Soft Matter

Cite this: *Soft Matter*, 2011, **7**, 9628

www.rsc.org/softmatter

PAPER

Guanidine-based polycarbonate hydrogels: from metal-free ring-opening polymerization to reversible self-assembling properties†

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Received 3rd April 2011, Accepted 25th May 2011
DOI: 10.1039/c1sm05581j

Bio-inspired stabilization of aliphatic polycarbonate-based hydrogels has been carried out by the metal-free ring-opening co-polymerization (ROP) of 6-membered cyclic carbonates containing, respectively, protected guanidine and carboxylic acid functions. Polyethylene glycol (PEG) bound to methylcarboxy trimethylene carbonate at each extremity was used as the cross-linker, and the copolymerizations were performed in CH_2Cl_2 for 24 h in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) and *N*-(3,5-trifluoromethyl)phenyl-*N*′-cyclohexylthiourea (TU), the catalyst and co-catalyst, respectively. Well-defined hydrogels of various compositions and presenting a high gel fraction were obtained. HR-MAS NMR has been successfully employed to validate our purification technique as well as to assess the selective de-protection of the guanidine and carboxylic acid functions. Evidence of self-assembling properties has been attested by differential scanning calorimetry (DSC), HR-MAS NMR analysis and swelling test experiments in aqueous buffered solutions at pH 4 and 8.

Introduction

Aliphatic polycarbonates in their diversities constitute an interesting class of materials with high diversity owing to their high biocompatibility, reliable (bio)degradability and low toxicity, making them very suitable for green technology and biomedical applications (scaffold for tissue engineering or drug carriers).[1,2] They are obtained either by copolymerization of carbon dioxide with epoxide or by ring-opening polymerisation (ROP) of cyclic carbonate monomers.[1,3,4] While the former approach is CO_2 consuming and can alleviate environmental concerns, the formation of a 5-membered cyclic by-product, the presence of ether linkage and the use of air-sensitive catalysts hamper the applicability of the latter approach from an up-scaled synthetic standpoint.[5,6]

Beside the facts that only few cyclic carbonates are commercially available, ring-opening polymerization is gaining increasing attention thanks to its versatility and mild reaction conditions. As a result, cationic,[7–9] anionic[10,11] and coordination–insertion approaches[12–18] have emerged as promising polymerization processes. However, several limitations have been observed as loss of CO_2, formation of ether linkages, broad molecular weight distribution, back-biting reactions leading to linear and cyclic polymer mixtures or large discrepancy between expected and experimental molecular weights.

In the recent years, the development of green catalysts for the ROP of lactones and cyclic carbonates has been spawned by the will to revolutionize polymer chemistry in order to fulfil the strict requirements of microelectronic or biomedical applications.[19,20] In this context, enzymatic catalysis of the ROP of cyclic carbonates has received great attention as a new methodology to produce biodegradable polymers with environmental benefits.[21,22] However, their low catalytic activities, lack of control over molecular weight distributions, the need of a highly stable support, their non-recyclability and their relatively high cost are not favouring their industrial development.[23,24] More recently, metal-free organic catalysts have been developed and applied successfully to the controlled ROP of lactones and cyclic carbonates. Successful organocatalysts include phosphazene,[25,26] *N*-heterocyclic carbene,[27–33] iodine trichloride,[34] bifunctional thiourea-amine,[35,36] amidine and guanidine-based catalysts[37–39] such as 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD), *N*-methyl TBD (MTBD), and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU). Amidine and guanidine-based catalysts were found particularly efficient for the controlled ROP of trimethylene carbonate (TMC)[40] as well as for other functionalized cyclic carbonates,[41] allowing to reach complex topologies such as block-[42,43] and graft-copolymers[44] presenting good control over molecular weights and their distribution. These catalytic systems appear as suitable alternatives to metal-based catalysts for producing biomaterials, and they have been investigated recently for the preparation of nanovectors,[45] nanoparticles[44] or hydrogels.[46,47] Hydrogels likewise constitute an emergent class of materials with high diversity thanks to their biocompatibility, their soft and

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1sm05581j

rubbery nature as well as their mechanical properties mimicking well natural tissues. Nevertheless, their development for tissue engineering or orthopaedic applications[48] has been limited because of the structural instability of aliphatic polycarbonates.[18] Such limitations can be addressed jointly by introducing functionalities in the network able to self-assemble through secondary interactions and by using living polymerization techniques. In this work, we take advantage of the natural self-assembling properties of guanidinium functions and carboxylic acids giving rise to cross-linked aliphatic polycarbonate-based hydrogels stabilized by secondary interactions. With these objectives in mind, we present the synthesis of functional cyclic carbonates bearing BOC-protected guanidines and *tert*-butyl-protected carboxylic acids, respectively, using the protocol revisited by Pratt *et al.*[41] and copolymerized by amidine-mediated ROP in the presence of poly(ethylene glycol)-α,ω-methylcarboxy trimethylene carbonate cross-linker (MTC-PEO-MTC). Selective de-protection of the above mentioned functions was carried out in trifluoroacetic acid (TFA) in solution in methylene chloride in order to investigate possible interactions occurring between guanidinium and carboxylic acid functions. It is demonstrated that ^{1}H HR-MAS NMR is a suitable and flexible tool not only to monitor the de-protection of the chemical groups considered but also to validate the purification technique investigated. Finally, evidence for self-assembling properties is assessed by DSC, HR-MAS NMR analysis in D_2O and swelling testing at pH 4 and 8.

Experimental part

Materials and instrumentation

α,ω-Hydroxyl polyethylene glycol (M_n = 2000 g mol^{-1}, Aldrich) was dried by three successive azeotropic distillations with anhydrous toluene. *N*-(3,5-Trifluoromethyl)phenyl-*N*′-cyclohexylthiourea (TU) was prepared as previously reported.[35] 2,2-Bis(methylol)propionic acid (bis-MPA, 98% Aldrich), benzyl bromide (98% Aldrich), *tert*-butyl bromoacetate (98% Aldrich), 1,3-di-boc-2-(2-hydroxyethyl)guanidine (>96%, Fluka), triphosgene (98% Aldrich), oxalyl chloride (99% Aldrich), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 99% Aldrich), cyclohexylamine (99% Aldrich), 3,5-bis(trifluoromethyl)phenyl isothiocyanate (98% Aldrich), Pd/C (Aldrich), trifluoroacetic acid (TFA, 99% Aldrich), and potassium hydroxide (KOH, Acros) were used as received. Pyridine (>99% Fiers), triethylamine (>99% Aldrich), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, >99% Fluka) and (−)-sparteine (>99% Aldrich) were all dried over BaO for 48 h, and the latter two compounds distilled under reduced pressure. Toluene, tetrahydrofuran and chloroform (p.a. Chemlab) were dried using activated aluminoxide column from a MBraun Solvent Purification System (MB-SPS-800), methylene chloride (CH_2Cl_2, HPLC grade, Fisher) was dried over CaH_2 for 48 h and distilled under reduced pressure, heptane (p.a. Chemlab) and ethyl acetate (Merck), and dimethylformamide (DMF, >99.8% Aldrich) were used as received.

