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Synthesis of pH-sensitive micelles from linseed oil using atom transfer radical polymerisation (ATRP)

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ABSTRACT

This paper reports the synthesis of an amphiphilic copolymer from linseed oils and its successive auto-association in water into pH-sensitive micelles. An original ATRP lipoinitiator is first designed from linseed oil in two steps. *tert*-butyl acrylate (*t*BA) polymerization is consequently initiated from this original initiator and amphiphilic copolymers are obtained after subsequent acidolysis of the P*t*BA block into poly(acrylic acid) (PAA). The ability of a lipid-*b*-PAA copolymer to auto-associate in water is finally investigated through different techniques (Fluorescence, Surface Tension, QELS). This copolymer forms well-defined micelles in acidic media with a low critical micellar concentration (cmc) of 7.6 mg L^{-1} and dissociates when the pH is raised above 7.

1. Introduction

By using nanoscale delivery vehicles, the pharmacological properties (e.g. solubility, circulating half-life) of drugs can be drastically improved, leading to safe and effective systems. Nanotechnologies, by addressing some of the shortcomings associated with potential drugs, have made a significant impact on the development of drug delivery systems [1]. Since liposomes were first described in the 1960s and proposed as carriers of proteins and drugs for disease treatment, they have been extensively studied and their efficiency as drug delivery systems has been demonstrated [2]. Liposomes can efficiently encapsulate both hydrophilic and hydrophobic drugs and their similarity with the constitution of cell membranes improve interactions between cells and the delivery device. There has been considerable interest in modification of liposomes surfaces to enhance their blood circulation time, obtain a controlled release [3], and introduce homing devices (antibody, receptor, ligand). Liposomes are now in the marketplace as pharmaceuticals, however liposomology still faces major deficiencies [4]. The relatively large size of liposomes is an inconvenient to access tumour with a small vasculature cutoff and due to their smaller size, micelles may have additional advantages as a tumour drug delivery system [5]. Developing new vectorisation systems of smaller size, typically micelles, but having the same affinity with cells membrane is then of great interest and we are consequently interested in amphiphilic block copolymers containing a hydrophobic block based on a lipid chain.

One simple strategy to obtain lipid-*b*-polymer architecture is to post-modify a preformed polymer chain. According to this strategy, the synthesis of poly(ethylene glycol)-*b*-phospholipids was reported in the literature [6]. These copolymers form micelles with a low critical micellar concentration (cmc) of 10^{-5} M and present interesting loading and release properties. Another synthetic approach is to use directly a lipid derivative as the initiator (or the transfer agent) to carry out the polymerization. This approach allows a better control of the final structure and avoids a subsequent purification step to eliminate unreacted polymeric chains. According to this strategy, the synthesis of lipid-based initiator/transfer agent for free radical polymerization [7–9], controlled radical polymerization (RAFT [10], NMP [11,12], ATRP [13–15]) and ring-opening polymerization [16] were reported. However, in all these studies, the so-called "lipid" block was issued from petroleum-based reactants (mainly aminoalkyl chains) and not from renewable resources. Furthermore, these studies were mainly developed by the lipid-membrane community [17,18], consequently the final copolymers were involved in liposomes formation or vesicles adsorption, and their ability to form micelles was not studied. Development of polymers from renewable resources is

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a growing field of interest [19,20], and a major concern of the scientific community. Our group aims at developing original bio-sourced lipopolymers directly from naturally occurring vegetable oils using ATRP. In that purpose, an original synthetic pathway in two main steps was chosen: (1) the vegetable oil is first chemically modified to introduce an initiating site for Atom Transfer Radical Polymerization (ATRP) leading to a lipoinitiator and (2) a synthetic poly(acrylic acid) hydrophilic block is added to the lipid block using ATRP of tert-butyl acrylate followed by subsequent acidolysis. If modified fatty acids have already been polymerized by ATRP [21–24], this is, to the best of our knowledge, the first report of an oil derivative used as an ATRP initiator.

Linseed oil was chosen as the starting material because of its large local bioavailability added to its low use in the food industry (indeed avoiding any market competition). Vegetable oils are constituted of triglycerides with a statistical mixture of fatty acids which are highly polyunsaturated ones in the case of linseed oil [25]. Obtaining well-defined systems from heterogeneous starting material is an interesting challenge to take up. Amongst the different possible synthetic approaches, fatty acids chains were chosen to constitute the hydrophobic block in order to reduce the heterogeneity of the final material (compared to a triglycerides-based copolymer) and to obtain small-sized micelles.

