

pH-responsive biodegradable amphiphilic networks

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Abstract

Copper-mediated azide–alkyne Huisgen’s 1,3-dipolar cycloaddition is a “click” reaction that was successfully used to prepare pH-responsive, amphiphilic and biodegradable networks. Indeed, this reaction proved to be very efficient in the “one pot” grafting of amino alkyne onto azide containing poly(ϵ -caprolactone) and the cross-linking of these chains by α,ω -dialkynyl poly(ethylene oxide). The pH-controlled release of guests hosted during the cross-linking step was illustrated with an entrapped model dye.

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1. Introduction

The copper-mediated Huisgen’s [3 + 2] cycloaddition of alkyne and azide is the cream of the crop of the class of “click” reactions [1–3] and is increasingly more popular in the tailoring of polymers [4,5]. Quite recently, “click” chemistry was used in combination with ring-opening polymerization of lactones and lactides to synthesize novel functional [6–8], star-shaped [9] and graft copolyesters [6,10,11]. Indeed, copolymers of poly(oxepan-2-one) [poly(ϵ -caprolactone) or PCL] substituted by many different functional groups, e.g., esters, amines, ammonium salts, acrylates, bromides, alcohols, were synthesized by Huisgen’s [3 + 2] cycloaddition of duly substituted alkynes onto PCL-*co*-P(α N₃ ϵ CL) (where α N₃ ϵ CL stands for 3-azidooxepan-2-one) [6–8]. Remarkably, when “click” reaction was carried out in organic solvents, such as *N,N*-dimethylformamide (DMF) and tetrahydrofuran (THF), at 35 °C chain degradation was minimized, if any. For instance, PCL-*graft*-PEO [where PEO stands for poly(ethylene oxide)] was synthesized by coupling an alkyne end-capped PEO onto PCL bearing pendant azides. The experimental conditions used were so mild that the grafting of PEO was also

very effective onto more sensitive PLA-*co*-P(α N₃ ϵ CL) copolyesters (LA stands for lactide or 3,6-dimethyl-1,4-dioxane-2,5-dione) [6]. More recently, a range of novel functional ϵ -caprolactones were made available by the direct cycloaddition of alkynes substituted by several functional groups, e.g., esters, unprotected alcohols, acrylates, ammonium salts, onto 5-azidooxepan-2-one without any detrimental ring-opening of the lactone [12]. Until now, only few examples of cross-linking by “click” chemistry were reported [13–17]. Hawker et al. prepared shell “click” cross-linked nanoparticles [13]. Networks were also prepared by intermolecular cross-linking [14,15]. Although Huisgen’s [3 + 2] cycloaddition is very promising in macromolecular chemistry, a special care must be taken anytime in handling low-molecular weight azides because of their very well-known propensity to explode spontaneously.

Nevertheless, no example is known for cross-linking of aliphatic polyesters by “click” chemistry. In this respect, Vert et al. cross-linked PCL by another chemistry that, however, shortened the chain length, i.e., metallation by lithium *N,N*-diisopropylamide followed by the addition of α,ω -dichloroformate PEO [18]. This paper aims at reporting on the synthesis of an amphiphilic aminated PCL/PEO network by “click” cycloaddition. The role of the amines is to impart pH sensitivity to the cross-linked amphiphile. The strategy relies on the following sequence of reactions: (i) synthesis of an aliphatic

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polyester substituted by chlorides by ring-opening polymerization of 3-chlorooxepan-2-one (α -chloro- ϵ -caprolactone or α Cl ϵ CL) [19], (ii) conversion of chlorides into azides by reaction with sodium azide [7], (iii) amination by reaction of part of the azides with *N,N*-dimethylprop-2-yn-1-amine, (iv) cross-linking by reaction of remaining azides with α,ω -dialkynyl PEO (Scheme 1).