^{1}H NMR spectra were recorded by using a Bruker AMX-300 or AMX-500 apparatus at room temperature in $CDCl_3$ or in DMSO-d_6 (30 mg per 0.6 mL). HR-MAS NMR spectra were acquired on a Bruker AVANCE II NMR spectrometer resonating at 500.08 MHz for ^{1}H nuclei, with a triple channel dedicated HR-MAS 500 SB BL4 probe for measuring ^{1}H, ^{117}Sn and ^{13}C nuclei with a z-gradient coil. The sample was prepared in a 4 mm ZrO_2 rotor (120 μL) in $CDCl_3$. The measurement was done at room temperature at a magic angle spinning rate of 5 KHz. The ^{1}H HR-MAS NMR spectra were obtained either in the standard one-pulse-acquire mode or in the diffusion-filtered mode. In particular, the diffusion-filtered HR-MAS ^{1}H spectra were acquired using the "Longitudinal Eddy Current Relaxation Delay with Bipolar Gradient Pulses" (LEDBPP), pulse sequence[49] available in the TOPSPIN 2.0 pulse sequence library of Bruker as "ledgp2s1d", with 70% of the maximum gradient intensity, using SIN.100 gradient pulse, according to a protocol previously described.[50] Size exclusion chromatography (SEC) was performed in $CHCl_3$ at 30 °C, using an Agilent 1200 chromatograph equipped with a degasser, an isocratic HPLC pump (flow rate = 1 mL min^{-1}), an auto-sampler (loop volume = 200 mL, solution concentration = 1 mg mL^{-1}), refractive-index and UV-Vis detectors, and three columns: a PL gel 10 mm guard column and two PL gel Mixed-B 10 mm columns (linear columns for separation of MW_{PS} ranging from 500 to 10^6 Dalton). Poly(styrene) standards were used for calibration. DSC measurements were performed under nitrogen flow using a DSC apparatus Q2000 from TA Instruments, in Tzero aluminium pan (heat–cool–heat mode, heating rate 10 °C min^{-1}, cooling rate 5 °C min^{-1}). FTIR analysis was carried out with a Tensor 27 FTIR spectrometer equipped with an ATR from Bruker. Spectra were recorded (from 4000 to 500 cm^{-1}) owing to a single reflection crystal system and a DTGS detector. MALDI mass spectra were recorded using a Waters QToF Premier mass spectrometer equipped with a nitrogen laser, operating at 337 nm with a maximum output of 500 J m^{-2} delivered to the sample in 4 ns pulses at 20 Hz repeating rate. Time-of-flight mass analyses were performed in the reflection mode at a resolution of about 10 000. The matrix, *trans*-2-[3-(4-*t*-butyl-phenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), was prepared in chloroform with a concentration of 20 mg mL^{-1}. The matrix solutions (1 μL) were applied to a stainless steel target and air dried. Polymer samples were dissolved in acetonitrile to obtain 1 mg mL^{-1} solutions. 1 μL aliquots of these solutions were applied onto the target area already charged with the matrix crystals, and then dried. Finally, 1 μL of a NaCl solution (2 mg mL^{-1} in acetonitrile/water, 1/1 v/v) was applied on the target plate. For recording the single-stage MALDI-MS spectrum, the quadrupole (rf-only mode) was set to pass ions from 3500 to 6500 Th, and all ions were transmitted to the pusher of the time-of-flight analyser where they were mass analyzed with 1 s integration time. Data were acquired in the continuum mode until acceptable averaged data were obtained.

Synthesis of trimethylenecarbonate-5-methyl-5-carboxyethylguanidine-1,3-*tert*-butyloxycarbonyl (MTC-GuaBOC) 1

In a flamed dried round bottom-flask under nitrogen, 5-methyl-2-oxo-[1,3]dioxane-5-carboxylic acid (MTC-COOH) (1.6 g, 10 mmol) was initially converted to MTC-Cl using standard procedures with oxalyl chloride (1.4 g, 11 mmol) in 22 mL of anhydrous THF.[51] After 1 hour reaction, the reactor is plunged in an ice bath and a solution of 1,3-di-boc-2-(2-hydroxyethyl)

guanidine (3 g, 10 mmol) and triethylamine (2 g, 20 mmol) in 20 mL of anhydrous THF was added dropwise over 30 min. The solution obtained was stirred for another 30 min prior to removing the ice bath, and subsequently the solution stirred for another 4 hours under nitrogen. The triethylamine salt formed was filtered off and the solvent evaporated under vacuum. The crude product was then solubilised in ethyl acetate and purified by filtration through silica column. Fractions of monomer identified by TLC were gathered and the solvent eliminated under vacuum to yield **1** as white crystals. Yield: 95%. ^{1}H NMR ($CDCl_3$) δ: 11.5 (s, 1H, NH), 8.65 (t, 1H, NH), 4.70 (d, 2H, CH_2), 4.31 (t, 2H, CH_2), 4.19 (d, 2H, CH_2), 3.73 (q, 2H, CH_2), 1.47 (s, 18H, CH_3), 1.36 (s, 3H, CH_3).

Synthesis of trimethylenecarbonate-5-methyl-5-carboxy-*tert*-butylacetate (MTC-*t*BAc) 2

In a round bottom flask, 2,2-bis(methylol)propionic acid (11.5 g, 86 mmol) and potassium hydroxide (4.8 g, 85 mmol) were dissolved in 70 mL of DMF. The medium was stirred at 100 °C for 1 h. Once the medium was homogeneous, 15 mL of *tert*-butyl bromoacetate (102 mmol) were added. After 15 h of reaction at 100 °C, the KBr salt was filtered off and DMF was evaporated under vacuum. The 2,2-bis(methylol)-3-methyl-3-carboxy-*tert*-butylacetate was then recovered by extraction with $CHCl_3$. The solvent was eliminated under vacuum to yield a light yellowish oil. Yield: 99%. ^{1}H NMR ($CDCl_3$) δ: 4.51 (s, 2H, CH_2), 3.71 (d, 2H, CH_2), 3.66 (d, 2H, CH_2), 2.44 (OH), 1.38 (s, 9H, CH_3), 1.10 (s, 3H, CH_3).

In a second step, 20 g of 2,2-bis(methylol)-3-methyl-3-carboxy-*tert*-butylacetate (81 mmol) and 250 mL of anhydrous CH_2Cl_2 and 80 mL of pyridine (486 mmol) were introduced in a dried round bottom flask maintained under nitrogen. The mixture was cooled down to −75 °C with an acetone/dry ice bath and a solution of triphosgene (12 g, 45 mmol) in 150 mL of CH_2Cl_2 was added dropwise in 30 minutes. The medium was allowed to return to room temperature and a saturated aqueous solution of NH_4Cl (200 mL) was added in order to quench triphosgene and stop the reaction. The organic layer was washed 2 times with 200 mL of a 1 M HCl aqueous solution and finally with 200 mL of saturated solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$ and the monomer recovered by evaporation of CH_2Cl_2 under vacuum. The resulting monomer **2** was recrystallized from diethyl ether. Yield: 28%. ^{1}H NMR ($CDCl_3$) δ: 4.71 (d, 2H, CH_2), 4.57 (s, 2H, CH_2), 4.21 (d, 2H, CH_2), 1.47 (s, 9H, CH_3), 1.41 (s, 3H, CH_3).

