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Novel Amphiphilic Mikto-Arm Star-Shaped Copolymers for the Preparation of PLA-Based Nanocarriers

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Summary: Three-arm (A_2B) and four-arm (A_2B_2) star-shaped copolymers based on biocompatible and biodegradable hydrophobic poly(ε-caprolactone) (PCL) (A arms) and biocompatible and bioeliminable hydrophilic poly(ethylene oxide) (PEO) (B arms) were synthesized by the coupling of an ω-azide terminated PEO chains with PCL chain bearing one (A_2B) or two (A_2B_2) alkyne functions at the middle of the chain by the copper mediated azide-alkyne cycloaddition (CuAAC). The amphiphilic behavior of these different stars was confirmed by micellization experiments in water followed by dynamic light scattering and transmission electron microscopy analyses. The efficiency to stabilize PLA nanoparticles was investigated in function of the stars structure.

Keywords: aliphatic polyesters; amphiphilic polymers; micelles; nanoparticles; star polymers

Introduction

Nowadays, an increasing interest was paid to the use of nanoparticles for the development of new drug delivery systems. Polymer micelles are well known nanoparticles composed of biocompatible and biodegradable amphiphilic copolymers already used for drug encapsulation. Indeed, many new drugs are poorly soluble in water, which leads to their rapid elimination from the body by enzymatic degradation or by metabolization. Drug encapsulation in nanometric particles improves their solubility in water, increasing the action time by controlling the drug release. Moreover, nanoparticles prevent the chemical and enzymatic degradation of the active principle and, when it is necessary, decrease its toxicity.[1,2]

Drug nanocarriers are characterized by colloidal suspensions of submicronic particles in an aqueous medium that can be directly injected in the blood stream.[3] They have to be composed of biocompatible polymers in order to prevent them from the effect of the stealth defense against foreign bodies. Various kinds of nanocarriers are described in the literature such like micelles,[2,4,5] nanoparticles[6,7] and liposomes.[8] Polymer micelles are supramolecular shell-core structure with a diameter of ten nanometers and are composed of amphiphilic block copolymers. By their supramolecular structure, polymer micelles are able to encapsulate a hydrophobic drug into their core, which allows increasing concentration to be reached in water.

Polymer nanoparticles are another type of polymer based nanocarrier generally prepared starting from hydrophobic and biodegradable biocompatible polymer, in which the drug is encapsulated. Their sizes are generally around one hundred nanometers. The use of amphiphilic copolymers is then required to stabilize such nanoparticles in aqueous media.

Aliphatic polyesters, such as poly(ε-caprolactone) (PCL) or poly(lactide)

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(PLA), are highly reported in the literature for the preparation of these nanocarriers. These polyesters are easily degradable by hydrolysis of the ester bonds leading to a decrease of the molecular weight. Short polymer chains are then removed by solubilization in the biological fluids or by phagocytosis. The degradation is catalyzed by acids or bases for PCL (PCL is known as an hydrolysable polymer) while enzymes catalyze the PLA degradation which is an actual biodegradable material.[9,10]

The poly(ethylene oxide) (PEO) is frequently used as hydrophilic block of amphiphilic structures used in vectorisation.[11,12] PEO is a neutral, hydrophilic, highly flexible and biocompatible polymer and is approved by the Food and Drug Administration (FDA) for intravenous applications. Contrary to aliphatic polyesters, PEO is not (bio)degradable but bioeliminable by renal filtration when the molecular weight remains lower than 20000 g/mol. Moreover, the flexibility of the PEO, due to the absence of bulky substituent and the easy rotation of the ether bonds, disfavors the adsorption of plasmic proteins at the surface of the nanocarrier, leading to nanocarriers of second generation[13] exhibiting prolonged circulation in the blood stream. Such carrier thus can accumulate in tumors by the EPR effect (Enhanced permeability and Retention Effect).[14,15]

The synthesis and applications of amphiphilic diblock copolymers based on PEO and PCL for pharmaceutical application are largely described in the literature due to their ability to form stable micelles[16,17] or to stabilize PLA nanoparticles in solution. Typically, when the molecular weight of the hydrophilic block (PEO) is higher than the hydrophobic block (PCL) ($HLB > 10$), the amphiphilic copolymers are generally prone to form spherical micelles. In contrast, when the molecular weight of the hydrophobic block is higher than the hydrophilic one ($HLB > 10$), the copolymer is rather used for nanoparticles stabilization. As already reported, the stabilization efficiency appears not only to depend on the copolymer composition (or hydrophilic/lipophilic balance, HLB) but also on the architecture of the copolymer. For example, palm-tree copolymers of PEO and PCL have been found more efficient than diblock copolymers of the same HLB for the stabilisation of PLA nanoparticles.[7]

In this paper, we focus on another type of architecture, i.e. star-shape copolymers with the aim to evidence another architecture effect on micellization and/or nanoparticles stabilization efficiency. Interestingly, these stars systems allow to vary the number of PEO arms (one or two) grafted on the mid-chain of a PCL backbone (Figure 1) and, to our knowledge, have so far not yet been investigated for nanomedecine. For this purpose, a novel strategy to synthesize well-defined three- and four-arm star-shaped amphiphilic copolymers based on PEO and PCL (Figure 1) has been first

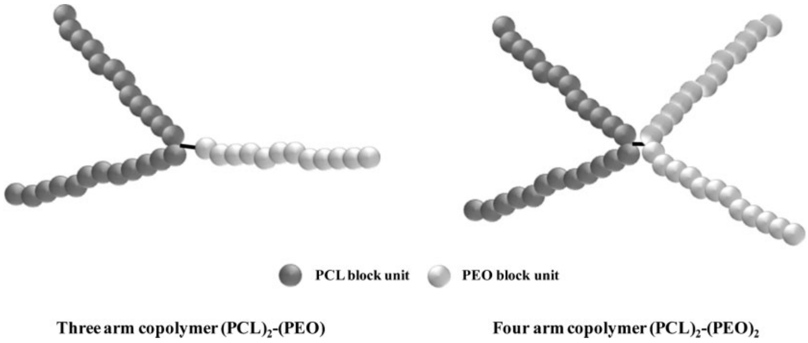


Figure 1.
Structures of the three- and four-arm star-shaped copolymers.

investigated and is reported in the present paper. Then, the micellization so as the efficiency to stabilize PLA nanoparticles of these star copolymers, have been studied in order to highlight the potential of such architecture to be used in drug delivery systems. Particularly, the effect of these star architectures on the micelles size and shape and on the nanoparticles stabilization efficiency will be discussed based on comparison to more conventional diblock and graft copolymers.

