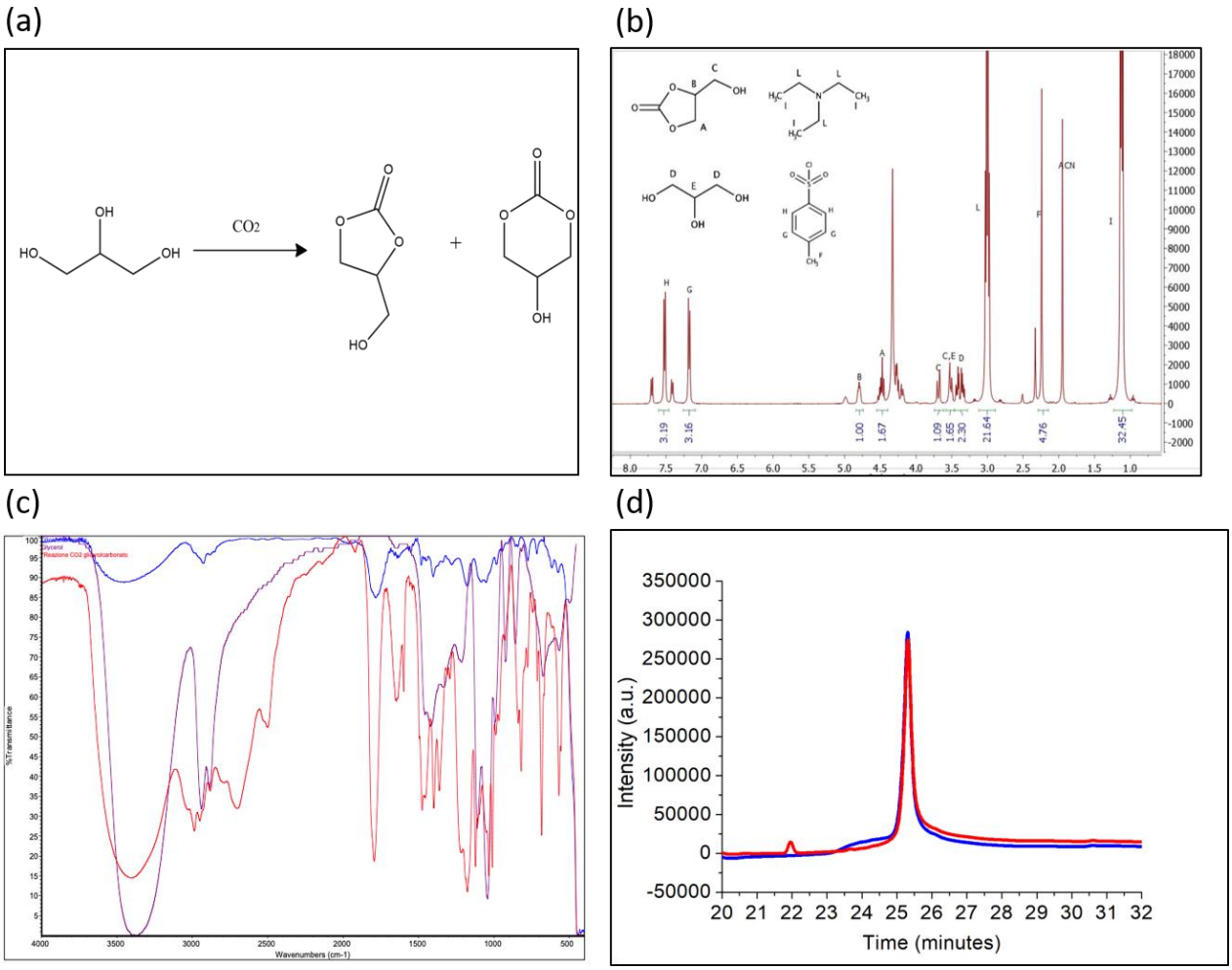

Figure 2. (a)Two possible products of the reaction between CO_2 and glycerol: glycerol carbonate and 5-hydroxy-1-3-dioxane-2one, (b) NMR spectrum recorded in deuterated water of the products. (c) FTIR spectra of the reaction products (red line) compared to glycerol (violet line) and commercially available glycerol carbonate (blue line). The characteristic peak of carbonate group at 1700 cm^{-1} is taken as reference for the success of the reaction. This peak is visible in the spectrum of both the product and the commercial glycerol carbonate, while it is missing in the spectrum of glycerol. (d) HPLC of commercially available glycerol carbonate (blue line) and the product obtained through the reaction between glycerol and CO_2 (red line).