Synthesis of poly(ethylene oxide)-α,ω-methylcarboxy trimethylene carbonate (MTC-PEO-MTC)

In a flame-dried round bottom flask kept under nitrogen and thermostatized at 0 °C in an ice bath, MTC-COOH (1.45 g, 9 mmol) and 5 mL of anhydrous THF were mixed. Once the medium was homogeneous, DCC (0.93 g, 4.5 mmol) preliminarily dissolved in a minimal amount of anhydrous THF was added to the medium. The immediate precipitation of *N*,*N*′-dicyclohexylurea in the medium indicates the formation of the anhydride. In a second flame-dried round bottom flask under nitrogen, 3 g of α,ω-hydroxyl polyethylene oxide $PEO(OH)_2$ (1.5 mmol) were introduced. The polymer was dried by 3 successive azeotropic distillations with anhydrous toluene prior to being dissolved in 50 mL of anhydrous THF at 40 °C. The polymer solution was then transferred to the anhydride solution under nitrogen by means of a stainless steel capillary. After 15 h of reaction at room temperature, the urea salt was removed by filtration and the PEO cross-linker is recovered by precipitation in 8 volumes of cold diethyl ether. Yield: 94%. ^{1}H NMR ($CDCl_3$) δ: 4.69 and 4.19 (d, 4H, 2 CH_2), 3.64 (t, 4*n*H, 2*n*CH_2), 1.35 (s, 3H, CH_3).

Synthesis of polycarbonate-based hydrogels by organocatalytic ROP

In a typical experiment, a glass vial in a glove box, equipped with a magnetic stirrer, was charged with 59 mg of **1** (0.13 mmol), 288 mg of **2** (0.1) (see Scheme 1), 300 mg of MTC-PEO-MTC (0.13 mmol) and 34 mg of TU (0.09 mmol). Reactants were dissolved in 0.5 mL of anhydrous CH_2Cl_2. In a second glass vial, 3 mg of 2,2-bis(methylol)-3-methyl-3-carboxy-benzyl (0.013 mmol) and 40 mg of DBU (0.26 mmol) were dissolved in 90 μL of CH_2Cl_2 to reach a final 1 M monomer concentration. Once the monomer mixture was homogeneous, the initiator solution was added quickly. The mixture was stirred for a few seconds and the magnetic stirrer was then removed from the medium. The polymerization was allowed to occur for 24 h, subsequently stopped by addition of few drops of benzoic acid solution in CH_2Cl_2. The hydrogel obtained was finally washed by swelling in a H_2O/THF (1/1 v/v) mixture in order to extract catalysts and soluble fractions and dried under vacuum until constant weight, $F_G = 92\%$.

Selective de-protection of guanidinium and carboxylic acid functions of the hydrogels with trifluoroacetic acid

In a typical experiment, a hydrogel slab was immersed in a CH_2Cl_2/TFA mixture (85/15 v/v) and left for 24 h on an orbital stirrer. The hydrogel was then thoroughly washed by swelling in H_2O/THF (1/1 v/v) in order to extract away *tert*-butanol and TFA and was finally dried under vacuum until constant weight.

Swelling experiments

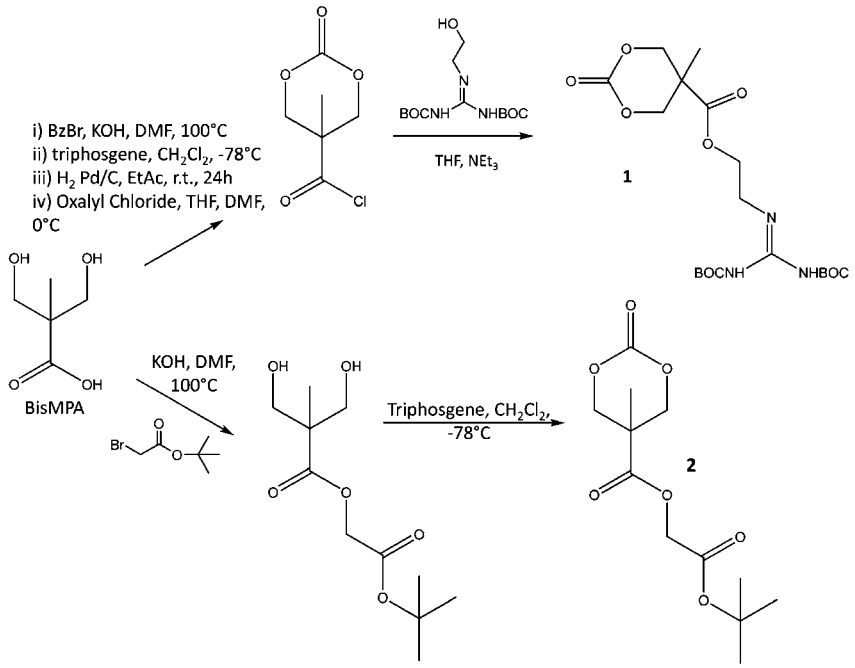
Dry hydrogel slabs were immersed in buffered aqueous solutions (pH 4 and pH 8) and withdrawn at determined time intervals, blotted with tissue paper to absorb excess surface water, and weighed. The swelling degree was determined at room temperature as a function of time using the following equation:

$$S\ (\%) = (m_w/m_d)/m_d$$

in which m_w and m_d represent the masses of the wet and dry gels, respectively.

Results and discussion

Six-membered cyclic carbonates bearing BOC-protected guanidine group **1** and *tert*-butoxy-protected carboxylic acid **2**, respectively, have been synthesized and characterized by ^{1}H NMR (Scheme 1 and Schemes S1 and S2 in the ESI†), using the



Scheme 1 Synthesis of trimethylenecarbonate-5-methyl-5-carboxyethylguanidine-1,3-*tert*-butyloxycarbonyl (MTC-GuaBOC) **1** and trimethylenecarbonate-5-methyl-5-carboxy-*tert*-butylacetate (MTC-*t*BAc) **2**.

protocol described by Pratt *et al.*[41,52] Poly(ethylene oxide)-α,ω-methylcarboxy trimethylene carbonate (MTC-PEO-MTC) cross-linker was obtained by esterification of the commercially available poly(ethylene oxide)-α,ω-hydroxyl (M_n = 2 kg mol^{-1}) by 5-methyl-2-oxo-[1,3]dioxane-5-carboxylic acid (MTC-COOH) preliminarily converted into anhydride using the *N*,*N*′-dicyclohexylcarbodiimide (DCC) coupling agent in anhydrous THF. After 15 h of reaction, the urea salt was removed by filtration and the PEO cross-linker recovered by selective precipitation in cold diethylether. ^{1}H NMR data in $CDCl_3$ reveal the presence of methylene oxocarbonate protons at 4.2 and 4.7 ppm while quantitative esterification of PEO was further evidenced by MALDI-TOF where only a population corresponding to [MTC-PEO-MTCNa]$^+$ was observed. No trace of $PEO(OH)_2$ or mono-esterified HO-PEO-MTC were detected in the mass spectrum (Scheme S3, ESI†).