Such study requires first the careful synthesis of well-defined star copolymers. Two similar routes were developed for the synthesis of the three-arm (A_2B) (Scheme 1) and four-arm (A_2B_2) (Scheme 2) star-shaped copolymers based on the copper catalyzed coupling of ω-azido-poly(ethylene oxide) with a poly(ε-caprolactone) chain bearing one or two alkyne groups at the mid-chain.

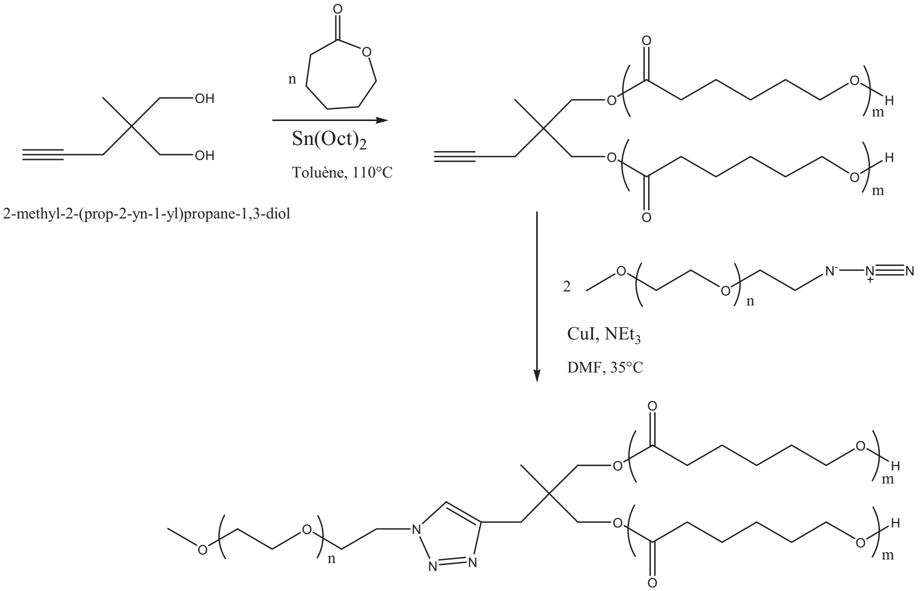
Experimental Part

Materials

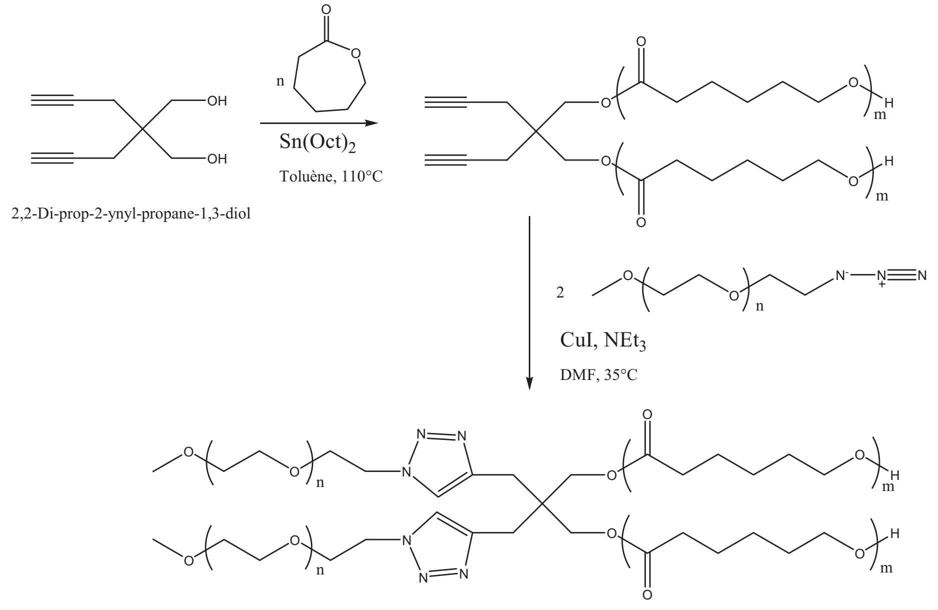
Monomethoxy poly(ethylene oxide) (M_n = 1000 and 2000 g/mol, MPEO-OH, Aldrich), *N,N*-dimethylformamide (DMF, Aldrich), dimethylsulfoxide (DMSO, Aldrich) methanol (Chem-lab), dichloromethane (CH_2Cl_2, Chem-lab), heptane (Chem-lab), diethyl ether (Chem-Lab), triethylamine (NEt_3, Aldrich), tin 2-ethylhexanoate ($SnOct_2$, Fluka), sodium azide (Aldrich), copper(I) iodide (CuI, Aldrich), poly(D,L-lactide) (PLA, Purac) were used as received. The synthesis of 2-methyl-2-(prop-2-yn-1-yl)propane-1,3-diol and 2-methyl-2-(prop-2-yn-1-yl)propane-1,3-diol were reported elsewhere.[18] ε-caprolactone (ε-CL) (Aldrich) was dried over calcium hydride at room temperature for 48 h and distilled under reduced pressure just before use. Toluene and dichloromethane were dried on molecular sieves under nitrogen.

Synthesis of the Alkyne Bearing Poly(ε-caprolactone)

The polymerization of ε-CL was carried out at 110 °C in toluene. Typically, 2-methyl-2-(prop-2-yn-1-yl)propane-1,3-diol or 2-methyl-2-(prop-2-yn-1-yl)propane-1,3-diol (0.304 g, 1.997 mmol) was transferred in a previously dried glass reactor. After three azeotropic distillations with toluene,



Scheme 1.
Synthesis strategy for the A_2B star copolymers.



Scheme 2.
Synthesis strategy for the A_2B_2 star copolymers.

ε-CL (4 mL, 35 mmol) and anhydrous toluene (10 mL) were sequentially added to the reactor through a rubber septum with a syringe equipped with a stainless steel capillary. The reactor was then thermostated at 110 °C before adding the catalyst (0.3 mL, 0.06 M $SnOct_2$ in toluene). After 5 h of polymerization, PCL was recovered by precipitation in cold heptane.[19]

PCL-(alkynes)$_1$: ^1H NMR($CDCl_3$) δ (ppm) = 1 (s, 3H, $C{-}C\underline{\boldsymbol{H}}_3$), 1.4 (m, 2H, $CO{-}CH_2{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}CH_2{-}O{-}$), 1.6 (m, 4H, $CO{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}O{-}$), 2 (m, 3H, $\underline{\boldsymbol{H}}C{\equiv}C{-}C\underline{\boldsymbol{H}}_2{-}$), 2.4 (t, 2H, $CO{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}O{-}$), 3.6 (t, 2H, terminated $-C\underline{\boldsymbol{H}}_2{-}OH$), 4.1 (t, 2H, $CO{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}O{-}$ and 2H, $-C\underline{\boldsymbol{H}}_2{-}O{-}CO{-}$).