2. Experimental section

2.1. Materials

THF was distilled over sodium/benzophenone and dichloromethane over CaH_2 prior to use. Linseed oil was kindly provided by Novance (Compiègne, France). *tert*-butyl acrylate (tBA, 99%) from Acros was distilled under vacuum and stored at 4 °C after purification. 2-bromo-2-methylpropionylbromide (98%), N,N,N′,N′,N″-pentamethylethylenetriamine (PMDETA, 99+%), anisole (99%), toluene (99%), Silica Gel (60–200 mesh), lithium aluminohydride, and triethylamine were purchased from Acros. Copper (I) bromide (99.99%) was purchased from Aldrich. Copper (II) bromide (99%) was purchased from Alfa Aesar.

2.2. Instrumentation

^1H and ^{13}C NMR spectra were recorded on a Bruker AC-P 300 MHz spectrometer. Chemical shifts are reported in ppm relative to the deuterated solvent resonances. Molecular weights and molecular weight distributions were measured using size exclusion chromatography (SEC) on a Varian PL-GPC50 device equipped with two mixed packed columns (PL gel mixed type C). The eluent used is dichloromethane at a flow rate of 1 mL min^{-1} at room temperature and poly(methyl methacrylate) standards were used for calibration. Fourier Transform InfraRed (FTIR) spectra were recorded on a Perkin Elmer Spectrum 2000 FTIR, equipped with a diamond ATR (Attenuated Total Reflection) device. Fluorescence measurements were recorded on a Cary Eclipse Varian spectrophotometer. Surface tension was measured using Krüss Tensiometer K12 with the SFT Wilhelmy plate method and the following parameters: detection speed: 6 mm/min, detection sensitivity = 0.01 g, immersion depth = 2 mm, values = 10, Acquistion = linear, times = 1000 s. The terminal conditions are values for mean = 5 values and standard deviation = 0 mN/m. QELS measurements were recorded on a Malvern Zetasizer. ESI-MS analyses were performed on an Esquire-LC ion-trap mass spectrometer equipped with an ESI source and the Esquire control 6.16 data system (Bruker Daltonics, Bremen, Germany).

2.3. Linseed oil reduction

A solution of linseed oil (2.9 g) in 45 mL of anhydrous THF was added dropwise to a mixture of lithium aluminohydride (2.1 g) in 45 mL of THF under inert atmosphere. The reaction media was stirred at room temperature overnight. After hydrolysis of the unreacted $LiAlH_4$ through water dropwise addition (~20 mL), the reaction media was filtered and THF was removed by evaporation. The resulting oil was consequently solubilized in dichloromethane and washed with a saturated sodium chloride aqueous solution. The organic layer was dried over anhydrous magnesium sulphate, and the solvent was removed by evaporation. The final alcohol was obtained as a yellowish oil with a yield of 89%. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.3 (m, −**H**C = C**H**−), 3.6 (*t*, −C**H$_2$**−OH), 2.75 (m, −HC = CH−C**H$_2$**−HC = CH−), 2.0 (m, −HC = CH−C**H$_2$**−CH_2), 1.5 (m, C**H$_2$**−CH_2OH), 1.25 (m, C**H$_2$** aliphatic), 0.9 (*t*, C**H$_3$** linolenic alcohol), 0.8 (m, C**H$_3$** other fatty alcohols). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 131.9–127.1 (−H**C** = **C**H−), 63.0 (−**C**H_2−OH), 32.7–20.5 (**C**H_2 aliphatic), 14.2 (**C**H_3 alkyl chain). MS [M-Li^+]: m/z = 271 (linolenic) 273 (linoleic), 275 (oleic), 277 (stearic).