In a second set of experiments, cross-linked hydrogels were produced by ring-opening copolymerization of **1**, **2** and MTC-PEO-MTC initiated by 2,2-bis(methylol)-3-methyl-3-carboxybenzyl in CH_2Cl_2 using DBU and TU as a catalyst and co-catalyst, respectively. The choice for this catalytic system was motivated by its high selectivity for ring-opening polymerization at the expense of non-desired transesterification reactions. This control stems from the high selectivity of this catalytic system toward the strained cyclic carbonate of the monomer relative to the acyclic carbonate and the ester moieties of the polymer chains. Therefore, very good control over macromolecular parameters can be achieved with molar molecular weight depending on the initial monomer to –OH initiator ratio. The initial $[\mathbf{1}+\mathbf{2}]_0/[\text{MTC-PEO-MTC}]_0/[\text{I}]_0/[\text{DBU}]_0/[\text{TU}]_0$ molar ratio was fixed to 90/10/1/20/7, which leads to a total degree of polymerization of 100. A molar fraction in cross-linker was fixed to 10 mol% for each sample while the ratio of **1** and **2** was modulated from 0 to 90 mol%. All polymerizations were carried out at room temperature in a sealed glass vial in the glove box with an initial monomer concentration of 1 mol L^{-1}. After 24 h of reaction, the catalyst is deactivated by addition of a few drops of benzoic acid solution in toluene and the swollen network disks were removed from their container. All polymer networks are finally purified by immersion in a solvent mixture of distilled water/THF (1/1 v/v) in order to release catalyst and soluble fractions (non-reacted monomers, cross-linker and polycarbonate oligomers containing or not some PEO grafts). Finally, gel fractions (F_G) of the hydrogels dried under vacuum at room temperature for 24 h were determined by weighing the insoluble parts (w_g) after solvent evaporation and knowing the initial weight (w_p) of co-monomers introduced: $F_G = w_g/w_p$. Table 1 gathers the initial molar composition and the gel fractions obtained for each sample. It turns out that, whatever the targeted composition, monolithic and transparent hydrogels with F_G as high as 92% or more were obtained. In order to validate our purification technique and make sure that no non-cross-linked monomers remained entrapped in the polymer network, diffusion-filtered HR-MAS ^{1}H NMR analysis spectra were recorded on each network swollen in $CDCl_3$. This technique proved very efficient to check that release of all non-grafted molecules was complete. Indeed, as demonstrated previously, application of an appropriate diffusion-filtered pulse sequence (Longitudinal Eddy Current Relaxation Delay with Bipolar Gradient Pulses" (LEDBPP), see Experimental section)[49,50] reveals for each purified hydrogel sample the absence of resonances arising from the monomer or catalyst species, the resonances of which are spectroscopically suppressed from the ^{1}H NMR spectrum on the basis of their translational mobility upon application of the above LEDBPP pulse sequence. As isotropic rotational mobility of the chemical moieties is a necessary

Table 1 Initial monomer composition and gel fraction (F_G) of the hydrogels obtained by ROP catalyzed by DBU/TU and initiated by 2,2-bis(methylol)-3-methyl-3-carboxy-benzyl in CH_2Cl_2 at r.t. for 24 h

Hydrogels	**1** (mol%)	**2** (mol%)	MTC-PEO-MTC(mol%)	F_G (%)
H_{90-0}	0	90	10	98
H_{80-10}	10	80	10	92
H_{60-30}	30	60	10	96
H_{40-50}	50	40	10	98
H_{0-90}	90	0	10	92

condition for HR-MAS NMR to be applicable,[49,50,53] rotationally mobility-refrained functions (*e.g.* methylene protons from the polycarbonate backbone) also merge into the baseline. Taking into account the almost quantitative gel fraction and the effective purification step as assessed by HR-MAS ^{1}H NMR, it can be stated that the final composition of the hydrogels is similar to the composition in the feed.

For the sake of clarity, all samples are depicted by the symbol H_{x-y} (with H for "Hydrogel") with in subscript the respective composition in monomer **1** (y) and **2** (x).

The transparency of the hydrogels in the dried state is noteworthy, even though the weight fraction in PEO is ranging from 38 to 44%, indicating that PEO is not able to crystallize within the network. This assumption was confirmed by DSC analysis in which all thermograms obtained after the second scan under inert atmosphere (N_2) revealed the absence of PEO melting point usually observed at 50 °C (Fig. 1). This absence of crystallinity can be ascribed to a homogeneous distribution of the PEO cross-linker within the polymer network.

In order to promote self-assembling within the network and strengthen the mechanical properties of the resulting hydrogels, de-protecting the guanidine and carboxylic acid functions is required. For this purpose, model de-protection reactions were first performed on the respective homopolymers in a CH_2Cl_2/TFA mixture solution (85/15, v/v) for 24 h at room temperature. The effectiveness of the de-protection of guanidine and carboxylic acid was evidenced by ^{1}H NMR analyses in DMSO-d_6 revealing that at most 1 mol% of the residual *tert*-butoxy methyl proton resonance at 1.48 ppm was left for the homopolycarbonate guanidine, while the homopolycarbonate carboxylic acid was found to be fully de-protected. It is worth mentioning that this de-protection is selective for *tert*-butoxy functions and does not affect the polycarbonate backbone, as further confirmed by ^{1}H NMR spectra of the homopolymers from which an excellent agreement between expected and experimental degrees of polymerizations was obtained. This procedure was applied to hydrogel slabs of known weight by immersion in CH_2Cl_2/TFA mixtures (85/15, v/v) (Scheme 2) for 24 h at room temperature and gentle shaking on an orbital shaker. The hydrogels were then thoroughly washed in a mixture of THF/H_2O (50/50 v/v) for 48 h at room temperature and dried under vacuum. De-protected hydrogels are denoted H_{x-y}dep where x and y stand for the molar composition in carboxylic acid and guanidine carbonate, respectively, and *dep* for de-protected. Diffusion filtered HR-MAS ^{1}H NMR spectra in $CDCl_3$ revealed the total absence of resonance arising from the rotationally mobile BOC and *tert*-butyl groups usually observed around 1.5 ppm, therefore confirming the complete de-protection of the gels. The weight of each de-protected sample is in satisfactory agreement with the expected one, evidencing not only the full de-protection of the

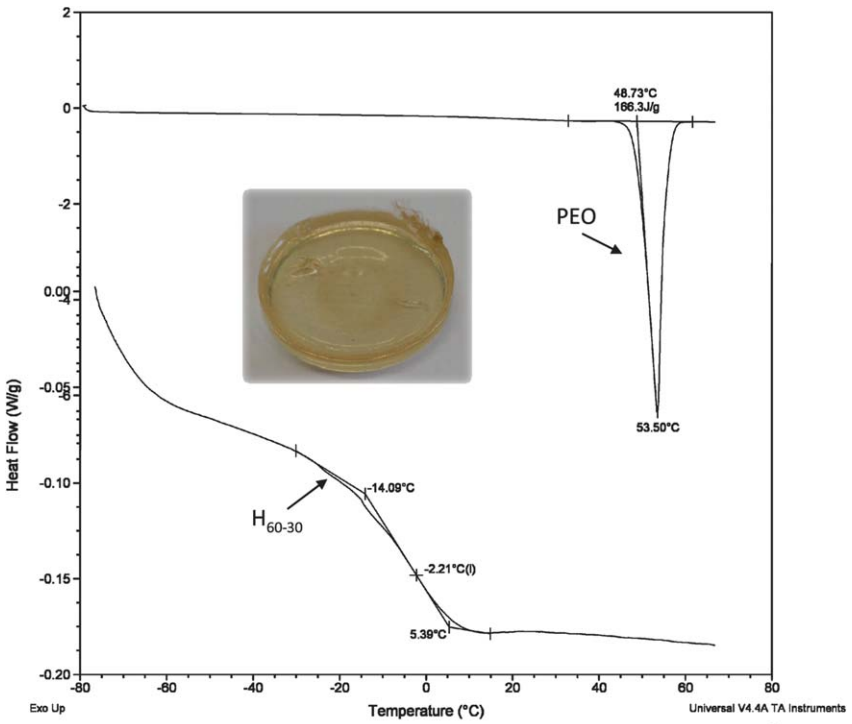
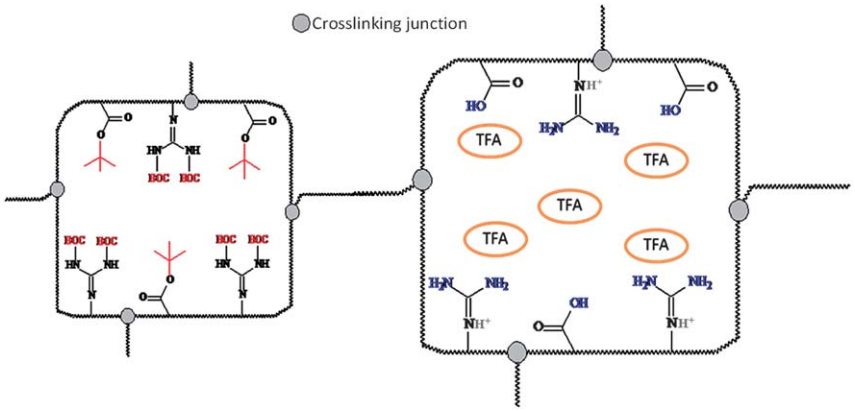


Fig. 1 DSC thermograms of PEO (2 K) and hydrogel H_{40-50} recorded after the 2nd scan under N_2.