PCL-(alkynes)$_2$: ^1H NMR ($CDCl_3$) δ (ppm) = 1.4 (m, 2H, $CO{-}CH_2{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}CH_2{-}O{-}$), 1.6 (m, 4H, $CO{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}O{-}$), 2 (m, 3H, $\underline{\boldsymbol{H}}C{\equiv}C{-}C\underline{\boldsymbol{H}}_2{-}$), 2.4 (t, 2H, $CO{-}C\underline{\boldsymbol{H}}_2{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}O{-}$), 3.6 (t, 2H, terminated $-C\underline{\boldsymbol{H}}_2{-}OH$), 4.1 (t, 2H, $CO{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}C\underline{\boldsymbol{H}}_2{-}O{-}$ and 2H, $-C\underline{\boldsymbol{H}}_2{-}O{-}CO{-}$).

Synthesis of the ω-Azido Terminated-Poly(ethylene oxide)

Monomethoxy poly(ethylene oxide) (1.5 g, 1.2 10^{-3} mol, $M_n = 1250$ g/mol) was transferred in a previously dried glass reactor and dried by three azeotropic distillations with anhydrous toluene. 15 mL of anhydrous dichloromethane were then added with an excess of *p*-toluenesulfonyl chloride (0.02 g, $1.2 \cdot 10^{-2}$ mol) and triethylamine (0.02 mL, $1.3 \cdot 10^{-2}$ mol). The reaction was carried out at room temperature for 48 hours. The organic phase was washed two times with saturated solution of $NH_4^+Cl^-$, one time with water before to be dried on anhydrous $MgSO_4$. The polymer was recovered by precipitation in cold diethyl ether. The ω-tosyl-poly(ethylene oxide) (1.2 g, $9.6 \cdot 10^{-4}$ mol, $M_n = 1250$ g/mol) was transferred in a previously dried glass reactor and dried by three azeotropic distillations of anhydrous toluene. 120 mL of *N,N*-dimethylformamide were added with an excess of sodium azide (0.09 g, $1.4\cdot10^{-3}$ mol). The reaction was carried out overnight at 30 °C. DMF was removed under reduce pressure. The polymer was dissolved in dichloromethane and was washed three times with water then, the ω-azido-PEO was extracted from the collected aqueous phases by using dichloromethane. Organic phases were combined and dried on anhydrous $MgSO_4$. The polymer was recovered by precipitation in cold diethyl ether.

ω-Tosyl-PEO: ^{1}H NMR ($CDCl_3$) δ (ppm) = 2.4 (s, 3H, $C\underline{\boldsymbol{H}}_3-C_6H_4SO_3-$), 3.4 (s, 3H, $C\underline{\boldsymbol{H}}_3-O-$), 3.6 (m, 4H, $-C\underline{\boldsymbol{H}}_2-C\underline{\boldsymbol{H}}_2-O-$), 4.1 (t, 2H, $-C\underline{\boldsymbol{H}}_2-O-SO_2-$), 7.3–7.8 (d, 4H, $-SO_2-C_6\underline{\boldsymbol{H}}_4-CH_3$).

ω-N_3-PEO: ^{1}H NMR ($CDCl_3$) δ (ppm) = 3.4 (s, 3H, $C\underline{\boldsymbol{H}}_3-O-$), 3.6 (m, 4H, $-C\underline{\boldsymbol{H}}_2-C\underline{\boldsymbol{H}}_2-O-$, $-C\underline{\boldsymbol{H}}_2-N_3$).

Synthesis of the Copolymers A_2B and A_2B_2 by CuAAC

The coupling of the ω-azido-poly(ethylene oxide) (0.4 g, 3.2 10^{-4} mol, $M_n = 1250$ g/mol) with the poly(ε-caprolactone) bearing the alkyne(s) (e.g. for PCL-bis-alkyne: 0.77 g, 3.2 10^{-4} mol, $M_n = 2400$ g/mol) was carried out in a glass reactor containing THF. CuI (0.7 mg, $3.2\cdot10^{-6}$ mol) and triethylamine (0.4 μL, $3.2\cdot10^{-6}$ mol) were added to the polymer solution and let reacted at 35°C during 4 hours. The star copolymers were recovered by two precipitations in cold heptane.

Three-arm star-shaped copolymer $(PCL)_2$-*b*-PEO: ^{1}H NMR ($CDCl_3$) δ (ppm) = 1 (s, 3H, $C-C\underline{\boldsymbol{H}}_3$), 1.4 (m, 2H, $CO-CH_2-CH_2-C\underline{\boldsymbol{H}}_2-CH_2-CH_2-O-$), 1.6 (m, 4H, $CO-CH_2-C\underline{\boldsymbol{H}}_2-CH_2-C\underline{\boldsymbol{H}}_2-CH_2-O-$), 2 (m, 3H, $\underline{\boldsymbol{H}}C\equiv C-C\underline{\boldsymbol{H}}_2-$), 2.4 (t, 2H, $CO-C\underline{\boldsymbol{H}}_2-CH_2-CH_2-CH_2-CH_2-O-$), 2.7 (s, 2H, triazole-$C\underline{\boldsymbol{H}}_2-$), 3.4 (s, 3H, CH_3-O-) 3.6 (m, 4H, PEO $-C\underline{\boldsymbol{H}}_2-C\underline{\boldsymbol{H}}_2-O-$), 3.9 (m, 2H, $-C\underline{\boldsymbol{H}}_2-O-CO-$ and 2H, $-C\underline{\boldsymbol{H}}_2-CH_2$-triazole), 4.1 (t, 2H, $CO-CH_2-CH_2-CH_2-CH_2-C\underline{\boldsymbol{H}}_2-O-$), 4.5 (m, $-CH_2-C\underline{\boldsymbol{H}}_2$-triazole), 7.7 (s, 1H, triazole($\underline{\boldsymbol{H}}$))