However, apart from particular reaction conditions, the hydrogen bonding between the hydroxyl group and the carbonate group promotes the ring opening and isomerisation of 5-hydroxy-1-3-dioxane-2-one to glycerol carbonate, that is much more stable.[22]

This consideration is supported by the NMR spectroscopy performed on our product. The NMR spectrum shown in **Figure 2(b)** confirms that there is no presence of 5-hydroxy-1-3-dioxane-2one while almost the 65% of glycerol converted into glycerol carbonate, as assessed according to **Equation 1**.

$$glycerol\ conversion = \frac{B}{B + \frac{D}{4}} \quad (1)$$

Where B is the area of the peak attributed to the proton on the secondary carbon in the glycerol carbonate, while D is the area of the peak of the four protons on the primary carbons in the glycerol, as shown in the NMR spectrum.

The formation of the carbonate group was further assessed by FTIR spectroscopy. In particular, the characteristic peak associated to the C=O stretching appears at 1700 cm^{-1} (red line in **Figure 2(c)**) and it is considered as a proof of glycerol carbonate formation**.** Indeed, it is possible to detect this characteristic peak in the FTIR spectrum of the commercial GC (blue line in **Figure 2(c)**) while it is absent in the glycerol (violet line in **Figure 2(c)**).

To assess the purity of the product, as well as to verify that tosyl chloride used as the coupling agent was no longer bounded to the available –OH group of the produced GC, the HPLC elution profile of our product was compared with the one obtained in the case of a commercially available GC. As shown in **Figure 2 (d),** the retention time of the two species is almost the same, with only a small impurity in the synthesized product, confirming the effectiveness of the protocol reported in this work in producing pure GC.

It is worth mentioning that in this work we conducted the synthesis of GC using refined glycerol as starting material. On the other hand, using the crude glycerol from the biodiesel production would avoid the expensive reagent purification. However, the main limitation in using such raw material is the high content in water, up to 25-35% w/w, and methanol, up to 15-20% w/w[23], which compromises the yield to GC at equilibrium. Then, a preliminary distillation is recommended for the use of crude glycerol.

3.2 Synthesis of Glycerol Carbonate-based Macromonomers

Once the GC was synthetized, the possibility of further functionalizing this molecule with a vinyl group in order to make it amenable for radical chemistry was explored. This would enable us to incorporate this molecule in functional polymers.

Two different macromonomers were synthesized starting from GC. GC acrylate (GCA) was synthesized from GC and acryloyl chloride. The reaction was straightforward and led to a high conversion (reaction scheme and full characterization in **Figure S1**).

On the other hand, the possibility of using GC as initiator in the ROP of lactide was explored. GC-PLA_2, GC-PLA_4, GC-PLA_6 and GC-PLA_8 were synthetized and studied *via* both NMR (**Figure S2 (a)**) and GPC (**Figure S2 (b)**). **Table 1** summarizes the properties of the produced carbonate-oligoesters, showing that GC can be conveniently incorporated into polyesters with high conversion and good control over the ROP. Indeed, the average DP evaluated through NMR is very close to the target value. A slight deviation is observed in the case of GC-PLA_8, for which we recorded similar DP and molecular weight distribution as for the GC-PLA_6.

Table 1. Characterisation of GC-PLAn.

Compound	GC conversion[a] (%)	Lactide conversion[a] (%)	DP[a]	Mn[b] (g/mol)	Đ[b] (-)
GC-PLA_2	82	93	2,67	538	1.03
GC-PLA_4	84	97	4,7	559	1.02
GC-PLA_6	95	91	6	931	1.14
GC-PLA_8	95	82	6	951	1.15

[a]From ^{1}H NMR in deuterated chloroform

[b]From GPC calibrated with polystyrene standards

These short oligoester chains can be further functionalized with a vinyl group in order to obtain hydrophobic and biodegradable macromonomers (GC-PLAn-A) suitable for the production of the hydrophobic core of our bio-based NPs. The full characterization of these macromonomers is reported

in **Figure S3.** GC-PLA$_2$-A was selected to produce the smallest possible nanoparticles since it has the shortest chain length.

3.3 Glycerol-Based Nanoparticles

After having obtained glycerol-based macromonomers, the aim was to synthesize biodegradable NPs from the self-assembly of amphiphilic block copolymers with a high content of bio-derived components. To synthesize these modular copolymers, RAFT polymerization was exploited, due to its high control over the polymer microstructure.[24],[25]

First, we synthetized hydrophilic poly(glycerol methacrylate) macro CTAs with different DPs, *i.e*. 5, 10, and 20. The monomer in this case was obtained by the hydrolysis of glycidyl methacrylate (full characterization in **Figure S4**). The average chain length of the macro CTAs, their molecular weight, and the monomer conversion were assessed by NMR and aqueous GPC and these properties are summarized in **Table 2**.