Scheme 2 De-protection of guanidine and carboxylic acid functions within the polymer networks by trifluoroacetic acid (TFA) in CH_2Cl_2 solution at room temperature.

aforementioned functionalities but also the absence of hydrogel damages in such an acidic environment. Differential scanning calorimetry (DSC) analyses as performed under nitrogen on the de-protected hydrogels revealed an increase of the glass transition temperature, T_g, indicating a modification of the nature of the hydrogel samples (Table 2).

The presence of selective interactions occurring inside the de-protected polymer networks has been first evidenced by differential scanning calorimetry (DSC). Indeed, it was already reported that macromolecular interactions in miscible polymer blends influence the composition dependence of the glass transition temperature, T_g, and the increment of heat capacity, ΔC_p.[54,55] In a non-interacting miscible system, the T_g and ΔC_p display a linear dependence on the composition. In strongly interacting blends, T_g and ΔC_p present a positive or a negative deviation from linearity, with the T_g lying no longer between those of the pure components. In this work, each hydrogel sample was characterized by DSC under nitrogen using a heat–cool–heat mode (heating rate: 10 °C min^{-1}; cooling rate: 5 °C min^{-1}). Each thermogram exhibits not only a single T_g, evidencing the good miscibility between the PEO cross-linker and the functional polycarbonate backbone, but also a positive deviation from linearity in the plot of T_g against composition, proving unambiguously the presence of segmental interactions occurring into the network (Fig. 2).[54,55] The maximum T_g value being reached around the stoichiometric composition of guanidine and carboxylic acid functions, it can be assumed that the positive T_g deviation is the result of secondary interactions bridging guanidine and carboxylic acid moieties.

Interestingly as revealed by Fig. 3, HR-MAS NMR analysis performed on de-protected hydrogels in D_2O reveals that upon increasing the guanidine composition, the oxocarbonyl carbonyl methylene proton signal shifts down from 4.77 to 4.68 ppm near the stoichiometric composition, which can reasonably be ascribed to a specific interaction between the aforementioned chemical groups.

Selective de-protection was also evidenced by swelling experiments in demineralised water and comparison of the swelling profile of the de-protected and protected hydrogels. Practically, dry hydrogel slabs were immersed in a large volume of demineralised water at room temperature and withdrawn at determined time intervals, blotted with tissue paper to absorb the

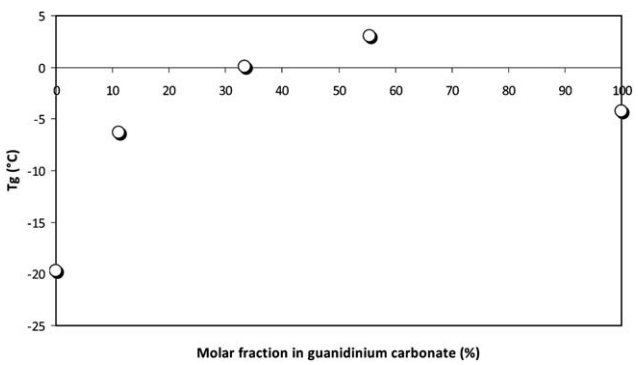


Fig. 2 Glass transition temperature (T_g) of the de-protected hydrogels against their molar composition.

Table 2 Glass transition temperature of the protected and de-protected hydrogels determined by DSC under nitrogen in a heat–cool–heat mode (heating rate: 10 °C min^{-1}, cooling rate 5 °C min^{-1}) and the conversion of de-protection of the polymer networks

Hydrogels	T_g/°C	De-protected hydrogels	T_g/°C	Conversion of de-protection[a] (%)
H_{90-0}	−22.4	H_{90-0}dep	−19.7	100
H_{80-10}	−19.3	H_{80-10}dep	−6.4	100
H_{60-30}	−2.3	H_{60-30}dep	0	100
H_{40-50}	−9.7	H_{40-50}dep	3	100
H_{0-90}	−23.8	H_{0-90}dep	−4.25	100

[a] As determined by diffusion filtered HR-MAS ^1H NMR in $CDCl_3$, see text.

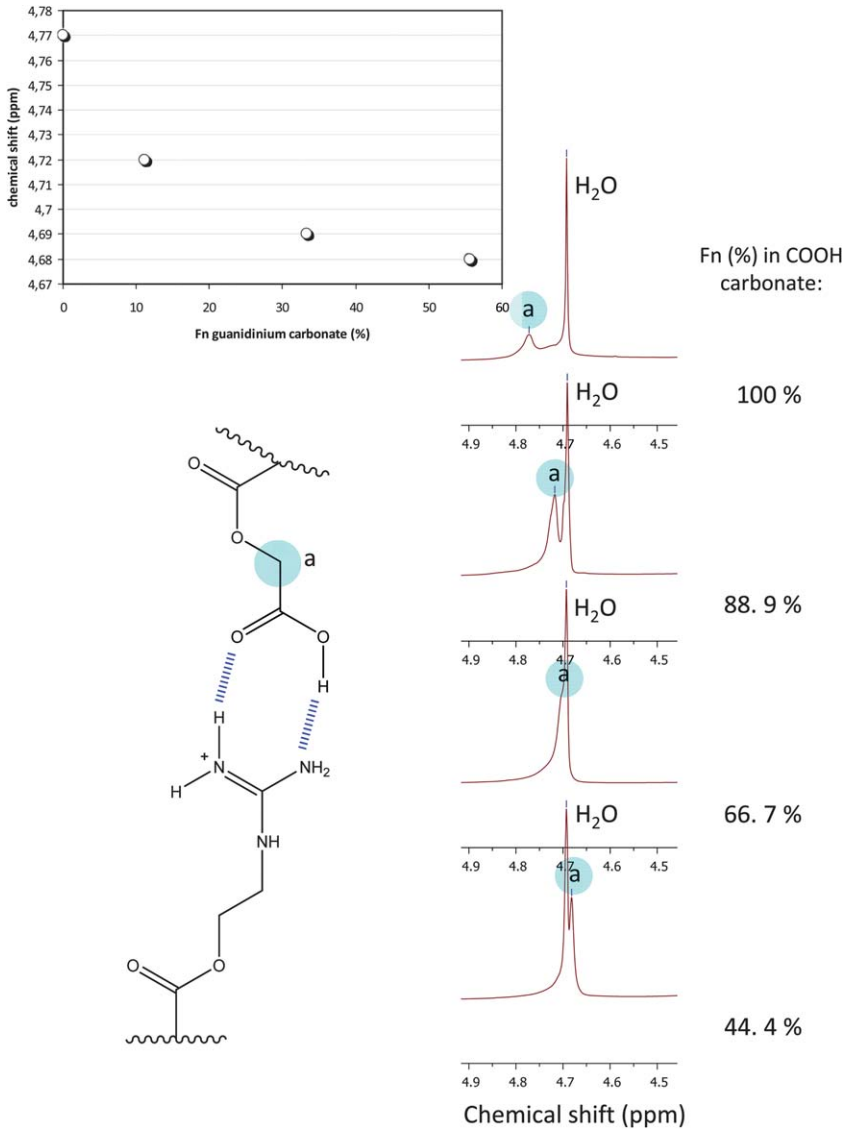


Fig. 3 Chemical shift of the oxocarbonyl carbonyl methylene proton (H_a) resonance with the molar composition of the gel in D_2O measured by HR-MAS NMR in a diffusion filter pulse sequence.

excess of water at surface and weighed. The swelling degree was determined as a function of time, using the following relationship:

$$S\,(\%) = (m_w - m_d)/m_d$$

with m_w and m_d the mass of the wet and dry gel, respectively.