Four-arm star-shaped copolymer $(PCL)_2$-*b*-$(PEO)_2$: ^{1}H NMR ($CDCl_3$) δ (ppm) = 1.4 (m, 2H, $CO-CH_2-CH_2-C\underline{\boldsymbol{H}}_2-CH_2-CH_2-O-$), 1.6 (m, 4H, $CO-CH_2-CH_2-CH_2-CH_2-CH_2-O-$), 2 (m, 3H, $\underline{\boldsymbol{H}}C\equiv C$-$C\underline{\boldsymbol{H}}_2$-), 2.4 (t, 2H, $CO-CH_2-CH_2-CH_2-CH_2-CH_2-O-$), 2.7 (s, 2H, triazole-$C\underline{\boldsymbol{H}}_2$-), 3.4 (s, 3H, CH_3-O-) 3.6 (m, 4H, PEO $-CH_2-CH_2-O-$), 3.9 (m, 2H, $-C\underline{\boldsymbol{H}}_2-O-CO-$ and 2H, $-C\underline{\boldsymbol{H}}_2-CH_2-$triazole), 4.1 (t, 2H, $CO-CH_2-CH_2-CH_2-CH_2-CH_2-O-$), 4.5 (m, $-C\underline{\boldsymbol{H}}_2-CH_2-$triazole), 7.7 (s, 1H, triazole($\underline{\boldsymbol{H}}$))

Micellisation in Water

Aqueous micelle dispersions of the star copolymers were obtained by addition of water to an organic solution. A stock solution of star copolymer was prepared with a well-defined concentration (1%) in DMF. Then, 20 mL of water (Milli-Q) were added to 5 mL of the organic solution under vigorous stirring for two hours. The solutions were dialyzed against water by using a cellulose dialysis membrane (Spectrapor, cut-off 3500) overnight.

Formation of Stabilized PLA Nanoparticles in Water

Nanoparticles of PLA were prepared by the nanoprecipitation technique[20] in presence of various amounts of three or four-arm star-shaped copolymers as already reported for diblock and palm-tree copolymers based on PEO and PCL.[7] Two stock solutions of PLA (16 mg/mL) or star copolymers (16 mg/mL) were prepared in DMSO. Various amounts of the star solution (10, 20, 30, 40, 50, 60 and 70 wt%) were added to 2 mL of PLA solution. Five volumes of phosphate buffer (pH = 7.4) were then added to the organic mixture under vigorous stirring for two hours. The solutions were dialyzed against water by using a cellulose dialysis membrane (Spectrapor, cut-off 6000–8000) overnight.

Characterization Techniques

Size exclusion chromatography (SEC) was carried out in THF at 45 °C at a flow rate of 1 mL/min with a SFD S5200 auto sampler liquid chromatograph equipped with a SFD refractometer index detector 2000. The PL gel 5 μm (10^5 Å, 10^4 Å, 10^3 Å, and 100 Å) columns were calibrated with polystyrene standards. For pure PCL samples, the molecular weights were corrected by Mark-Houwink relation: $M_n(PCL) = 0.259Mn(PS)^{1.073}$. ^1H NMR spectra were recorded in $CDCl_3$ at 400 MHz in the FT mode with a Bruker AN 400 apparatus at 25 °C. The infrared spectra were recorded with a Perkin-Elmer FT-IR 1720X. The IR samples were prepared by slow evaporation of a star solution, in THF, onto NaCl windows. Dynamic light scattering measurements were performed using a Beckman Coulter Delsa Nano C Particle analyzer and the data were treated by the Delsa Nano UI 2.21 software. All the measurements were carried out at 25 °C at a measuring angle of 165°. The samples for transmission electron microscopy (TEM) were prepared by slow evaporation of the solutions after DLS analysis on a formvar-coated copper grid. The excess of solution was removed with a filter paper. The samples were analyzed with a Philips CM100 microscope equipped with an Olympus camera and transferred to a computer equipped with the Megaview system.

Results and Discussion

2-methyl-2-(prop-2-yn-1-yl)propane-1,3-diol and 2-methyl-2-(prop-2-yn-1-yl)propane-1,3-diol are two diols already used in polyurethane chemistry so that their synthesis is already described in the literature.[18] These two diols have been used here as difunctional initiator for the ring opening polymerization of ε-CL in order to reach both targeted mono-alkyne-PCL (PCL-$(alkyne)_1$) and bis-alkyne-PCL (PCL-$(alkyne)_2$), respectively. The polymerization performed in anhydrous toluene at 110 °C in presence of conventional tin 2-ethylhexanoate as catalyst with the same monomer to initiator ratio led to two poly(ε-caprolactone)s having the macromolecular characteristics reported in Table 1.

As an example, the ^1H NMR spectrum for the bis-alkyne-PCL is shown in Figure 2 allowing determination of the molecular weight by the comparison of the intensity of the characteristic peaks of the alkyne at 2 ppm (peak **a** and **b**, Figure 2) with the protons at 4.2 ppm of the ε-CL unit (peak **h**, Figure 2) according to equation 1:

$$M_n = \frac{I_h/2}{I_{a\,and\,b}/3} \times 114 \tag{1}$$

The ^1H NMR spectrum of PCL-$(alkyne)_1$ (not shown) is quite similar excepted the presence of an additional signal at 1 ppm corresponding to the CH_3 group present on the initiator and gives the data reported in Table 1. For both polymers, a good agreement between the theoretical and experimental molecular weights is observed. Moreover, the polydispersity indexes as determined by SEC are very low. These PCLs exhibiting one or two alkyne groups at the middle of the chain will be used further to anchor one or two PEO arms by CuAAC to get the two targeted stars. This requires first the functionalization of PEO chain-end with an azide group.

Table 1.
Macromolecular characteristics of the obtained PCL-$(alkyne)_1$ and PCL-$(alkyne)_2$.

	DP_{th}[a]	$M_{n,\ th}$[b]	DP_{exp} (^1H NMR)[c]	$M_{n,\ exp}$ (^1H NMR)[d]	DP_{exp} (SEC)[e]	$M_{n,\ exp}$ (SEC)[f]	M_w/M_n (SEC)[g]
PCL-$(alkyne)_1$	18	2000	18.5	2100	17	1900	1.10
PCL-$(alkyne)_2$	18	2000	21	2400	23	2600	1.17

[a] Theoretical value for the polymerization degree of PCL calculated by the [monomer] /[initiator] ratio.
[b] Theoretical value for the molecular weight (g/mol) of PCL.
[c] Polymerization degree determined of PCL by ^1H NMR.
[d] Molecular weight (g/mol) of PCL determined by ^1H NMR.
[e] Polymerization degree of PCL determined by SEC.
[f] Molecular weight (g/mol) of PCL measured by SEC (universal calibration).
[g] Polydispersity index determined by SEC.