Table 2. Molecular weight and polydispersity index of poly(glycerol methacrylate) with DP 5, 10, 20 from GPC and NMR analysis.

mCPA	M_n[a](g/mol)	Đ[a] (-)	DP[b]	M_n[c] (g/mol)	Conversion[b] (%)
5	997	1.4	8	1272	>99%
10	1494	1.4	16	2545	>99%
20	1975	1.3	24	3819	>99%

[a]From GPC calibrated with PEG standards

[b]From 1H NMR in deuterium oxide

[c]Calculated from the DP

Owing to the high control of the RAFT polymerization, it was possible to obtain polymers with narrow molecular weight distribution (*i.e.* <1.4), as shown also in **Figure 3**, DP close to the target, and high monomer conversion (as reported in **Figure S5**).

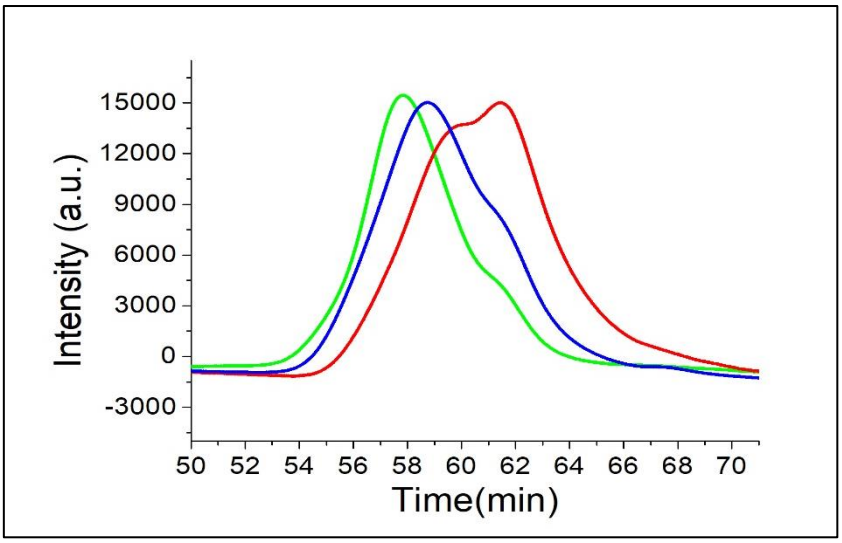

Figure 3. GPC chromatograms of poly(glycerol methacrylate) with DP 5 (red), 10 (blue), and 20 (green). The shift of the peaks towards lower retention times confirms the molecular weight increase.

The hydrophilic poly(GM) macro CTAs were then chain extended with either GCA or GC-PLA2-A to obtain glycerol-based amphiphilic block copolymers. While poly(GM-GCA) are mostly made of glycerol-derived products, in poly(GM-GCPLA$_2$-A) a chain of PLA was added to the side chains. Indeed, lactic acid is notoriously obtained from renewable resources[26], so this contributes in introducing bio-based components in our polymers. A library of block copolymers at different lengths for the hydrophobic block was synthesized and their properties are summarized in **Table 3**.

From ^{1}H NMR spectroscopy (**Figure S6**) we demonstrated that high conversion of the monomer (>90%) was achieved in all cases. In addition, the DP of the hydrophobic chain was similar to the target, proving the good control over the reaction.

A set of NPs made up of copolymers with different DP of both hydrophilic and hydrophobic block was produced *via* nanoprecipitation and studied via DLS.

Firstly, it was demonstrated that narrowly distributed NPs (PDI<0.2, see **Table 3**) could be obtained from the self-assembly of amphiphilic block copolymers mainly constituted of glycerol. Then, it was investigated how the polymer composition, and in particular the lengths of the hydrophilic and the hydrophobic block, affect the NP size. Indeed, poly(GM-GCA)-based NPs only showed a small stability range. Therefore, it was decided to focus mainly on the NPs obtained from poly(GM-GCPLA$_2$-A), which showed improved stability at different copolymer microstructures. This could be due to a different arrangement that the copolymer chains assume in water with the addition of a spacer between the backbone and the glycerol carbonate, which is highly hydrophobic.

Table 3. Conversion, size, PDI and percentage of polymer coming from a bio-based material (glycerol, CO_2 and lactic acid) of the produced nanoparticles. The percentages are calculated taking into account how many grams of product are bio-based over 100 total grams.

Compound	DP hydrophilic	DP hydrophobic	Conversion (%)	Bio-based content (% w/w)	Size (nm)	PDI (-)
GM-GCA	20	15	99	65	30.87 ± 0.22	0.13 ± 0.01
GM-GCA	20	20	96	65	66.41 ± 1.20	0.21 ± 0.01
GM-GCPLA$_2$A	10	5	98	68	51.32 ± 0.75	0.31 ± 0.02
GM-GCPLA$_2$A	10	10	94	72	88.43 ± 0.54	0.19 ± 0.01
GM-GCPLA$_2$A	10	20	98	75	153.1 ± 1.84	0.20 ± 0.01
GM-GCPLA$_2$A	10	30	88	76	217.6 ± 0.33	0.26 ± 0.01
GM-GCPLA$_2$A	20	15	99	70	30.6 ± 0.20	0.16 ± 0.01
GM-GCPLA$_2$A	20	20	99	72	46.5 ± 0.60	0.15 ± 0.01
GM-GCPLA$_2$A	20	30	99	73	67.9 ± 1.40	0.15 ± 0.01
GM-GCPLA$_2$A	20	40	97	75	102.2 ± 0.70	0.18 ± 0.01