2.4. Lipoinitiator 1 synthesis

Fatty alcohol **1** (1.20 g) was mixed with triethylamine (1.9 mL, 3 eq.) and anhydrous dichloromethane (30 mL) under argon atmosphere. The mixture was cooled by an ice-water bath and the 2-bromoisobutyryl bromide (1,1 mL, 2 eq.) was added dropwise under stirring. The mixture was stirred at room temperature during 4 h. Hydrochloric acid aqueous solution (10%, 15 mL), sodium hydrogenocarbonate aqueous solution (10%, 15 mL) and saturated sodium chloride aqueous solution (15 mL) were consequently used to wash the dichloromethane solution. The organic layer was dried over anhydrous magnesium sulphate, and filtered. After removing the solvent by evaporation, a column chromatography was performed to purify the product (diethylether/cyclohexane: 5/95). The final product was obtained as a colourless liquid with a yield of 65%. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.3 (m, 2H, −**H**C = C**H**−), 4.1 (*t*, 2H, −C**H$_2$**−OCO), 2.75 (m, −HC = CH−C**H$_2$**−HC = CH−), 2.0 (m, −HC = CH−C**H$_2$**−CH_2), 1.85 (s, Br(C**H$_3$**)$_2$C−), 1.6 (m, C**H$_2$**−CH_2OCO), 1.25 (m, C**H$_2$** aliphatic), 0.9 (*t*, C**H$_3$** linolenic chain), 0.8 (m, C**H$_3$** other chains). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 171.7 (**C**=O), 131.9–127.0 (−H**C** = **C**H−), 66.1 (−**C**H_2−OCO), 55.9 (**C**−Br), 31.8–22.6 (**C**H_2 aliphatic), 30.7 (Br(**C**H_3)$_2$C−), 14.1 (**C**H_3 alkyl chain). MS [M-Li+]: m/z = 421 (linolenic), 423 (linoleic), 425 (oleic), 427 (stearic).

2.5. tBA ATRP

A Schlenk tube was loaded with copper (I) and copper (II) bromide, capped with a rubber septum, and cycled three times between vacuum and argon to remove oxygen. In another Schlenk tube, toluene, lipoinitiator **1**, anisole (internal reference) and *t*BA were introduced according to the following ratios: [*t*BA]:[(1)]:[Cu(I)Br]:[Cu(II)Br_2]:[PMDETA] = 50 : 1 : 0.5 : 0.025 : 0.525, toluene: 75% v/v, anisole: 5% v/v, 60 °C. The resulting solution was degassed by three freeze–pump–thaw cycled and was added to the Cu(I)Br and Cu(II)Br_2 contained in the first Schlenk tube via a cannula. The Schlenk tube was placed in an oil bath thermostated at the polymerization temperature. At t = 0, the ligand was added. Aliquots were taken periodically via a degassed syringe to follow the kinetic of the polymerization process. The aliquots were diluted with THF followed by filtration through a basic alumina column prior to analysis by SEC. The final polymer **2** was isolated by filtration through a basic alumina column followed by solvent evaporation.

2.6. Lipid-b-poly(tert-butyl acrylate) acidolysis

Trifluoroacetic acid (TFA) (2 equiv. according to *tert*-butyl acrylate units) was added to a solution of copolymer **2** in dichloromethane. The reaction mixture was stirred overnight until the de-*tert*-butylated polymer precipitated. Polymer **3** was isolated by filtration and washed with dichloromethane prior to drying.

2.7. Conductimetry – determination of the number of repeating unit

1 mL of an hydrochloric acid solution (0.1 mol L^{-1}) was added to the solution of amphiphilic copolymer **3** (1 g L^{-1}) in order to stress the beginning of the carboxylic acid units titration (through the slope gradient change). The solution was then titrated using a solution of sodium hydroxide (0.1 mol L^{-1}) and the titration is followed by conductimetry. Calculation details are provided in the supporting information (S4).

2.8. Determination of the iodine value

This iodine value was determined according to the Wijs method. A known amount of lipoinitiator 2 (0.1 g) is introduced in 20 mL of dichloromethane and 20 mL of Wijs solution is added. The solution is stirred during 1 h in the dark. Then 100 mL of water and 20 mL of potassium iodide solution ($C = 10$ g/L) are introduced. This mixture is titrated with a solution of sodium thiosulfate ($C = 0.2$ mol/L). Details are provided in the supporting information (S3).

2.9. Fluorescence measurements

A pyrene stock solution (4×10^{-3} M – 50 mL) in acetone was prepared and 50 μL were introduced in a 500 mL volumetric flask (to obtain a final pyrene concentration of 4.10^{-7} M after dilution). Acetone was allowed to evaporate and a buffer solution containing 0.1 M of NaCl was added (pH 4.6: 10^{-2} M sodium acetate/acetic acid, pH 7: 10^{-2} M sodium phosphate/disodium phosphate, pH 10: 10^{-2} M sodium bicarbonate/sodium carbonate). This stock solution was used to prepare the copolymer **3b** solutions ranging from 1 mg L^{-1} to 1 g L^{-1}. The solution, prepared by successive dilution from a stock solution of 1 g L^{-1} were stirred overnight prior to measurement. Excitation spectra of pyrene were recorded between 330 and 360 nm (bandwidth = 1.5 nm) at a fixed emission wavelength (λEm = 374 nm, bandwidth = 5 nm).