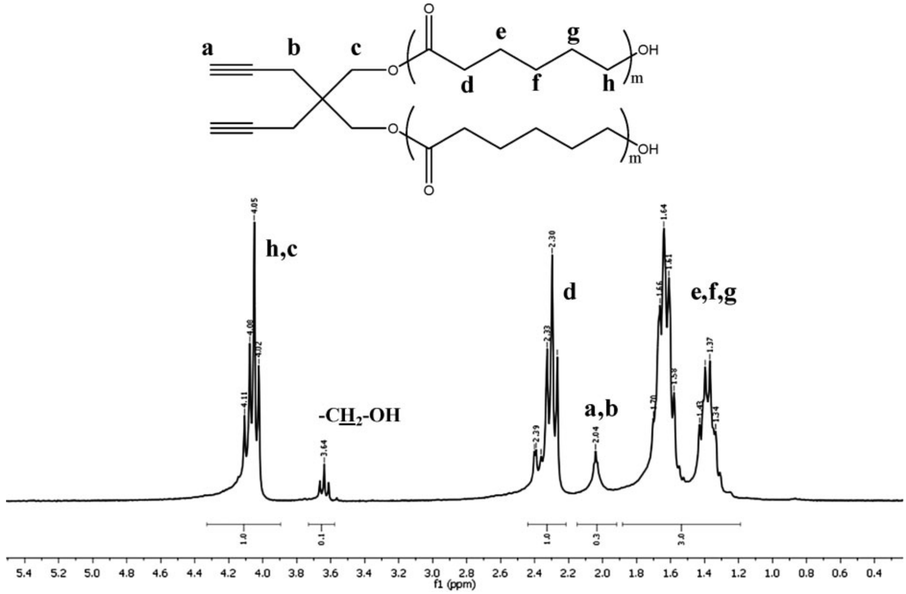


Figure 2.
^{1}H NMR spectrum of the PCL-(alkyne)$_2$ in CDCl$_3$.

The conversion of the hydroxyl chain end of two α-methoxy-ω-hydroxy-PEO ($M_n(PEO_A) = 1000$ g/mol and $M_n(PEO_B) = 2000$ g/mol) into azide was performed by a two-step reaction. First, the hydroxyl end-group was converted into tosylate, a better leaving group. The ^{1}H NMR spectrum corresponding to tosyl-PEO$_A$ is shown Figure 3-a. The functionalization yield was determined by comparison of the intensity of the methoxy protons at 3.3 ppm and the protons of the tosyl group at 7.8 ppm. A quantitative conversion was obtained with high yield (90%). Azide end-

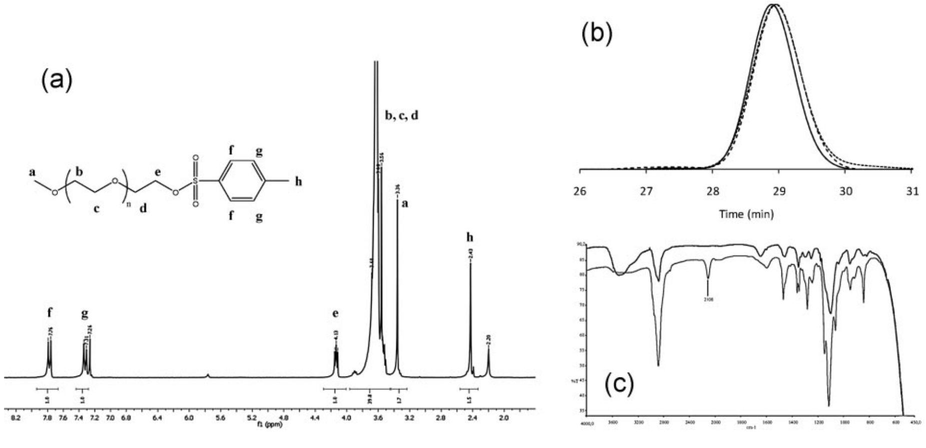


Figure 3.
(**a**) ^{1}H NMR spectrum of the ω-tosyl-PEO$_A$ ($M_n = 1250$ g/mol) in CDCl$_3$, (**b**) SEC traces in THF of ω-hydroxy-PEO$_B$ ($M_n = 2100$ g/mol) (▬ ▬), ω-tosyl-PEO$_B$ (▬▬▬) and ω – azido-PEO$_B$ (▪▪▪▪▪▪) and (**c**) FITR spectrum of ω-tosyl-PEO$_B$ (black) and ω – azido-PEO$_A$ (grey).

group was then obtained by reaction of the tosylate terminated PEO with an excess of sodium azide. A quantitative conversion was also confirmed by a complete disappearance of the signals of the aromatic protons of the tosyl group at 2.4, 7.3 and 7.8 ppm and of the $-CH_2-$ protons at the α-position of the tosyl at 4.2 ppm. The presence of the azide group was confirmed by FTIR analysis by the appearance of a characteristic absorption band at 2106 cm^{-1} (Figure 3-c). Except for the chain-end functionality, the mild conditions used during both reactions kept unchanged the PEO chains as shown by the SEC elugrams recorded before and after reactions (Figure 3-b). The same conclusions can be drawn for the functionalization of PEO_B even if the Mn is doubled.

The coupling reaction between the PCL-$(alkyne)_1$ or PCL-$(alkyne)_2$ with the $\omega-$azido-$PEO_{A\ or\ B}$ was performed by CuAAC in THF in presence of 10 mol% of CuI and NEt_3. The progress of the reaction was followed by the decrease of the intensity of the azide band in the IR spectrum. After four hours of reaction, the intensity of the azide band was low and remained constant in time. This observation was explained by the presence of a small excess of $\omega-$azido-PEO during the reaction even if reaction in stoechiometric conditions was targeted. The star copolymer obtained after precipitation was first analyzed by SEC which confirmed by the shift of the SEC trace to lower elution volume after the coupling reaction, the quantitative conversion of PCL-$(alkyne)_1$ and PCL-$(alkyne)_2$ in star copolymers of expected three-arm $(PCL)_2$-PEO_B (Figure 4-c) and four-arm $(PCL)_2$-$(PEO)_2$ (Figures 4-a and 4-b). A small amount of $\omega-$azido-PEO was still detected after purification mainly from the catalyst by precipitation in cold heptane. Indeed, this solvent does not allowed the separation of the residual $\omega-$azido-PEO from the star. Nevertheless, the presence of this small amount of free PEO chains in the star samples was not deleterious for the formation of micelles or nanoparticles as it will be shown later.