The size of the poly(GM-GCPLA$_2$-A)-based NPs linearly increases with the length of the hydrophobic portion. In fact, the longer the hydrophobic portion the bigger the NP size (**Figure 4**). Comparing instead the NP size with the length of the hydrophilic portion, we found that the NPs with 20 units of glycerol methacrylate are smaller than the corresponding NPs with 10 units at each DP of the hydrophobic block. This trend has been previously explained in our group[27]. In fact, it was reported that the NP size has an inverse proportionality with the superficial area covered by hydrophilic chains (A_{Cov}) in the case of macro RAFT surfmers (*i.e.* reactive surfactants). Indeed, this is the case for glycerol methacrylate, comprising a hydrophobic methacrylate group and a hydrophilic glycerol portion. Therefore, longer GM chains cover a higher portion of the NP surface, thus leading

to an overall decrease in the NP size, as experienced for poly(GM20-GCPLA$_2$-A)-based NPs compared to poly(GM10-GCPLA$_2$-A)-based NPs showed in **Figure 4**.

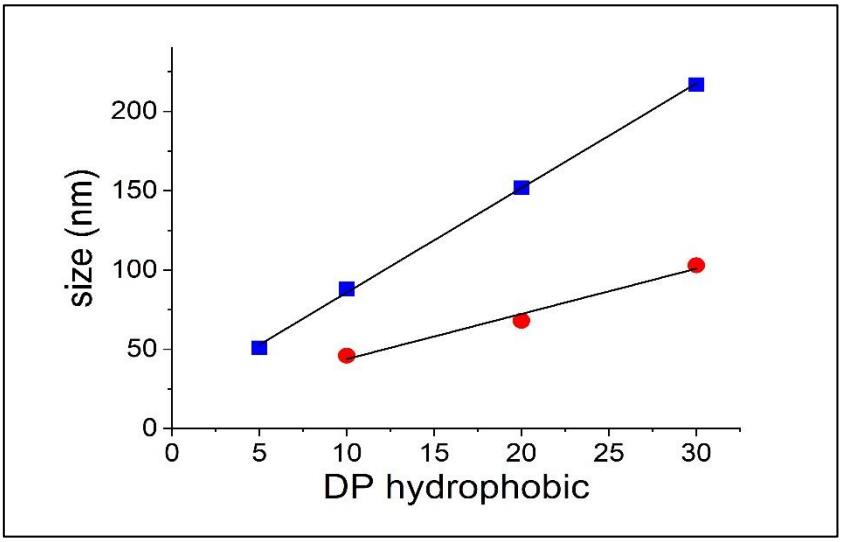

Figure 4. Size as a function of the DP of the hydrophobic block for NPs obtained from poly(GM10-GCPLA2-A) (■) and poly(GM20-GCPLA2-A) (●). The data are the average of three measurements with the error bars showing the polydispersity. The lines are the best fitting of the experimental data.

With these NPs, the amount of bio-derived products with respect to the oil-based one (that comes from the necessity of adding an acrylate group through acryloyl chloride) is shown in **Table 3**, and it is always higher than 65% w/w. This confirmed the possibility of obtaining tuneable NPs with a high bio-based content that enable the reconversion of both glycerol and CO_2.

As already explained in the introduction, glycerol carbonate and poly(lactic acid) are expected to degrade in aqueous environment through the hydrolysis of the ester bonds, leaving behind water soluble degradation products[26]. Therefore, the degradation of the produced glycerol-based NPs was studied, which is important to avoid the accumulation of plastics in the environment. **Figure 5** shows the degradation behaviour at different pH and in the short-term period, that is the first few hours after the particles have been dispersed in an acidic/alkaline solution. In particular, the NP degradation was tracked by measuring the relative scattering intensity (RSI), which is the intensity of the scattered light at the generic time *t* compared to the value at time zero. This parameter is proportional to the NP concentration and size at the sixth power[28]. Therefore, a decrease in this parameter over time testifies the progressive dissolution of the NPs. Both GCA-based and GCPLA-A-based NPs degrade much faster at alkaline pH rather than at acidic pH. In general, at alkaline pH there is an initial small period of time in which the scattering intensity increases, here the NPs become more hydrophilic but

still assembled and so their size increases due to the swelling[29],[30]. Then, at very alkaline pH, there is an abrupt decrease of the curve while at pH 12 the decrease is much smoother. At acidic pH, only swelling is noticed within 12 hours. Even if the behaviour of the two NPs is similar, poly(GM-GCPLA2-A)-based NPs seem to be more susceptible to hydrolysis, probably because both hydrolysis of the ester bonds and CO_2 release from GCA occur at the same time. This is more evident at pH 3 since the poly(GM-GCPLA2-A) particles swell more than the poly(GM-GCA).