2.10. QELS measurements

A copolymer **3** solution (1 g L^{-1}) was prepared in a buffer solution containing 0.1 M of NaCl (pH 4.6: 10^{-2} M sodium acetate/acetic acid, pH 7: 10^{-2} M sodium phosphate/disodium phosphate, pH 10: 10^{-2} M sodium bicarbonate/sodium carbonate), filtered on a 0.45 μm cellulose filter and introduced in the Zetasizer device.

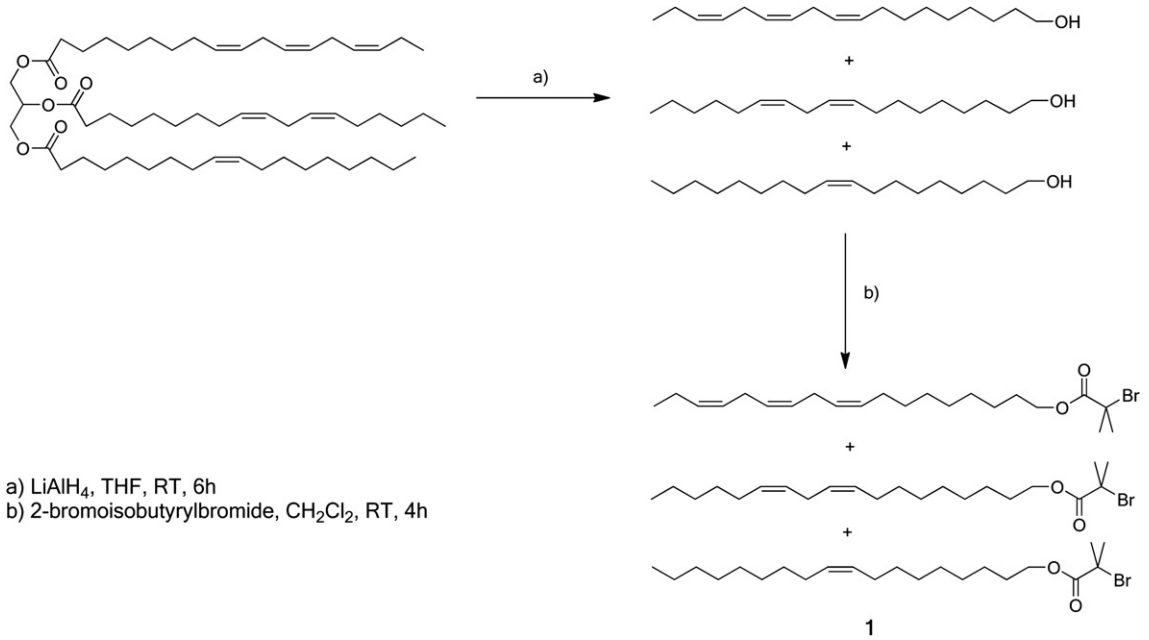
2.11. Surface tension

Copolymer **3** solutions (1 mg L^{-1} to 1 g L^{-1}) were prepared by successive dilution from a copolymer stock solution (1 g L^{-1}) in a buffer solution containing 0.1 M of NaCl (pH 4.6: 10^{-2} M sodium acetate/acetic acid, pH 7: 10^{-2} M sodium phosphate/disodium phosphate, pH 10: 10^{-2} M sodium bicarbonate/sodium carbonate).

3. Results and discussion

3.1. Lipoinitiator synthesis

As a natural product, the composition of linseed oil varies with the source. The starting linseed oil was characterized by Mass Spectrometry and ^{1}H NMR (Sup. inf. S1) to determine the ratio of each fatty acid according to a previously reported method [25]. This linseed oil is composed of 54.0% linolenic acid, 9.5% linoleic acid, 23.4% oleic acid and 13.1% of saturated acid. Linseed oil was first reacted with lithium aluminohydride to reduce the triglyceride ester functions and obtain fatty alcohols (Scheme 1 – for clarity purpose, the starting triglyceride has been depicted as a 1:1:1 mixture of linolenic, linoleic and oleic acids, but one has to keep in mind that it is in fact a complex mixture of



Scheme 1. Lipoinitiator(s) synthesis from linseed oil (for clarity purpose, the starting triglyceride has been depicted as a 1:1:1 mixture of linolenic, linoleic and oleic acids, but one has to keep in mind that it is in fact a complex mixture of different fatty acids).