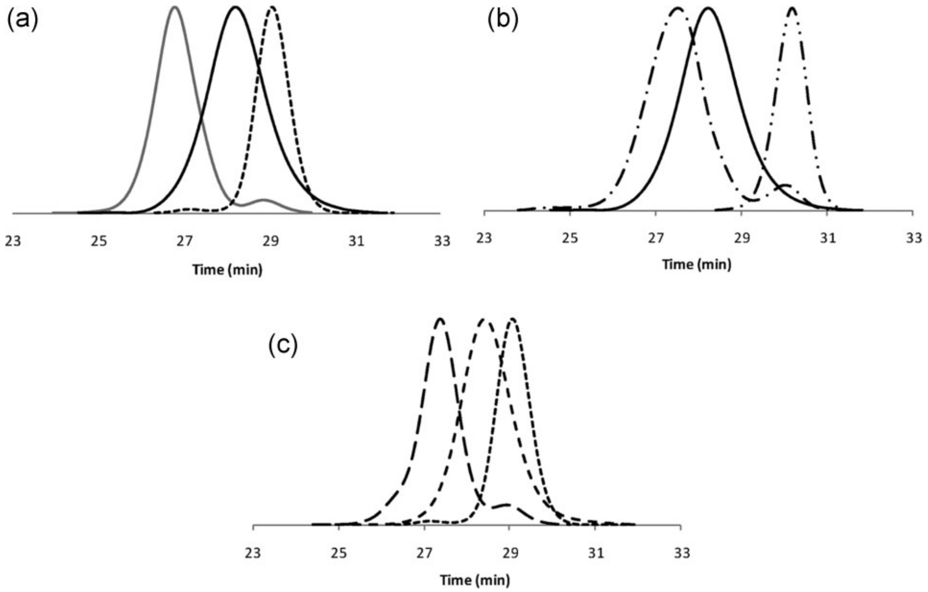


Figure 4.
SEC traces recorded for the coupling between the PCL-$(alkyne)_1$ and PCL-$(alkyne)_2$ with $\omega-$azido-PEO: **a**: $(PCL)_2$-$(PEO_B)_2$ (━━━), PCL-$(alkyne)_2$ (━━━), $\omega-$azido-PEO_B ($M_n = 2100$ g/mol) (••••••); **b**: $(PCL)_2$-$(PEO_A)_2$ (— · —), PCL-$(alkyne)_2$ (━━━), $\omega-$azido-PEO_A ($M_n = 1250$ g/mol) (— · · ·); **c**: $(PCL)_2$-PEO_B (— —), PCL-$(alkyne)_1$ (– – –), $\omega-$azido-PEO_B ($M_n = 2100$ g/mol) (••••••).

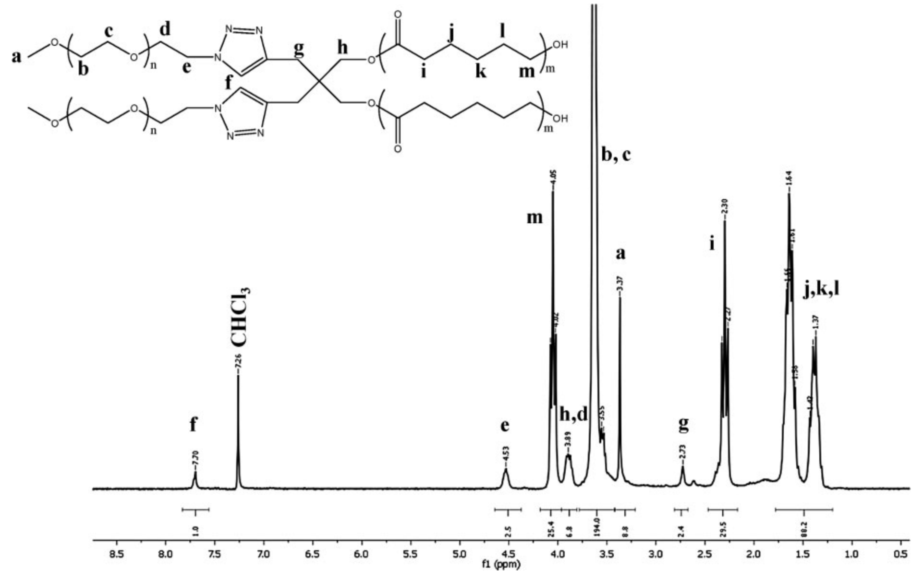


Figure 5.
^{1}H NMR spectrum of the $(PCL)_2$-$(PEO_A)_2$ amphiphilic copolymer in $CDCl_3$.

^{1}H NMR also confirmed the coupling thanks to the appearance of a new signal at 7.7 ppm, characteristic of the proton of the triazole ring (peak f, Figure 5) and at 4.5 ppm and 2.7 ppm, corresponding to two CH_2 next to triazole ring (peak e and g, Figure 5). A ratio of 0.96 between the intensity of the two $-CH_2-$ groups next to the triazole ring that belong to the PCL (peak g, Figure 5) and PEO arms (peak e, Figure 5), confirmed the quantitative coupling reaction. The excess of $\omega-$azido-PEO could not be determined by ^{1}H NMR since the signal of the CH_2 next to the azide is covered by the large signal of the proton of the PEO.

The Table 2 summarizes the different characteristics of the stars obtained by this coupling method and for which the behavior in aqueous media was investigated. For each copolymer, the length of the PCL chain remained constant. In order to keep the same HLB for the three-arm and four-arm stars, two azido-PEO of $M_n = 2100$ and

Table 2.
Characteristics of the stars synthesized by the developed strategy.