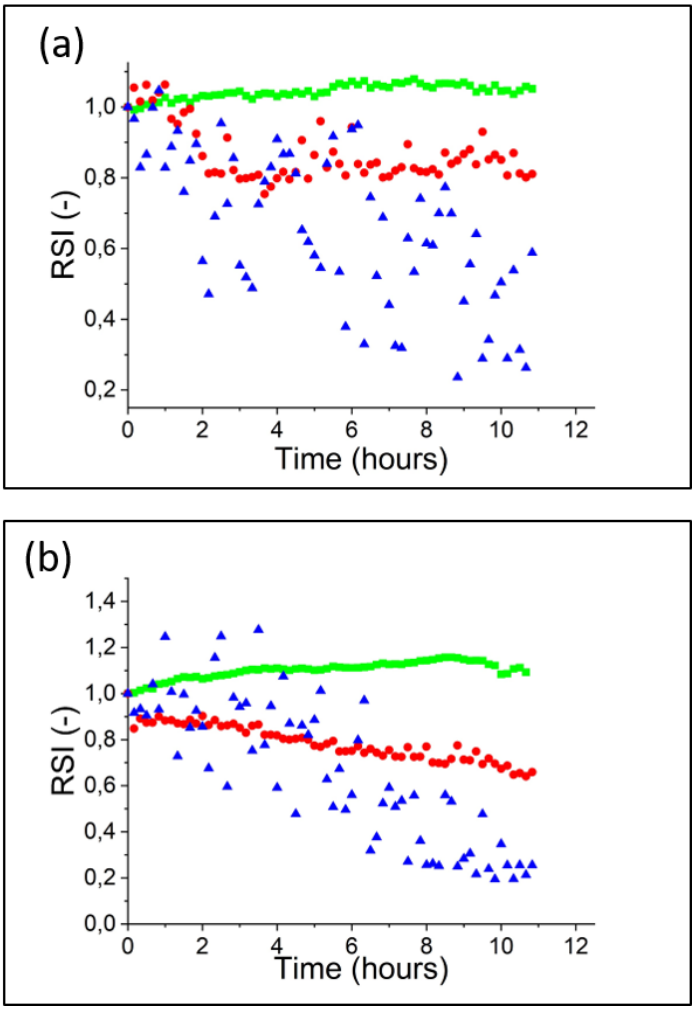

Figure 5. Short-term degradation of: (a) poly(GM20-GCA10)-based NPs and (b) poly(GM20-GCPLA2-A10)-based NPs. The study was conducted at three different pH: 3 (■), 12 (●) and pH 14 (▲). The degradation was estimated via DLS analysis measuring the scattering intensity. The degradation was calculated setting as one the scattering intensity of the NPs before the addition of the acid/base.

The degradation behaviour was assessed also in the long-term period by tracking the scattering intensity of the NPs over 20 days (**Figure 6**). While at pH 14 scattering intensities very closed to zero are reached already after 1 day, thus testifying the almost complete dissolution of the NPs, at pH 12 the degradation rate is much slower. The scattering intensity decreases to the 60% of the original value only after 20 days. Finally, at pH 3, the degradation proceeded very slowly and we only

recorded an increase in the scattering intensity most likely due to the NP swelling. To assess if the matter was the creation of crosslinks between the particles or the slow degradation, the residual polymer after degradation was studied through NMR. In **Figure S7** it is visible that the peaks corresponding to glycerol carbonate and PLA are still present, meaning that the degradation did not occur yet, while at pH 12 the two peaks disappeared totally.