All tests were performed in triplicate. Fig. 4 presents the swelling profile of gels H_{40-50} and H_{40-50}dep in deionised water at r.t. As clearly evidenced, the de-protection of carboxylic acid and guanidinium functions led to a significant increase of water uptake, *i.e.*, S_{eq}: 175.7%, when compared to the protected hydrogel (S_{eq}: 62.4%), owing to the de-protection of highly hydrophilic groups.

In order to rule out possible hydrolysis of carbonate links of hydrogels, that could likewise be responsible for water uptake increase, the reproducibility of the swelling behaviour of hydrogels H40-50 in deionised water was assessed. As displayed in Fig. 5, the gel presents exactly the same swelling profile and reaches the same degree of swelling at equilibrium, demonstrating the stability of the network towards hydrolysis in deionised water.

Self-assembling properties between carboxylic acid and guanidinium functions have also been evidenced by swelling test, both in acetate buffer (pH 4, ionic strength: 0.20) and in phosphate buffer (pH 8, ionic strength: 0.27). In Fig. 6 the swelling degree at equilibrium reached pH 4 and pH 8 and is plotted as a function of the hydrogel composition.

As expected, the hydrogel containing only carboxylic acid functions (H_{90-0}dep) presents a higher degree of swelling at equilibrium in the basic condition. Indeed, at pH 4 and therefore below the pK_a of the carboxylic acid (pK_a: 4.75), all acidic functions are protonated and can form H-bonds in between themselves. These secondary interactions constitute additional cross-linking points, triggering a reduced swelling degree (S_{eq} H_{90-0}dep = 71%, Scheme 3A). In contrast, at pH 8, all functions

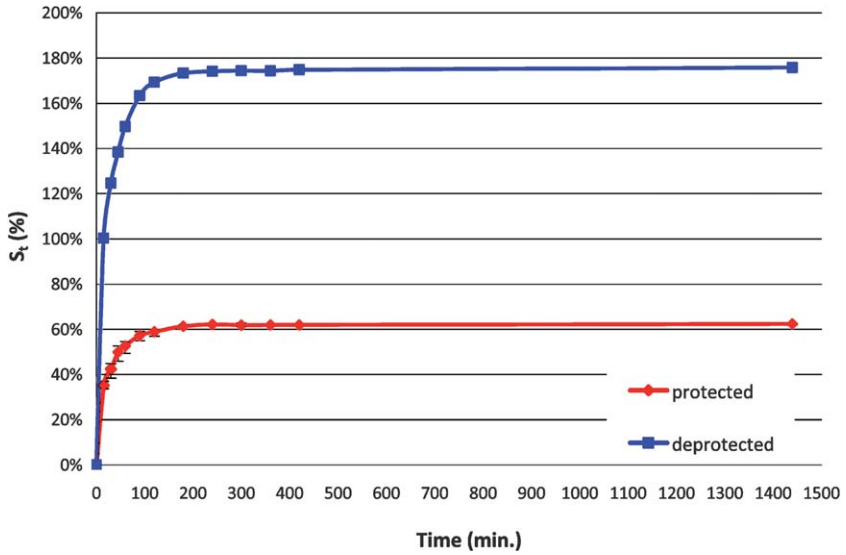


Fig. 4 Swelling profile of H_{40-50} (red curve) and H_{40-50}dep (blue curve) in deionised water at r.t. Mean relative error over $S_t = \pm 4\%$.

are in anionic carboxylate form. The resulting electrostatic repulsion occurring between negative charges is responsible for the gel expansion and the significantly larger water uptake (S_{eq} H_{90-0}dep = 680%, Scheme 3B). The hydrogel containing 100% of guanidine functions (H_{0-90} dep) displays a very important degree of swelling at pH 4, at which all guanidine moieties are protonated to guanidinium cations. Similarly to the gel H_{90-0}dep, the gel H_{0-90}dep swells considerably to alleviate the electrostatic repulsion between cationic charges (S_{eq} = 1221%). At pH 8, a clear decrease of S_{eq} is measured (H_{0-90}dep : S_{eq} = 586%). While the reported pK_a value of guanidine ions is 13.6,[56] no value for guanidinium alkyl derivatives is, to the best of our knowledge, available in the literature. Acetyl guanidine is meanwhile characterized by a pK_a of 8.3.[56] Therefore, and based on the hydrogel behaviour, it can be assumed that the pK_a is close to 8–9. It is stressed that the hydrogels were found to be very unstable at pH 8 resulting in H_{0-90} dep to completely degrade within 24 h. This was ascribed to the hydrolysis of carbonate links under basic conditions. Between these two extreme compositions, interesting swelling behaviour of the gels has been observed. At pH 4, carboxylic acid functions are protonated, allowing H-bonding to occur between acidic functions and possibly also with neighbouring PEO fragments. As the molar composition in guanidinium cations increases, carboxylic acid functions can establish H-bonds with guanidinium ions as well, explaining the smooth increases of S_{eq}. Beyond the stoichiometric composition, the additional cationic guanidinium moieties are not stabilized by H-bonding and the electrostatic repulsions present in the network contribute to the large increase of S_{eq}. At pH 8, the carboxylic acid functions are fully de-protonated to carboxylates

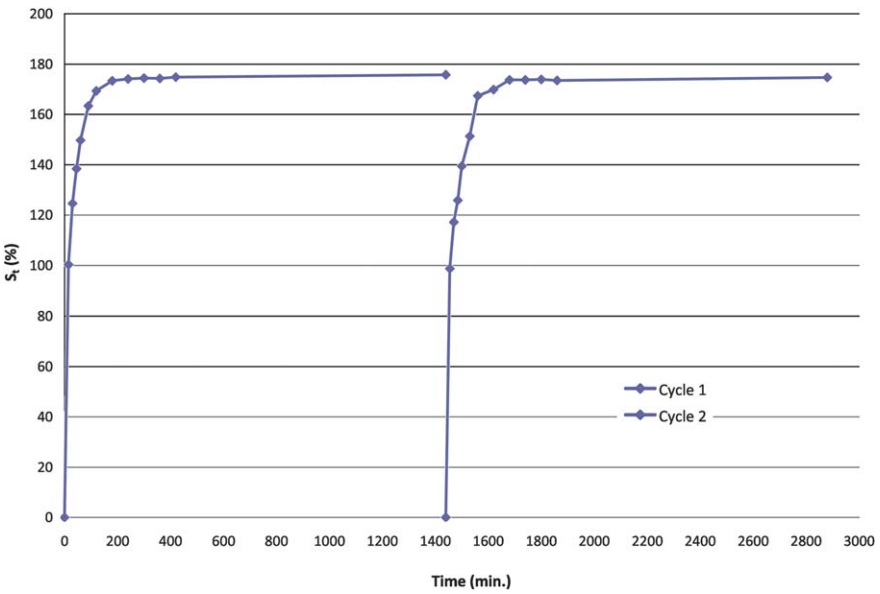


Fig. 5 Reproducibility of the swelling behaviour of hydrogel H_{40-50}dep in deionised water. Mean relative error over $S_t = \pm 4\%$.