Copolymers	PCL Arm content		PEO Arm content		M_w/M_n (SEC)^e	HLB^f
	DP_{exp} (^{2}H NMR)^a	$M_{n,\ exp}$ (^{2}H NMR)^b	DP_{exp} (^{2}H NMR)^c	$M_{n,\ exp}$ (^{2}H NMR)^d		
$(PCL)_2$-$(PEO_B)_1$	18.5	2100	48	2100	1.13	10
$(PCL)_2$-$(PEO_A)_2$	21	2400	56	2500	1.16	10.2
$(PCL)_2$-$(PEO_B)_2$	21	2400	96	4200	1.19	12.7

[a] Polymerization degree of PCL determined by ^{1}H NMR for PCL-$(alkyne)_1$ and PCL-$(alkyne)_2$ before coupling reaction.
[b] Molecular weight (g/mol) of PCL determined by ^{1}H NMR for PCL-$(alkyne)_1$ and PCL-$(alkyne)_2$ before coupling reaction.
[c] Polymerization degree of PEO determined by ^{1}H NMR for the $\omega-$azido-PEO before coupling reaction.
[d] Molecular weight (g/mol) of PEO determined by ^{1}H NMR for the $\omega-$azido-PEO before coupling reaction.
[e] Polydispersity index measured by SEC after coupling reaction.
[f] Hydrophilic-Lypophilic Balance (HLB) determined by $20x\left(1-\frac{M_nPCL}{M_n\ TOTALE(PCL+PEO)}\right)$.

Table 3.
DLS data for micelles of the various copolymers in water.

Copolymers	D_h, a_{DD} (nm) [a]	PDI [b]
$(PCL)_2$-(PEO_B)i–*4200 g/mol*	18.6 nm	0.15
$(PCL)_2$-$(PEO_A)_2$–*4900 g/mol*	18.4 nm	0.17
$(PCL)_2$-$(PEO_B)_2$–*6600 g/mol*	19.9 nm	0.13

Apparent hydrodynamic diameter [a] and PDI [b] determined by DLS.

1250 g/mol, were respectively introduced to get the star. In addition, a third four-arm star copolymer has been synthesized with a higher HLB but with PEO arms of identical length to the three-arm star. The influence of the architecture and of the hydrophilic arms length of the star copolymers on the objects formed in solution was so studied.

The micellization of the well-defined star-shaped copolymers in water was achieved by dissolving the copolymer into DMF, a good solvent of all arms of the copolymer followed by the rapid addition of water, a selective solvent of the hydrophilic arms. After two hours of stirring, the solution was dialyzed against water to remove the DMF. The size and the polydispersity data of the objects in solution were collected by DLS and are listed in Table 3. Small particles of around 20 nanometers are observed and no significant influence of the molecular weight or the architecture of the copolymers is underlined. The spherical morphology of the micelles was confirmed by TEM analysis (Figure 6).

Nanoparticles of PLA were prepared by the nanoprecipitation technique by the addition of increasing amounts of copolymers (from 10 to 70% in weight) to a constant volume of PLA in DMSO (concentration of 16 mg/mL). The efficiency of these copolymers to stabilize nanoparticles is presented in the Table 4. For each copolymer synthesized in this work, a concentration of 30%weight is required to stabilize the PLA nanoparticle solution. Nevertheless, the better stabilization was obtained with the four-arm star-shaped copolymer $(PCL)_2$-$(PEO_B)_2$ with the HLB of 12.7 (the most hydrophilic copolymer used), whose the longer PEO chains prevent the coalescence of the nanoparticles (Figure 7-c). For the two copolymers of the same HLB (about 10), the three-arm star-shaped copolymer (Figure 7-a) exhibited a less efficient stabilization of the PLA nanoparticles by comparison of the quantity of PLA precipitated for the three-arm and four-arm star-shaped copolymers (Figure 7-b). Indeed, as depicted on the drawings of Table 4, the three-arm star-shaped copolymer could present a lower density of PEO chain at the surface of the PLA nanoparticles than the four-arm star-shaped copolymer with the same HLB. This observation is in line with already published results comparing diblock and palm-tree copolymers.[7]

The star-shaped copolymers prepared during this work have a comparable efficiency to stabilize PLA nanoparticles, compared to graft amphiphilic copolymers PCL-*g*-PEO. Indeed, 33 wt% of graft copolymers are required to stabilize PLA nanoparticles solution but didn't require a high HLB (a HLB of 7.5 is enough to

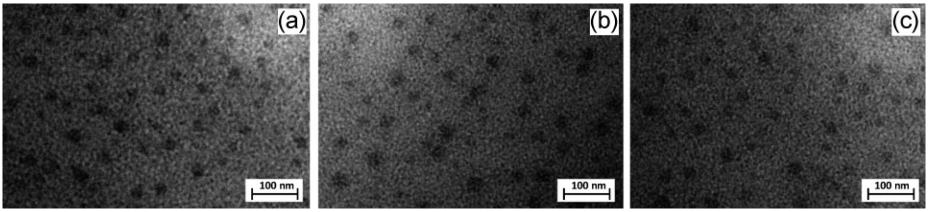
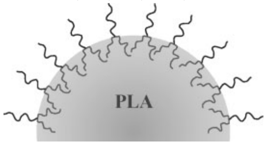
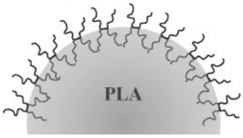
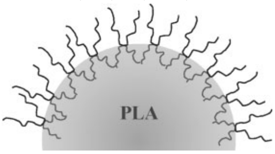


Figure 6.
TEM images of micelles obtained for copolymers: a - $(PCL)_2$-PEO_B (HLB = 10), b - $(PCL)_2$-$(PEO_A)_2$ (HLB = 10.2) and c - $(PCL)_2$-$(PEO_B)_2$ (HLB = 12.7).

Table 4.
Characterization of the stabilized nanoparticles

Star Copolymers			Nanoparticles		
	M_{nTOT}	HLB	wt% copolymers[a]	$D_{h\cdot app}$(nm)[b]	PDI[c]
(PCL)$_2$–(PEO$_B$)$_1$ 	4200 g/mol	10	10	*PLA precipitation*	
			20	*PLA precipitation*	
			30	165 ± 1	0.16
			40	163 ± 1	0.16
			50	160 ± 1	0.11
			60	152 ± 2	0.13
			70	151 ± 1	0.11
(PCL)$_2$–(PEO$_A$)$_2$ 	4900 g/mol	10.2	10	*PLA precipitation*	
			20	*PLA precipitation*	
			30	217 ± 4	0.17
			40	207 ± 2	0.19
			50	201 ± 3	0.15
			60	198 ± 1	0.13
			70	210 ± 1	0.11
(PCL)$_2$–(PEO$_B$)$_2$ 	6600 g/mol	12.7	10	*PLA precipitation*	
			20	*PLA precipitation*	
			30	208 ± 2	0.17
			40	191 ± 1	0.18
			50	214 ± 3	0.15
			60	196 ± 1	0.14
			70	203 ± 1	0.15