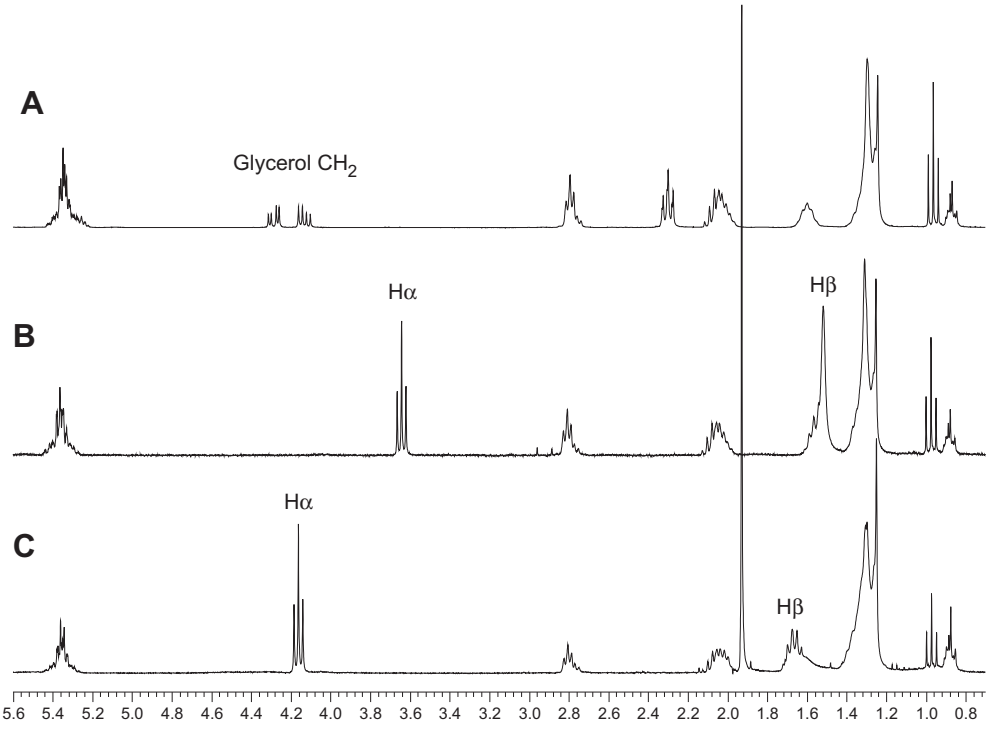


Fig. 1. NMR spectra of A) linseed oil, B) fatty alcohol and C) lipoinitiator 1.

different fatty acids as mentioned previously). NMR analysis (Fig. 1B) of the fatty alcohol shows the disappearance of the CH_2 glycerol moiety signals (δ = 4.1 ppm) and the appearance of signals corresponding to the protons in α and β positions of the hydroxyl function ($\delta_{H\alpha}$ = 3.6 ppm and $\delta_{H\beta}$ = 1.5 ppm). FT-IR Analysis (Sup. inf. S2) shows the disappearance of the ν(C═O) signal at 1730 cm^{-1} and the appearance of a ν(O–H) band at 3320 cm^{-1}.

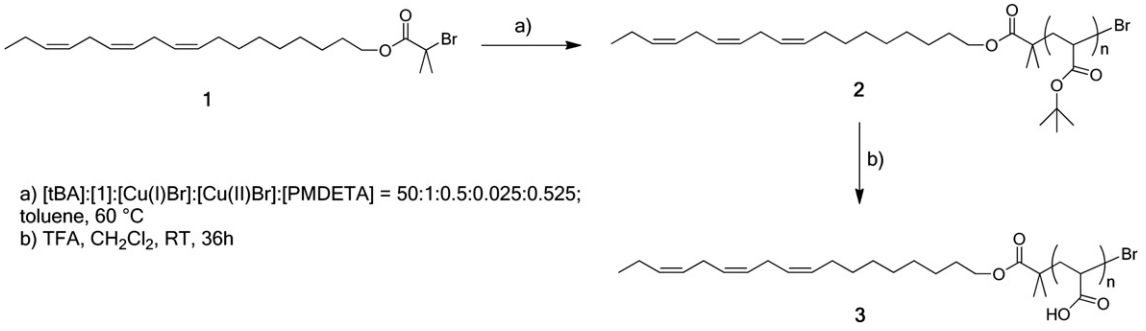
The hydroxyl extremity was subsequently reacted with 2-bromoisobutyryl bromide to introduce the ATRP initiating moiety. The ^1H NMR spectrum (Fig. 1C) of the final product shows the shift of the signals corresponding to the protons in α and β positions of the introduced ester function ($\delta_{H\alpha}$ = 4.1 ppm and $\delta_{H\beta}$ = 1.6 ppm) and the appearance of the *gem*-dimethyl signal at 1.85 ppm. This structure is confirmed by ^{13}C NMR. The appearance of a ν(C═O) signal at 1735 cm^{-1} is also observed in FTIR (sup. inf. S2) simultaneously to the ν(O–H) signal disappearance.