This strategy is actually very flexible for tuning the properties of these pH-sensitive, amphiphilic and biodegradable networks. Indeed, the length of both the hydrophobic PCL chains and the hydrophilic PEO chains can be predetermined in a very large range because their synthesis is living/controlled. Moreover, the content of both the amines and the cross-linkers can be changed at will by the composition of the PCL-*co*-P(α N₃ ϵ CL) copolymer and the stoichiometry of the azide/*N,N*-dimethylprop-2-yn-1-amine reaction.

2. Experimental section

2.1. Materials

Toluene (Chem-lab), tetrahydrofuran (THF; Chem-Lab), dichloromethane (CH₂Cl₂; Chem-lab), *N,N*-dimethylformamide (DMF; Aldrich), pent-4-ynoic acid (Aldrich), *N,N'*-dicyclohexylcarbodiimide (DCC; Aldrich), *N,N*-dimethylpyridin-4-amine (DMAP; Aldrich), α,ω -diOH PEO (*M_w* 2000 g/mol; Fluka), *N,N*-dimethylprop-2-yn-1-amine (Aldrich), copper(I) iodide (Aldrich), triethylamine (Aldrich) were used as received. Poly(α N₃ ϵ CL) was synthesized according to the procedure of Riva et al. [7]. 1,1'-(Methanediyl)dibenzene-4,1-diyl)bis(3-{bis[2-(diethylamino)ethyl]amino}pyrrolidine-2,5-dione), a model yellow dye, was prepared by Michael

reaction of *N*-(2-aminoethyl)-*N*-[2-(diethylamino)ethyl]-*N,N'*-diethylethane-1,2-diamine and 1,1'-(methanediyl)dibenzene-4,1-diyl)bis(1*H*-pyrrole-2,5-dione) according a procedure reported elsewhere [20].

2.2. Preparation of α,ω -dialkynyl PEO

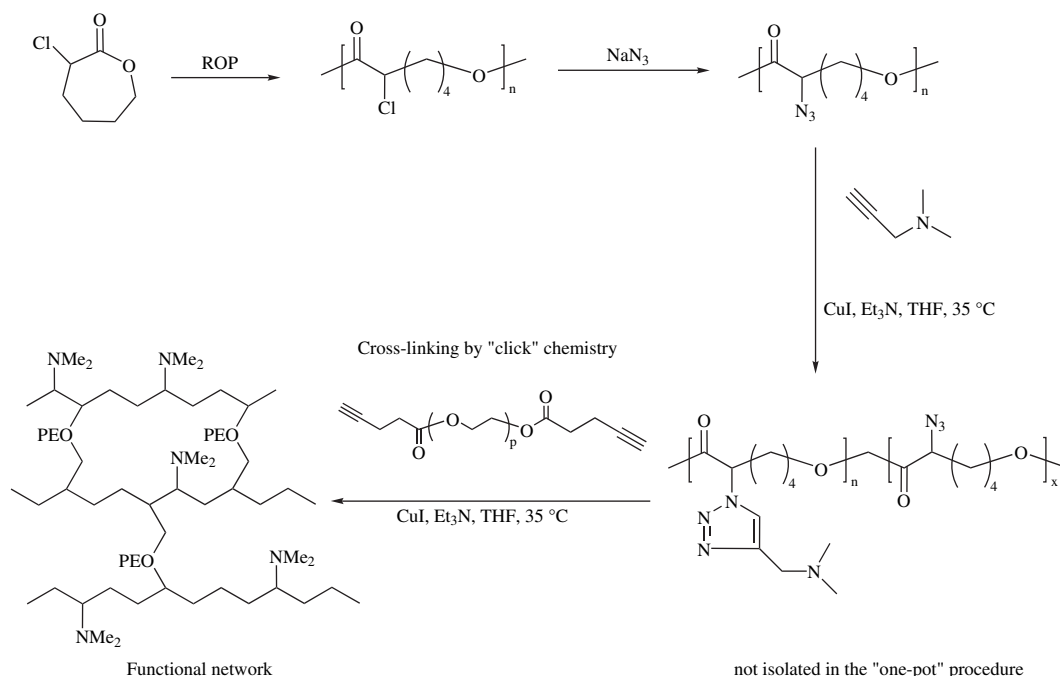
α,ω -DiOH PEO (1.0 g, *M_n* = 2000), 251 mg of DCC and 20 mg of DMAP were added into a previously dried glass reactor and dried by azeotropic distillation of dry toluene (3 times 5 ml). 4-Pentynoic acid (125 mg) dissolved in 10 ml of CH₂Cl₂ was added. The mixture was stirred overnight at room temperature. The *N,N'*-dicyclohexylurea byproduct was removed by filtration. α,ω -Dialkynyl PEO was precipitated in cool diethyl ether and collected by filtration at -20 °C (yield: 48%).