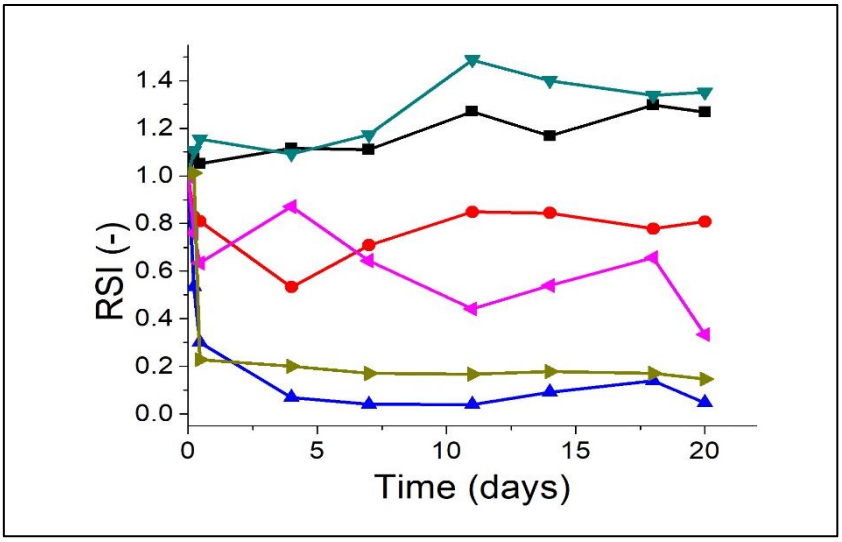

Figure 6. Long-term degradation of the particles at different pH measured through relative scattering intensity (RSI) variation. Poly(GM-GCA) (■) and poly(GM-GCPLA2-A) (▼) at pH 3, poly(GM-GCA) (●) and poly(GM-GCPLA2-A) (◄) at pH 12, poly(GM-GCA) (▲) and poly(GM-GCPLA2-A) (►) at pH 14.

3.4 Glycerol-Based Nanoparticles for Controlled Drug Release

Since both glycerol and glycerol carbonate are considered as safe by the FDA, we explored the possibility of using the NPs obtained from glycerol-based copolymers for the controlled release of hydrophobic drugs. In particular, we loaded the poly(GM20-GCPLA$_2$-A10)-based NPs with pyrene, used as a drug mimic molecule[31], and tracked its release over time. The test was conducted at acidic (pH = 3), alkaline (pH = 14) and neutral pH (*i.e.* 7.4 using a PBS solution to buffer the system). The pyrene release profiles are shown in **Figure 7**.

An initial burst release accounting for the release of 50% of pyrene can be observed in the first 2 hours. This fast release may be attributed to the pyrene localized on the surface of the NPs and hence more prone to diffuse in the aqueous buffer. After this initial stage, a slower sustained release of the molecule was achieved in all of the conditions. In this stage, the fastest release is obtained at pH 14, probably assisted by the degradation of the NPs at alkaline pH, thus reaching almost the 90% of

pyrene released after 48 hours. On the other hand, the particles at pH 3 only reached the 60% of released pyrene, since they take more than 21 days to degrade. In PBS, the release is smoother than the one at pH 14, but finally reaching the same percentage after 48 hours.

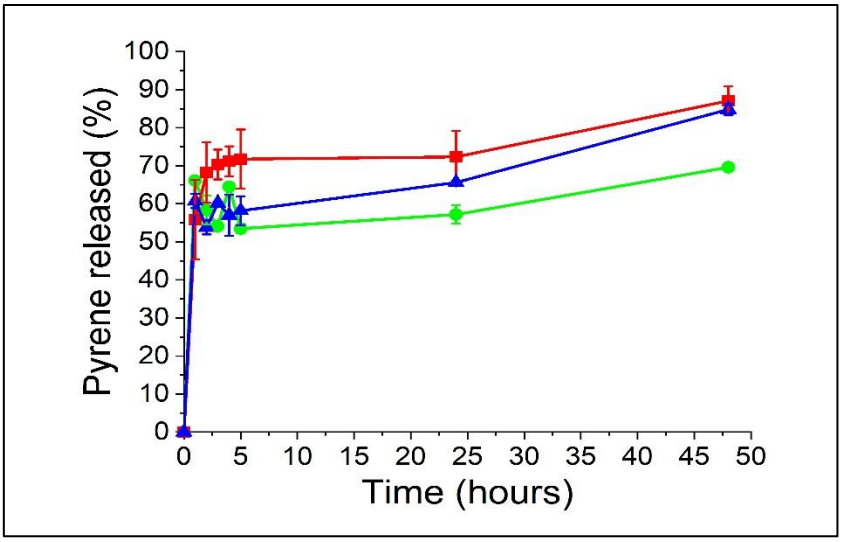

Figure 7. Release of pyrene from poly(GM10-GCPLA2A10)-based NPs at pH 3 (●), pH 7.4 (PBS, ▲) and pH 14 (■).

4. Conclusions

In this work, we propose a way to valorise glycerol and CO_2, well-known by-products of anthropogenic processes, which are urgently pushing the community in finding a way for their exploitation. In particular, we showed a strategy for the incorporation of these products in functional amphiphilic copolymers. More in details, glycerol and CO_2 were converted into glycerol carbonate through a one-pot synthesis conducted under mild reaction conditions. Starting from glycerol and glycerol carbonate as building blocks, we produced amphiphilic block copolymers able to self-assemble in water by RAFT polymerization. We showed that narrowly dispersed NPs with tuneable size and a bio-based content higher than 65% w/w could be produced from these glycerol-based copolymers. For these NPs, we studied the degradation in aqueous environments, which is an important feature to avoid the accumulation of plastics. While at very alkaline pH the degradation is fast and the complete dissolution of the NPs is observed already after 1 day, at milder conditions it could take more than 20 days for the complete NP degradation. Finally, since both glycerol and glycerol carbonate are considered as safe by the regulatory agencies, we demonstrated the feasibility of using this kind of NPs as drug delivery systems. Indeed, we showed that the NPs could grant a sustained release of pyrene, a small hydrophobic molecule chosen as a drug mimic molecule.