The average number of unsaturations per molecules was investigated at each step by NMR and by titration using the Wijs method. Details are provided in the supporting information (S3). Both methods give consistent results with average values of 6 unsaturations/molecule for linseed oil (containing 3 fatty acid chains) and 2 unsaturations/molecule for fatty alcohols and lipoinitiator **1**, proving the double bond conservation during the lipoinitiator synthesis.

Synthesis of lipoinitiator **1** with a good yield in two easy steps is then demonstrated. As previously mentioned, the alkyl chains nature is not homogeneous so that lipoinitiator **1** is a mixture of C_{18} chains containing various numbers of unsaturations (between 0 and 3), the average value being 2 unsaturations/chain. This is, to the best of our knowledge, the first reported synthesis of a lipoinitiator directly from renewable resources.

3.2. Amphiphilic copolymers synthesis

Lipoinitiator **1** was then engaged in ATRP to synthesize a hydrophilic block and obtain amphiphilic copolymers. *tert*-Butyl acrylate (*t*BA) was chosen as the monomer because it's an hydrophobic monomer allowing to conduct the polymerization in an organic media compatible with the initiator **1** lipo-affinity. This choice avoids then any compatibility issues between the initiator and the monomer. Furthermore, hydrophobic poly(*tert*-butyl acrylate) easily give access through acidolysis to hydrophilic PAA, commonly accepted as a bioadhesive and safe polymer [26]. Lipoinitiator **1** was first engaged in copper mediated



Scheme 2. Synthesis of amphiphilic lipid-b-poly(acrylic acid) copolymers from lipoinitiator 1 (for clarity purpose only the major aliphatic chain is represented).