¹H NMR (CDCl₃): 4.25 (t, *J* = 5 Hz, CH₂OC(=O)-, chain ends), 3.6 (s, O-CH₂CH₂O); 2.6 (m, *J* = 8 Hz, C(=O)-CH₂-CH₂-C_{acetylenic}, chain ends); 2.5 (m, *J* = 2 Hz and 7 Hz, C(=O)-CH₂-CH₂-C_{acetylenic}, chain ends); 1.95 (t, *J* = 2 Hz, C(H)_{acetylenic}, chain end). The attribution of the peaks at 2.5 ppm and 2.6 ppm was carried out by 2D COSY NMR spectroscopy (not shown).

IR (cm⁻¹): 3413, 2918, 1643, 1456, 1350, 1257, 1095, 950, 844.

2.3. General procedure for cross-linking of poly(α N₃ ϵ CL-*co*- ϵ CL) with α,ω -dialkynyl PEO

In a glass round-bottom flask reactor were added 600 mg (0.035 mmol of polymer chains and 1.4 mmol of pendant azide) of poly(α N₃ ϵ CL-*co*- ϵ CL) (30 mol% of α N₃ ϵ CL, *M_n* = 17 000),



Scheme 1. General strategy for the synthesis of aminated amphiphilic networks by "click" chemistry.

0.28 g (0.14 mmol) of α,ω -dialkynyl PEO ($M_n = 2000$; 8 cross-links per PCL chain), 36 mg (0.14 mmol) of CuI, 14 mg (0.14 mmol) of triethylamine and 5 ml of THF. After complete dissolution at 35 °C, the solution was transferred into a 1 ml propylene hypodermic syringe used as a mold. The top of the syringe was closed with a parafilm before being placed into an oven at 35 °C. The gel was formed after 1 h, the bottom part of the syringe was cut away with a razor blade and the gel was pushed out of the syringe body with a piston. The gel was repeatedly extracted with 100 ml of dichloromethane (6 times for 40 min each) and then dried in vacuo until constant weight (typically 48 h). When prepared in a syringe, 4 cm long rods with a diameter of 3.5 mm were made available.

2.4. Grafting of *N,N*-dimethylprop-2-yn-1-amine onto poly($\alpha N_3\epsilon CL$) and gelification

Poly($\alpha N_3\epsilon CL$) ($M_n = 10000$, 190 mg, 0.019 mmol of polymer chains and 1.23 mmol of pendant azide) and 30 mg (0.36 mmol) of *N,N*-dimethylprop-2-yn-1-amine were dissolved in 2 ml of THF. CuI (9 mg, 4 mol% with respect to the azide) was added to the reaction mixture that was stirred at 35 °C. The progress of the reaction was monitored by IR spectroscopy. After 150 min the intensity of the azide band (2107 cm^{-1}) did not decrease anymore. When 70% of the azides were converted into amines, the polyester was less soluble, and 0.5 ml of DMF was added to keep the solution homogeneous. A sample was picked out (0.5 ml) for the sake of analysis. The THF solution containing 0.015 mmol of functionalized polyester (cfr supra) was then added with 120 mg (0.06 mmol) of α,ω -dialkynyl PEO ($M_n = 2000$; 8 cross-links per PCL chain) and poured into a syringe used as a mold. The gel was formed after 2 h at 35 °C and recovered as a rod (length = 4 cm, diameter = 3.5 mm) that was repeatedly extracted with 100 ml of dichloromethane (6 times for 40 min each). The gel was then dried in vacuo until constant weight (typically 48 h).