[a] wt% of copolymer in the (PLA + copolymer) mixture dissolved in DMSO (c = 16 mg/ml).
[b] Apparent diameter determined by DLS.
[c] PDI determined by DLS.

stabilized nanoparticles). However, amphiphilic diblock copolymers PEO-*b*-PCL are less efficient stabilizers compared to star-shaped copolymer since higher amount of copolymers (about 50 wt% for a HLB of 7.5) or higher HLB value (33 wt% for a HLB of 13) are required to stabilize PLA. The results obtained for the star-shaped copolymer with the longer PEO chain are close to the results for the amphiphilic diblock copolymer. However, only the macromolecular architecture is compared but another point to take into account is the molecular weight. A higher molecular weight will probably favor the stabilization of a PLA nanoparticles suspension. In case of the synthesized stars, low molecular weight appears to give efficient stabilization, while higher molecular weight of about 15 000 g/mol are rather required for the diblock copolymers.[7]

As a rule, the nanoparticles have a diameter of about 150–200 nm, whatever the macromolecular architecture and the

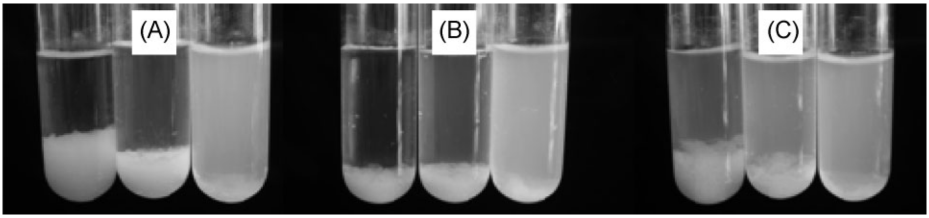


Figure 7.
Co-precipitation of PLA with increasing amount of 10 wt%, 20 wt% and 30 wt% (from left to right) copolymers: A - (PCL)$_2$-PEO$_B$ (HLB = 10), B - (PCL)$_2$-(PEO$_A$)$_2$ (HLB = 10.2) and C - (PCL)$_2$-(PEO$_B$)$_2$ (HLB = 12.7).

amount of polymeric stabilizer. The obtained sizes are comparable to those observed with graft or block copolymers based on PEO and PCL. For the three-arm star-shaped copolymers, the size of the PLA nanoparticles seemed to decrease when the amount of polymer increases. No significant improvement was observed with the increase of the HLB value for the four-arm star-shaped copolymers with an average size of 200 nm.

Conclusion

Well-defined three and four-arm star-shaped copolymers were successfully synthesized via an original pathway based on the coupling reaction between a PCL bearing one or two alkyne groups at the middle of the chain with an ω-azido-poly(ethylene oxide). Moreover, the synthesis strategy elaborated could be easily adapted to the synthesis of a variety of star-shaped copolymers by adapting the nature of the alkyne initiated monomer or/and the nature of ω-azido-functional polymer. The ability of these star copolymers to form micelles in aqueous solution was assessed by DLS and TEM which did not evidence a strong effect of the numbers and length of the hydrophilic arms on the size and shape of the micelles that remain spherical with a diameter around 20 nm. Besides micellization, the efficiency of these star copolymers to stabilize PLA nanoparticles has been studied and compared to more conventional diblock and graft copolymers. Star-shaped copolymers appeared to exhibit an intermediate behavior between these two other architectures in terms of PLA stabilization efficiency. These novel star copolymers, that were obtained via a quite simple synthesis route, have thus quite promising future in the elaboration of drug delivery systems.

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[1] K. Kataoka, A. Harada, Y. Nagasaki, *Advanced Drug Delivery Reviews* **2001**, *47*, 113.

[2] G. S. Kwon, T. Okano, *Advanced Drug Delivery Reviews* **1996**, *21*, 107.

[3] G. Barratt, G. Couarraze, P. Couvreur, C. Dubernet, E. Fattal, R. Gref, D. Labarre, P. Legrand, G. Ponchel, C. Vauthier, *Polymeric Biomaterials* **2002**, 753.

[4] I. Astafieva, X. F. Zhong, A. Eisenberg, *Macromolecules* **1993**, *26*, 7339.

[5] M. C. Jones, J. C. Leroux, *European Journal of Pharmaceutics and Biopharmaceutics* **1999**, *48*, 101.

[6] S. Gautier, N. Grudzielski, G. Goffinet, S. Henry De Hassonville, L. Delattre, R. Jérôme, *Journal of Biomaterial Science Polymer Edition* **2001**, *12*, 429.

[7] J. Rieger, C. Passirani, J.-P. Benoit, K. Van Butsele, R. Jerome, C. Jerome, *Advanced Functional Materials* **2006**, *16*, 1506.

[8] E. Forssen, M. Willis, *Advanced Drug Delivery Reviews* **1998**, *29*, 249.

[9] C. Jerome, P. Lecomte, *Advanced Drug Delivery Reviews* **2008**, *60*, 1056.

[10] A.-C. Albertsson, I. K. Varma, *Advances in Polymer Science* **2002**, *157*, 1.

[11] J. H. Lee, H. B. Lee, J. D. Andrade, *Progress in Polymer Science* **1995**, *20*, 1043.

[12] P. Vermette, L. Meagher, *Colloids Surf., B* **2003**, *28*, 153.

[13] P. Couvreur, *Annales Pharmaceutiques Francaises* **2001**, *59*, 232.

[14] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, *Journal of Controlled Release* **2000**, *65*, 271.

[15] Y. Takakura, M. Hashida, *Critical reviews in oncology/hematology* **1995**, *18*, 207.

[16] H. M. Aliabadi, S. Elhasi, A. Mahmud, R. Gulamhusein, P. Mahdipoor, A. Lavasanifar, *International Journal of Pharmaceutics* **2007**, *329*, 158.

[17] P. Vangeyte, S. Gautier, R. Jerome, *Colloids and Surfaces, A: Physicochemical and Engineering Aspects* **2004**, *242*, 203.

[18] L. Billiet, D. Fournier, F. Du Prez, *Journal of Polymer Science, Part A: Polymer Chemistry* **2008**, *46*, 6552.

[19] R. Riva, W. Lazzari, L. Billiet, F. Du Prez, C. Jerome, P. Lecomte, *Journal of Polymer Science, Part A: Polymer Chemistry* **2011**, *49*, 1552.

[20] 0274961 (**1988**), invs.: H. Fessi, F. Puissieux, J-. P. Devissaguet; Eur. Patent