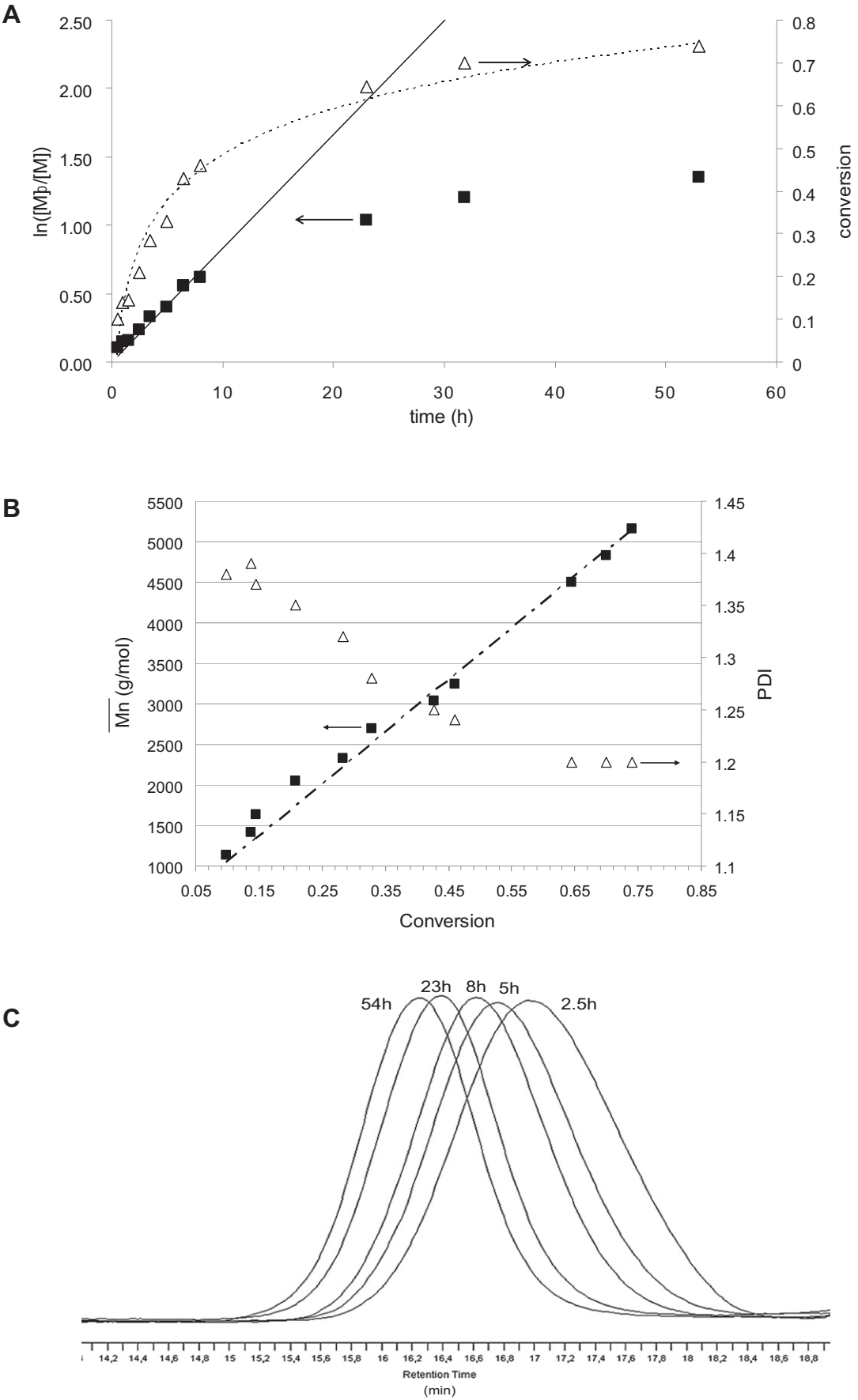


Fig. 2. Kinetic study of tBA ATRP initiated by lipoinitiator 1:[tBA]:[1]:[Cu(I)Br]:[Cu(II)Br2]:[PMDETA] = 50:1:0.5:0.025:0.525, toluene (75% v:v), 60 °C. (A) Kinetic plot, (B) Evolution of the number average molecular weights (– theoretical, ■ experimental) and the polydispersity indexes (△) *vs.* monomer conversion and (C) GPC traces.

ATRP of *t*BA with a Cu(I)Br/Cu(II)Br_2/PMDETA (*N,N,N′,N′,N″*-pentamethyldiethylenetriamine) catalytic system at 60 °C in toluene (Scheme 2).

The $\ln([M]_0/[M])$ *vs.* time plot (Fig. 2A) is linear during the first 10 h showing a first order kinetic compatible with a constant concentration of active species until 50% conversion. The number average molecular weight *vs.* conversion plot provides good agreement between experimental and theoretical molecular weights (even above 50% conversion) and the polydispersity index progressively decreases with the conversion (Fig. 2B).

The non-linear plot for $\ln([M]_0/[M])$ *vs.* time above 50% conversion indicates a decrease in the propagating species concentration which could be attributed to termination reactions involving the lipoinitiator unsaturation sites (by chain–chain coupling). However, termination reactions should be accompanied by a deviation between the experimental and the theoretical molecular weights, a PDI increase and the appearance of shoulders on the GPC traces (Fig. 2C), and none of these phenomena was observed.

Thus, the non-living character of the polymerization is apparently not accompanied by a loss of molecular weights control and lipoinitiator **1** enables a good control of the final copolymer composition and architecture.

A range of lipid-*b*-PtBA copolymers **2** with different lengths of the P*t*BA block were then synthesized by varying the initial [monomer]/[initiator] ratio and the final conversion (Table 1). The theoretical polymerization degree of the P*t*BA block is ranging from 4 to 47 and there is a good agreement between theoretical and experimental number average molecular weights further proving the interest of ATRP to control the hydrophilic block length.

To obtain the final amphiphilic copolymers, an acidolysis using trifluoroacetic acid (TFA) is performed to deprotect the *tert*-butyl moieties. We focused our attention on copolymer **3c** (issued from **2c** acidolysis), presenting a theoretical molecular weight of 1634 g mol^{-1} ($\overline{M_{n,\mathrm{theo}}} = M_{\mathrm{lipoinitiator1}} + \overline{X_{n,\mathrm{theo}}} \times M_{\mathrm{AA}}$) and a hydrophilic/hydrophobic balance of 75/25 (wt/wt). Consequently, this copolymer should easily disperse in water and self-assemble into small and relatively monodisperse micelles [27]. The final lipid-*b*-PAA copolymer **3c** was titrated with a sodium hydroxide solution followed by pH-metry and conductimetry to determine the copolymer pKa and the average number of acrylic acid (AA) unit per chain. A pKa value of 5.7, in really good agreement with the literature value of 5.8 is measured [28]. An average value of 19 AA unit/chain is also obtained from this titration (see detailed calculation in supporting information S4). This really good agreement between the experimental $\overline{X_n} = 19$ and the theoretical $\overline{X_n} = 17$ calculated from the monomer conversion, further proves the good control of the polymerization.