2.5. Swelling experiments

Dry gel (30 mg) was immersed either in a solution of phosphate buffer or into the organic solvent. Three phosphate buffers (pH = 5.5, 6.95 and 9; 0.1 M) and two organic solvents (CH_2Cl_2 and DMF) were used. The sample was periodically removed from the solution with pincers, blotted with a filter paper and carefully weighed. Then, the sample was again immersed in the buffer.

2.6. Characterization

Size exclusion chromatography (SEC) was carried out in THF at 45 °C at a flow rate of 1 ml/min with a SFD S5200 autosampler liquid chromatograph equipped with an SFD refractometer index detector 2000. PL gel 5 μm (10^5 \AA , 10^4 \AA , 10^3 \AA and 100 \AA) columns were calibrated with either polystyrene or PEO standards. ^1H NMR spectra were recorded in CDCl_3 at 400 MHz in the FT mode with a Bruker AN

400 apparatus at 25 °C. Infrared spectra were recorded with a Perkin–Elmer FT-IR 1720X. The gels were analyzed by FT-IR ATR spectroscopy using gallium germanide ATR crystal.

3. Results and discussion

The synthesis of poly($\alpha N_3\epsilon CL$) was previously reported [7] and will not be detailed in this paper. Briefly, poly($\alpha N_3\epsilon CL$) was synthesized by ring-opening polymerization of $\alpha\text{Cl}\epsilon\text{CL}$ initiated by 2,2-dibutyl-2-stanna-1,3-dioxepane (DSDOP) [19] followed by reaction with sodium azide [7]. Very minor chain degradation was observed during the substitution reaction, which increased slightly the polydispersity index [7]. In this work, poly($\alpha N_3\epsilon CL$) with $M_n = 10000$ and $M_w/M_n = 1.6$ was synthesized.

The hydroxyl end groups of α,ω -diOH PEO ($M_n = 2000$) were esterified by pent-4-ynoic acid in THF at room temperature for 24 h, in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) and *N,N*-dimethylpyridin-4-amine (DMAP). New ^1H NMR resonances were observed at 1.9 ppm (alkyne proton), at 2.5 ppm (CH_2CH_2 -alkyne) and at 4.25 ppm ($\text{CH}_2\text{O}-\text{CO}-$) (Fig. 1). The experimental molecular weight ($M_n = 2000$), calculated from the relative intensity of the resonances at 4.25 ppm (for the end groups) and 3.6 ppm (for the $-\text{CH}_2\text{O}-$ groups), is in close agreement with the original value ($M_n = 2000$), which testified to the completeness of the esterification of the PEO chain ends.

Poly($\alpha N_3\epsilon CL$) ($M_n = 10000$) was reacted with α,ω -dialkynyl-PEO ($M_n = 2000$) in a 1/4 molar ratio under the mild conditions that were previously reported as non-degrading for PCL (THF, triethylamine, 35 °C) [6,7]. Gelation was observed within 10 min and no unreacted PEO could be extracted from this network that contained 44 wt% of PEO.

The amphiphilicity of the network was illustrated by swelling experiments in dichloromethane and in water at pHs 5.5 and 9.0, respectively (Fig. 2). Expectedly, no significant influence of the pH on the swelling of the network was observed in water.