Supporting Information: Supplemental data are available free of charge at the Publisher's website. This include the NMR characterization of the monomers and polymers synthesized in this work.

Conflict of Interests: The authors declare no conflict of interests for this work.

References

[1] N. Hernández, R.C. Williams, E.W. Cochran, The battle for the "green" polymer. Different approaches for biopolymer synthesis: bioadvantaged vs. bioreplacement, Org. Biomol. Chem. 12 (2014) 2834–2849. https://doi.org/10.1039/C3OB42339E.

[2] Y. Zhu, C. Romain, C.K. Williams, Sustainable polymers from renewable resources, Nature. 540 (2016) 354–362. https://doi.org/10.1038/nature21001.

[3] P. Le Feuvre, Biofuels, Transp. Biofuels. (n.d.).

[4] C.A.G. Quispe, C.J.R. Coronado, J.A. Carvalho, Glycerol: Production, consumption, prices, characterization and new trends in combustion, Renew. Sustain. Energy Rev. 27 (2013) 475–493. https://doi.org/10.1016/j.rser.2013.06.017.

[5] H. Zhang, M.W. Grinstaff, Recent advances in glycerol polymers: Chemistry and biomedical applications, Macromol. Rapid Commun. 35 (2014) 1906–1924. https://doi.org/10.1002/marc.201400389.

[6] M. Gurgel, A. Vieira, M. Altenhofen, L. Oliveira, M.M. Beppu, Natural-based plasticizers and biopolymer films : A review, Eur. Polym. J. 47 (2011) 254–263. https://doi.org/10.1016/j.eurpolymj.2010.12.011.

[7] I. Boileau, J. et al Mori, Emollients for eczema What emollient should I use ?, DermNet. (2019) 6–8.

[8] D. Baum, Glycerol Lubricant : Potential Source of Error in Commercially Prepared Blood Specimen Tubes, (1967) 73–74.

[9] O. Valerio, T. Horvath, C. Pond, M. Misra, A. Mohanty, Improved utilization of crude glycerol from biodiesel industries: Synthesis and characterization of sustainable biobased polyesters, Ind. Crops Prod. 78 (2015) 141–147. https://doi.org/10.1016/j.indcrop.2015.10.019.

[10] J.C.J. Bart, N. Palmeri, S. Cavallaro, Valorisation of the glycerol by-product from biodiesel

production, Biodiesel Sci. Technol. (2010) 571–624. https://doi.org/10.1533/9781845697761.571.

[11] J.L.J. Van Velthoven, L. Gootjes, D.S. Van Es, B.A.J. Noordover, J. Meuldijk, Poly(hydroxy urethane)s based on renewable diglycerol dicarbonate, Eur. Polym. J. 70 (2015) 125–135. https://doi.org/10.1016/j.eurpolymj.2015.07.011.

[12] P.D. Pham, S. Monge, V. Lapinte, Y. Raoul, J.J. Robin, I. Charles, G. Montpellier, E. Inge, Review Article Various radical polymerizations of glycerol-based monomers, (2013) 28–40. https://doi.org/10.1002/ejlt.201200202.

[13] B. Nohra, L. Candy, J.F. Blanco, Y. Raoul, Z. Mouloungui, Synthesis of high-molecular-weight multifunctional glycerol polyhydroxyurethanes PHUs, Molecules. 21 (2016) 6–8. https://doi.org/10.3390/molecules21091220.

[14] J. Rotta, P.D. Pham, V. Lapinte, R. Borsali, E. Minatti, J. Robin, Synthesis of Amphiphilic Polymers Based on Fatty Acids and Glycerol-Derived Monomers – A Study of Their Self-Assembly in Water, (n.d.) 131–139.

[15] M. Lelovsky, A. Kaszonyi, Glycerol carbonate from bioglycerol, Int. Pet. Conf. (2009).

[16] R. Bai, H. Zhang, F. Mei, S. Wang, T. Li, Y. Gu, G. Li, One-pot synthesis of glycidol from glycerol and dimethyl carbonate over a highly efficient and easily available solid catalyst NaAlO<inf>2</inf>, Green Chem. 15 (2013) 2929–2934. https://doi.org/10.1039/c3gc40855h.

[17] J.R. Ochoa-Gómez, O. Gómez-Jiménez-Aberasturi, C. Ramírez-López, M. Belsué, A brief review on industrial alternatives for the manufacturing of glycerol carbonate, a green chemical, Org. Process Res. Dev. 16 (2012) 389–399. https://doi.org/10.1021/op200369v.