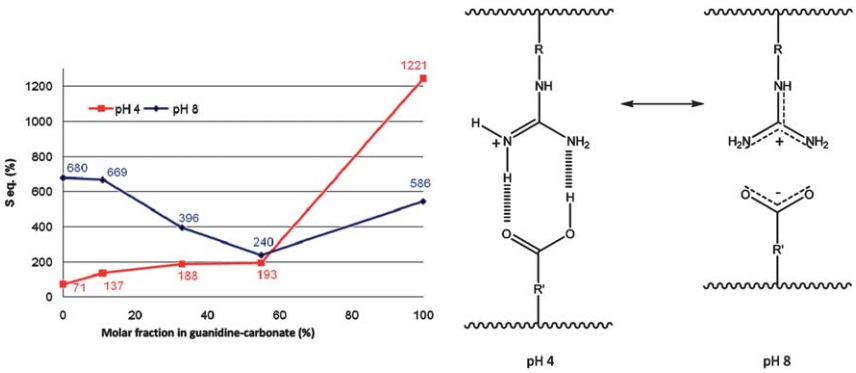
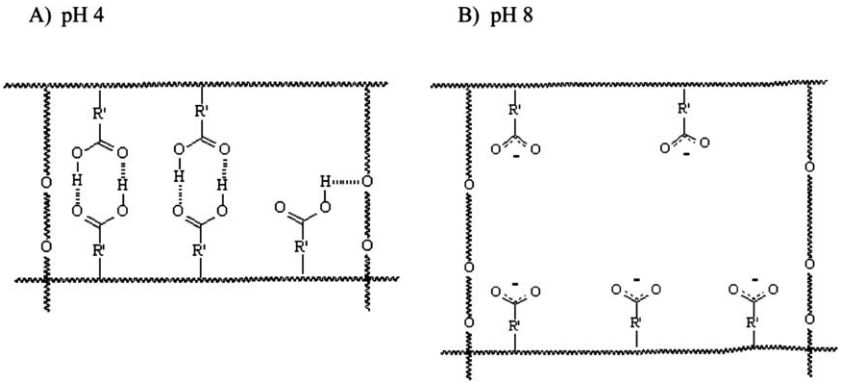


Fig. 6 Effect of the molar composition of de-protected hydrogels on the degree of swelling at equilibrium (S_{eq}) at pH 4 (red curve) and pH 8 (blue curve). Mean relative error over $S_t = \pm 6\%$.



Scheme 3 Schematic representation of the swelling behaviour of carboxylic acid-containing hydrogels at (A) pH 4 and (B) pH 8.

even though guanidine functions are partially protonated. When the hydrogel composition tends to the stoichiometric 1/1 ratio, cationic and anionic charges are in balance and lead to neutralization of the hydrogel, and therefore to a decrease of the degree of swelling. These swelling tests in acidic and basic conditions demonstrate not only the adaptive properties of these hydrogels but also confirm the presence of secondary interactions occurring inside the polymer networks.

Conclusion

The synthesis of well-defined polycarbonate hydrogels containing lateral groups able to self-assemble through secondary interactions has been carried out with success by metal-free ring-opening polymerization. HR-MAS NMR was found to be an efficient tool to discriminate between rotationally mobile groups like *tert*-butyl functions and constrained functions as the polycarbonate backbone. Even better, this technique enabled us to assess our purification protocol of hydrogels since no signal arising from translationally mobile non-reacted monomers or free catalyst was detected. Selective de-protection of *tert*-butyl and BOC groups was achieved successfully in TFA/CH_2Cl_2 (15/85) solution without any alteration of the polycarbonate backbone as supported by NMR. Finally, self-assembling of carboxylic acid and guanidine functions has been fully evidenced first by DSC analysis where a positive deviation from linearity of the plot of T_g *versus* composition was observed, proving unambiguously the presence of segmental interactions occurring within the network. Moreover, it appeared that the maximum of the deviation was close to the 1/1 stoichiometric composition in carboxylic acid and guanidine functions. This self-assembling property was further supported by HR-MAS NMR in D_2O with a composition dependent shift of the methylene oxocarbonyl carbonyl proton resonance. Last but not least, swelling testing in buffer aqueous solution of pH 4 and pH 8 allowed us to provide some support to the stabilisation of the network either by H-bonding or by electrostatic interactions. This work opens the way to new polycarbonate-based materials with improved performances allowing development of new materials for tissue engineering or drug delivery systems.

Acknowledgements

This work was supported by the European Commission and Region Wallonne (FEDER Program) in the frame of Materia Nova and OPTI^{2}MAT program of excellence, by the Interuniversity Attraction Pole program of the Belgian Federal Science Policy Office (PAI 6/27) and FNRS-FRFC. Dr Pascal Gerbaux, FNRS Research associate, and Dr Julien De Winter, FNRS fellow, are thanked for the mass analysis experiments. R.W. is indebted to the Fund for Scientific Research-Flanders (FWO, Belgium) for financial support (Grant G.0064.07) and to the Research Council (Onderzoeksraad) of the Vrije Universiteit Brussel for financial support (Concerted Research Action, Grant GOA31 and other grants).