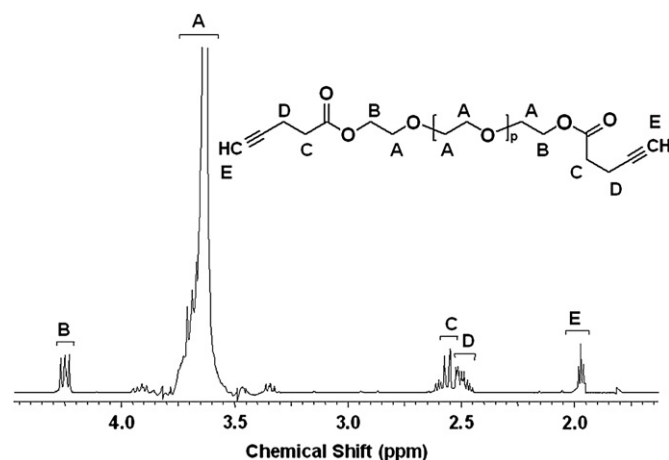


Fig. 1. ^1H NMR spectrum of α,ω -dialkynyl PEO.

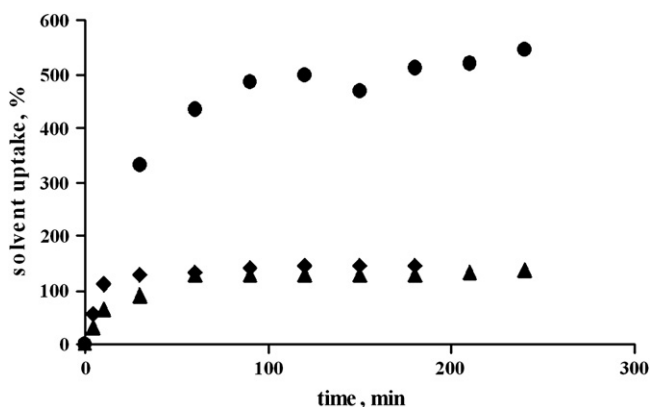


Fig. 2. Swelling behavior of the PCL/PEO network in dichloromethane (●) and in water at pHs 5.5 (◆) and 9.0 (▲) at 25 °C.

PCL with a lower content of azide was also prepared by copolymerization of $\alpha N_3\epsilon CL$ (30 mol%) with ϵCL . Gelation was again promoted by “click” cross-linking of poly($\alpha N_3\epsilon CL$ -*co*- ϵCL) with α,ω -dialkynyl PEO although it took 1 h for gelation to occur.

In order to impart pH sensitivity to the network, tertiary amines were attached onto PCL prior to cross-linking. Poly($\alpha N_3\epsilon CL$) ($M_n = 10\,000$; $M_w/M_n = 1.6$) was reacted with 30 mol% and 70 mol% of *N,N*-dimethylprop-2-yn-1-amine in THF at 35 °C in the presence of copper(I) iodide and triethylamine [7], respectively. After 150 min, a sample was picked out and analyzed by 1H NMR and SEC (DMF, polystyrene calibration). The amine content of PCL was close to expectation, i.e., 32 mol% vs 30 mol% and 65 mol% vs 70 mol%, respectively. The apparent molecular weight (SEC in DMF) was much higher than the original M_n of PCL ($\sim 35\,000$ vs 10 000) and the polydispersity index was 1.5 for the two samples.

The amine containing copolyesters were immediately reacted with α,ω -dialkynyl PEO (80 mol% of PEO), thus without intermediate isolation and purification. Moreover, copper iodide previously used for the grafting of amines remained

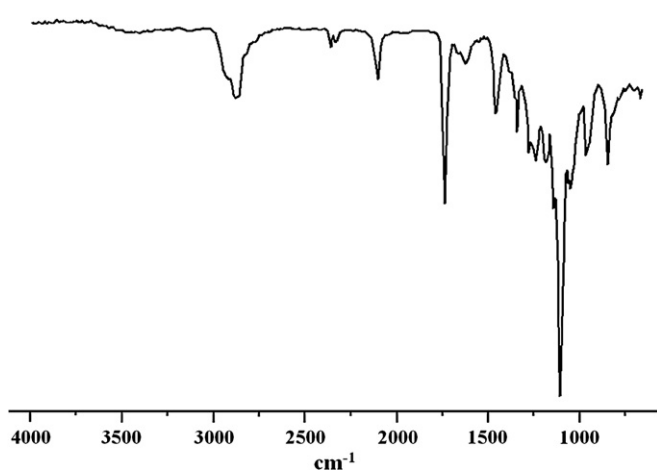


Fig. 3. FT-IR ATR spectrum for the amphiphilic network obtained by gelation of the copolyester containing 70 mol% of amine.