[18] M. Aresta, A. Dibenedetto, F. Nocito, C. Pastore, A study on the carboxylation of glycerol to glycerol carbonate with carbon dioxide: The role of the catalyst, solvent and reaction conditions, Atmos. Environ. 41 (2007) 407–416. https://doi.org/10.1016/j.atmosenv.2006.07.033.

[19] M.O. Sonnati, S. Amigoni, E.P. Taffin De Givenchy, T. Darmanin, O. Choulet, F. Guittard, Glycerol carbonate as a versatile building block for tomorrow: Synthesis, reactivity, properties and applications, Green Chem. 15 (2013) 283–306. https://doi.org/10.1039/c2gc36525a.

[20] O. Gómez-Jiménez-Aberasturi, J.R. Ochoa-Gómez, A. Pesquera-Rodríguez, C. Ramírez-

López, A. Alonso-Vicario, J. Torrecilla-Soria, Solvent-free synthesis of glycerol carbonate and glycidol from 3-chloro-1,2-propanediol and potassium (hydrogen) carbonate, J. Chem. Technol. Biotechnol. 85 (2010) 1663–1670. https://doi.org/10.1002/jctb.2478.

[21] T.M. McGuire, E.M. López-Vidal, G.L. Gregory, A. Buchard, Synthesis of 5- to 8-membered cyclic carbonates from diols and CO2: A one-step, atmospheric pressure and ambient temperature procedure, J. CO2 Util. 27 (2018) 283–288. https://doi.org/10.1016/j.jcou.2018.08.009.

[22] M. Tryznowski, Z. Zołek-Tryznowska, A. ̄widerska, P.G. Parzuchowski, Synthesis, characterization and reactivity of a six-membered cyclic glycerol carbonate bearing a free hydroxyl group, Green Chem. 18 (2016) 802–807. https://doi.org/10.1039/c5gc01688f.

[23] L.R. Kumar, R. Kaur, S.K. Yellapu, Microorganisms With Wastes as Raw Materials, 2nd ed., Elsevier Inc., 2019. https://doi.org/10.1016/B978-0-12-816856-1.00027-0.

[24] U. Capasso Palmiero, M. Sponchioni, N. Manfredini, M. Maraldi, D. Moscatelli, Strategies to combine ROP with ATRP or RAFT polymerization for the synthesis of biodegradable polymeric nanoparticles for biomedical applications, Polym. Chem. 9 (2018) 4084–4099. https://doi.org/10.1039/c8py00649k.

[25] M. Sponchioni, U. Capasso Palmiero, N. Manfredini, D. Moscatelli, RAFT copolymerization of oppositely charged monomers and its use to tailor the composition of nonfouling polyampholytes with an UCST behaviour, React. Chem. Eng. 4 (2019) 436–446. https://doi.org/10.1039/c8re00221e.

[26] E. Castro-aguirre, F. Iñiguez-franco, H. Samsudin, X. Fang, R. Auras, Poly (lactic acid) — Mass production , processing , industrial applications , and end of life ☆, Adv. Drug Deliv. Rev. 107 (2016) 333–366. https://doi.org/10.1016/j.addr.2016.03.010.

[27] U.C. Palmiero, A. Agostini, S. Gatti, M. Sponchioni, V. Valenti, L. Brunel, D. Moscatelli, RAFT macro-surfmers and their use in the ab initio RAFT emulsion polymerization to decouple nanoparticle size and polymer molecular weight, Macromolecules. 49 (2016) 8387–8396. https://doi.org/10.1021/acs.macromol.6b01827.

[28] C. Colombo, L. Dragoni, S. Gatti, R.M. Pesce, T.R. Rooney, E. Mavroudakis, R. Ferrari, D. Moscatelli, Tunable degradation behavior of PEGylated polyester-based nanoparticles obtained through emulsion free radical polymerization, Ind. Eng. Chem. Res. 53 (2014) 9128–9135. https://doi.org/10.1021/ie4036077.

[29] M. Sponchioni, U.C. Palmiero, D. Moscatelli, HPMA-PEG Surfmers and Their Use in Stabilizing Fully Biodegradable Polymer Nanoparticles, Macromol. Chem. Phys. 218 (2017) 1–12. https://doi.org/10.1002/macp.201700380.

[30] M. Sponchioni, L. Morosi, M. Lupi, U. Capasso Palmiero, Poly(HPMA)-based copolymers with biodegradable side chains able to self assemble into nanoparticles, RSC Adv. 7 (2017) 50981–50992. https://doi.org/10.1039/c7ra11179g.

[31] M. Sponchioni, P. Rodrigues Bassam, D. Moscatelli, P. Arosio, U. Capasso Palmiero, Biodegradable zwitterionic nanoparticles with tunable UCST-type phase separation under physiological conditions, Nanoscale. 11 (2019) 16582–16591. https://doi.org/10.1039/c9nr04311j.