References

1 G. Rokicki, *Prog. Polym. Sci.*, 2000, **25**, 259.
2 L. B. Lu and K. L. Huang, *J. Polym. Sci., Part A: Polym. Chem.*, 2005, **43**, 2468.
3 B. Ochiai and T. Endo, *Prog. Polym. Sci.*, 2005, **30**, 183.
4 D. J. Darensbourg, *Chem. Rev.*, 2007, **107**, 2388.
5 M. Kroger, C. Folli, O. Walter and M. Doring, *Adv. Synth. Catal.*, 2006, **348**, 1908.
6 R. C. Jeske, J. M. Rowley and G. W. Coates, *Angew. Chem., Int. Ed.*, 2008, **47**, 6041.
7 H. R. Kricheldorf, R. Dunsing and A. S. I. Albet, *Makromol. Chem.*, 1987, **188**, 2453.
8 A. C. Albertsson and M. Sjoling, *J. Macromol. Sci., Part A: Pure Appl. Chem.*, 1992, **29**, 43.
9 T. Ariga, T. Takata and T. Endo, *Macromolecules*, 1997, **30**, 737.
10 J. Matsuo, K. Aoki, F. Sanda and T. Endo, *Macromolecules*, 1998, **31**, 4432.
11 H. Keul, R. Bächer and H. Höcker, *Makromol. Chem.*, 1986, **187**, 2579.
12 H. R. Kricheldorf, J. Jenssen and I. Kreisersaunders, *Makromol. Chem.*, 1991, **192**, 2391.
13 K. R. Carter, R. Richter, H. R. Kricheldorf and J. L. Hedrick, *Macromolecules*, 1997, **30**, 6074.
14 P. Dobrzynski and J. Kasperczyk, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 3184.
15 M. Helou, O. Miserque, J. M. Brusson, J. F. Carpentier and S. M. Guillaume, *ChemCatChem*, 2010, **2**, 306.
16 H. R. Kricheldorf and A. Stricker, *Macromol. Chem. Phys.*, 1999, **200**, 1726.
17 H. R. Kricheldorf, G. Behnken, G. Schwarz, P. Simon and M. Brinkmann, *J. Macromol. Sci., Part A: Pure Appl. Chem.*, 2009, **46**, 353.
18 T. F. Al-Azemi and K. S. Bisht, *Polymer*, 2002, **43**, 2161.
19 N. E. Kamber, W. Jeong, R. M. Waymouth, R. C. Pratt, B. G. G. Lohmeijer and J. L. Hedrick, *Chem. Rev.*, 2007, **107**, 5813.
20 D. Bourissou, S. Moebs-Sanchez and B. Martin-Vaca, *C. R. Chim.*, 2007, **10**, 775.
21 K. S. Bisht, Y. Y. Svirkin, L. A. Henderson, R. A. Gross, D. L. Kaplan and G. Swift, *Macromolecules*, 1997, **30**, 7735.
22 I. K. Varma, A. C. Albertsson, R. Rajkhowa and R. K. Srivastava, *Prog. Polym. Sci.*, 2005, **30**, 949.
23 J. Feng, F. He and R. X. Zhuo, *Macromolecules*, 2002, **35**, 7175.
24 R. A. Gross, B. Kalra and A. Kumar, *Appl. Microbiol. Biotechnol.*, 2001, **55**, 655.
25 L. Zhang, F. Nederberg, J. M. Messman, R. C. Pratt, J. L. Hedrick and C. G. Wade, *J. Am. Chem. Soc.*, 2007, **129**, 12610.
26 L. Zhang, F. Nederberg, R. C. Pratt, R. M. Waymouth, J. L. Hedrick and C. G. Wade, *Macromolecules*, 2007, **40**, 4154.
27 E. F. Connor, G. W. Nyce, M. Myers, A. Mock and J. L. Hedrick, *J. Am. Chem. Soc.*, 2002, **124**, 914.
28 G. W. Nyce, T. Glauser, E. F. Connor, A. Mock, R. M. Waymouth and J. L. Hedrick, *J. Am. Chem. Soc.*, 2003, **125**, 3046.
29 O. Coulembier, A. P. Dove, R. C. Pratt, A. C. Sentman, D. A. Culkin, L. Mespouille, P. Dubois, R. M. Waymouth and J. L. Hedrick, *Angew. Chem., Int. Ed.*, 2005, **44**, 4964.
30 O. Coulembier, B. G. G. Lohmeijer, A. P. Dove, R. C. Pratt, L. Mespouille, D. A. Culkin, S. J. Benight, P. Dubois, R. M. Waymouth and J. L. Hedrick, *Macromolecules*, 2006, **39**, 5617.
31 O. Coulembier, L. Mespouille, J. L. Hedrick, R. M. Waymouth and P. Dubois, *Macromolecules*, 2006, **39**, 4001.
32 S. Csihony, D. A. Culkin, A. C. Sentman, A. P. Dove, R. M. Waymouth and J. L. Hedrick, *J. Am. Chem. Soc.*, 2005, **127**, 9079.
33 N. E. Kamber, W. Jeong, S. Gonzalez, J. L. Hedrick and R. M. Waymouth, *Macromolecules*, 2009, **42**, 1634.
34 O. Coulembier, F. Meyer and P. Dubois, *Polym. Chem.*, 2010, **1**, 434.
35 A. P. Dove, R. C. Pratt, B. G. G. Lohmeijer, R. M. Waymouth and J. L. Hedrick, *J. Am. Chem. Soc.*, 2005, **127**, 13798.
36 R. C. Pratt, B. G. G. Lohmeijer, D. A. Long, P. N. P. Lundberg, A. P. Dove, H. B. Li, C. G. Wade, R. M. Waymouth and J. L. Hedrick, *Macromolecules*, 2006, **39**, 7863.
37 B. G. G. Lohmeijer, R. C. Pratt, F. Leibfarth, J. W. Logan, D. A. Long, A. P. Dove, F. Nederberg, J. Choi, C. Wade, R. M. Waymouth and J. L. Hedrick, *Macromolecules*, 2006, **39**, 8574.
38 R. C. Pratt, B. G. G. Lohmeijer, D. A. Long, R. M. Waymouth and J. L. Hedrick, *J. Am. Chem. Soc.*, 2006, **128**, 4556.
39 L. Zhang, R. C. Pratt, F. Nederberg, H. W. Horn, J. E. Rice, R. M. Waymouth, C. G. Wade and J. L. Hedrick, *Macromolecules*, 2010, **43**, 1660.
40 F. Nederberg, B. G. G. Lohmeijer, F. Leibfarth, R. C. Pratt, J. Choi, A. P. Dove, R. M. Waymouth and J. L. Hedrick, *Biomacromolecules*, 2007, **8**, 153.
41 R. C. Pratt, F. Nederberg, R. M. Waymouth and J. L. Hedrick, *Chem. Commun.*, 2008, 114.
42 L. Mespouille, F. Nederberg, J. L. Hedrick and P. Dubois, *Macromolecules*, 2009, **42**, 6319.
43 S. Tempelaar, L. Mespouille, Ph. Dubois and A. P. Dove, *Macromolecules*, 2011, **44**, 2084.
44 K. Fukushima, R. C. Pratt, F. Nederberg, J. P. K. Tan, Y. Y. Yang, R. M. Waymouth and J. L. Hedrick, *Biomacromolecules*, 2008, **9**, 3051.
45 C. B. Cooley, B. M. Trantow, F. Nederberg, M. K. Kiesewetter, J. L. Hedrick, R. M. Waymouth and P. A. Wender, *J. Am. Chem. Soc.*, 2009, **131**, 16401.
46 F. Nederberg, V. Trang, R. C. Pratt, S. H. Kim, J. Colson, A. Nelson, C. W. Frank, J. L. Hedrick, P. Dubois and L. Mespouille, *Soft Matter*, 2010, **6**, 2006.
47 F. Nederberg, V. Trang, R. C. Pratt, A. F. Mason, C. W. Frank, R. M. Waymouth and J. L. Hedrick, *Biomacromolecules*, 2007, **8**, 3294.
48 S. Anseth Kristi, C. Svaldi Dina, T. Laurencin Cato and R. Langer, in *Photopolymerization*, American Chemical Society, 1997, vol. 673, p. 189.
49 R. Warrass, J. M. Wieruszeski and G. Lippens, *J. Am. Chem. Soc.*, 1999, **121**, 3787.
50 S. Iqbal, F. Rodriguez-Llansola, B. Escuder, J. F. Miravet, I. Verbruggen and R. Willem, *Soft Matter*, 2010, **6**, 1875.
51 P. A. Wender, E. Kreider, E. T. Pelkey, J. Rothbard and C. L. VanDeusen, *Org. Lett.*, 2005, **7**, 4815.
52 D. P. Sanders, K. Fukushima, D. J. Coady, A. Nelson, M. Fujiwara, M. Yasumoto and J. L. Hedrick, *J. Am. Chem. Soc.*, 2010, **132**, 14724.
53 J. C. Martins, F. A. G. Mercier, A. Vandervelden, M. Biesemans, J. M. Wieruszeski, E. Humpfer, R. Willem and G. Lippens, *Chem.–Eur. J.*, 2002, **8**, 3431.
54 M. Song, D. J. Hourston, H. M. Pollock and A. Hammiche, *Polymer*, 1999, **40**, 4763.
55 T. K. Kwei, *J. Polym. Sci., Polym. Lett. Ed.*, 1984, **22**, 307.
56 A. Albert, R. Goldacre and J. Phillips, *J. Chem. Soc.*, 1948, 2240.