active and thus, no additional catalyst was needed for the cross-linking step. Expectedly, gelation was slower as a result of the lower azide content. Indeed, 50 min and 75 min were needed for gelation of the copolyesters containing 30 mol% and 70 mol% of amine, respectively, to be observed. FT-IR ATR analysis of the networks after THF elimination showed

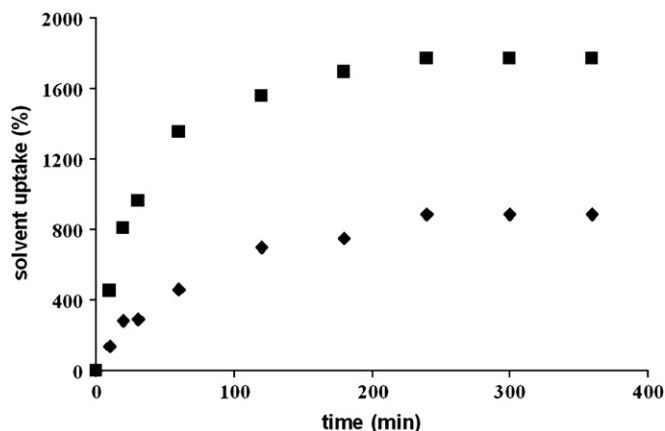


Fig. 4. Swelling behavior of the PCL/PEO network with 30 mol% of pendant amine onto PCL in DMF (◆) and dichloromethane (■) at 25 °C.

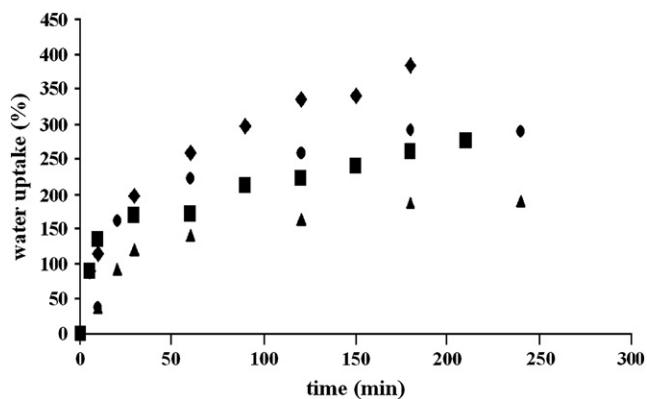


Fig. 5. Swelling behavior of the PCL/PEO network with 30 mol% of amine onto PCL at pHs 5.5 (◆) and 9.0 (■) (first cycle) and pHs 5.5 (●) and 9.0 (▲) (second cycle) at 25 °C.

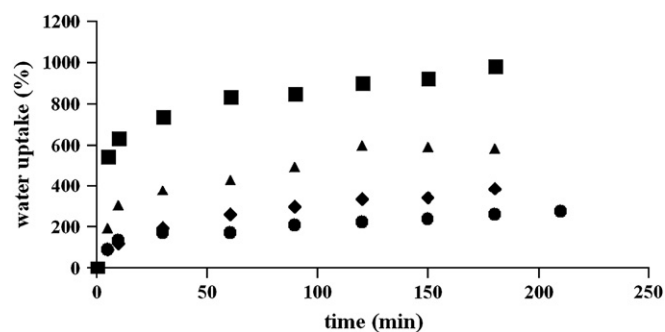


Fig. 6. Swelling behavior of the PCL/PEO network with 30 mol% of amine onto PCL at pHs 5.5 (◆) and 9.0 (●) and with 70 mol% of amine onto PCL at pHs 5.5 (■) and 9.0 (▲) at 25 °C.



Fig. 7. Hydrogel rod prepared by “click” cross-linking in a syringe followed by extraction of the copper catalysts by 0.1 M aqueous EDTA solution.

the vibrations typical of the triazole ring at 1533 cm^{-1} although unreacted azide was left (Fig. 3).

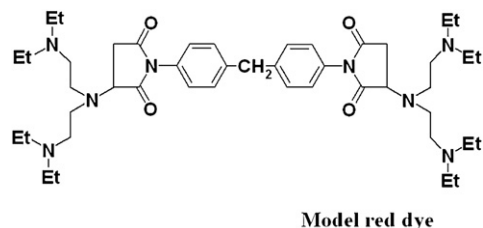
The swelling of the network prepared from PCL with 30 mol% of amine was investigated in DMF and in dichloromethane (Fig. 4) and in water at two pHs 9.0 and 5.5 (Fig. 5). Remarkably, the water uptake was higher at pH 5.5 than at pH 9.0 as a result of the protonation of the pendant amines. After drying, the network was again swollen by water at the same pHs as before (Fig. 5). The swelling was clearly reversible, even though the swelling curves were not typically superposable.

The swelling of the same PCL/PEO network although prepared with PCL containing 70 mol% of amines was substantially increased in water as shown in Fig. 6. Not only the higher hydrophilicity of the constitutive polyester chains can explain this observation, but also a lower effective cross-linking density because less azides were left on the PCL chains for cross-linking by difunctional PEO.

Hydrogel rods were prepared by “click” cross-linking in a hypodermic syringe used as a mold. These gels were completely green because of the copper catalyst they contained. However, after extraction with 0.1 M aqueous ethylene diamine tetraacetic acid (EDTA) solution for 3 days [14], the gels turned out to be quasi colorless (Fig. 7).

Last but not least, the pH sensitivity of the amine containing gels makes them promising hosts for the controlled release of low-molecular weight guests. For the sake of demonstration, 1,1'-(methanediyl)benzene-4,1-diyl)bis(3-{bis[2-(diethylamino)ethyl]amino}pyrrolidine-2,5-dione), a model yellow dye, was incorporated into an amphiphilic PCL/PEO network

during the cross-linking reaction. After drying, the network was swollen in water at pHs 1.0 and 7.0. At pH 1.0, the dye was slowly released as assessed by the yellow color of the aqueous phase after 5 h (Supplementary material). In contrast, the aqueous phase remained colorless at neutral pH within the same period of time. It must be noted that the color of the dye – and thus of the hydrogel rod – changed with pH (Supplementary material).



4. Conclusions

Copper-mediated Huisgen's [3 + 2] cycloaddition proved to be very efficient for the “one pot” grafting of amino alkynes onto azide containing PCL and the cross-linking of these chains by α,ω -dialkynyl PEO. pH-responsive, amphiphilic and biodegradable networks were accordingly prepared. This strategy is very versatile because the molecular weight of the constitutive PCL and PEO chains can be predetermined in a very large range by controlled/living polymerization techniques. The content of azides can also be changed at will, merely by copolymerization of the azide containing ϵ CL with different amounts of ϵ CL.

Finally, it is possible to attach additional functional groups to the network by “click” cycloaddition and to tune accordingly the chemical properties and the biological activity of the networks. “Click” chemistry turned out to be very efficient to graft functional groups (amines in this work) and to perform the cross-linking by a “one pot” procedure. The molar ratio of the amino alkyne and the alkyne end-capped PEO is an additional parameter that controls the degree of cross-linking and the pH sensitivity of the networks.

The pH-controlled release of guests entrapped during the cross-linking step was illustrated by using a model dye. Because “click” chemistry tolerates a variety of functional groups without needing protection/deprotection reactions, these groups could be attached to the network instead of (or in addition to) the tertiary amines. Accordingly, a large variety of functional amphiphilic networks can be envisioned, in order to fit the requirements of specific and diverse applications.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.polymer.2007.12.029](https://doi.org/10.1016/j.polymer.2007.12.029).

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