3.3. Micelles characterization

The final copolymer **3c** is soluble in water and lead to uncoloured solution that tends to foams when stirred (Fig. 3A). Addition of lipid-*b*-PAA copolymer **3c** to a mixture of water and dichloromethane leads after shaking to a fine dispersion, stable over days (Fig. 3B). The surfactant properties of our original biosourced copolymer **3** are then qualitatively observable.

In order to better characterize the auto-association properties of copolymer **3c** in water, excitation fluorescence measurements using pyrene as a probe were conducted (the pyrene was excited with $\lambda_{\mathrm{Ex}} = 330–360$ nm and its emission was then recorded at $\lambda_{\mathrm{Em}} = 374$ nm). Depending on the pyrene surroundings, the maximum emission is obtained for different excitation wavelengths, shifting from 333 nm to 338 nm when going from hydrophilic to hydrophobic surroundings. The formation of micelles is then easily observable from a shift of the excitation wavelength corresponding to the maximum emission of pyrene [29]. Pyrene excitation spectra were recorded for a range of copolymer concentration going from 1 mg L^{-1} to 1 g L^{-1} and the ratios of the emission intensity for $\lambda_{\mathrm{Ex}} = 338$ nm (I_{338}) over $\lambda_{\mathrm{Ex}} = 333$ nm (I_{333}) was plotted *vs.* the copolymer concentration (Fig. 4). The critical micellar concentration (cmc) corresponds to the point where the maximum wavelength begins to change due to pyrene incorporation in the micelles hydrophobic core *i.e.* a I_{338}/I_{333} ratio change. This experiment was carried out in buffer solutions (10^{-2} M) at 3 different pHs (4.6, 7 and 10) to investigate the pH influence on the copolymer auto-association ability, and in presence of 0.1 M of NaCl to shield the electrostatic repulsion between the carboxylate moieties. At pH 4.6 the formation of hydrophobic clusters is detected at 8 mg L^{-1} (*i.e.* 5.3×10^{-6} mol L^{-1}). As reported in the literature, pyrene fluorescence spectrum is not significantly modified in presence of pure PAA even for low ionization degree [30,31]. Consequently, the detected hydrophobic clusters can't be attributed to pyrene solubilization into the PAA segments of the unimers but definitely corresponds to micelles formation. The cmc value is more than tenfold increased at pH 7, whereas at pH 10 no cluster formation is detected anymore until 1 g L^{-1}. This behaviour can be explained by the modification of the copolymer hydrophilic/hydrophobic balance when the pH is increased. At pH 4.6, the polymeric chain is mainly on the carboxylic acid form, and micelles are obtained at low concentrations. When the proportion of deprotonated moieties increases, the copolymer hydrophilicity also increases shifting the cmc towards higher concentrations.

However, as pyrene has been reported to be inefficient to monitor micelles formation at pH = 11 in the case of fluorocarbon-modified PAA [32], QELS measurements (size distribution by number) have

Table 1
Characterization of the lipid-*b*-PtBA copolymers.

Ref.	[Monomer]/[initiator]	Conv. (%)	$\overline{X_{n,\mathrm{theo}}}$[a]	$\overline{M_{n,\mathrm{theo}}}$[b] (g mol^{-1})	$\overline{M_{n,\mathrm{exp}}}$[c] (g mol^{-1})	PDI[c]
2a	50	8	4	926	1100	1.25
2b	100	14	14	2080	2230	1.50
2c	50	34	17	2590	2700	1.27
2d	50	44	22	3290	3170	1.28
2e	100	37	37	5146	5100	1.32
2f	100	47	47	6426	7200	1.26

[a] $\overline{X_{n,\mathrm{theo}}} = [\mathrm{monomer}]/[\mathrm{initiator}] \times \mathrm{conversion}$.
[b] $\overline{M_{n,\mathrm{theo}}} = M_{\mathrm{lipoinitiator1}} + \overline{X_{n,\mathrm{theo}}} \times M_{\mathrm{tBA}}$.
[c] determined by SEC using poly(methyl methacrylate) standard calibration.

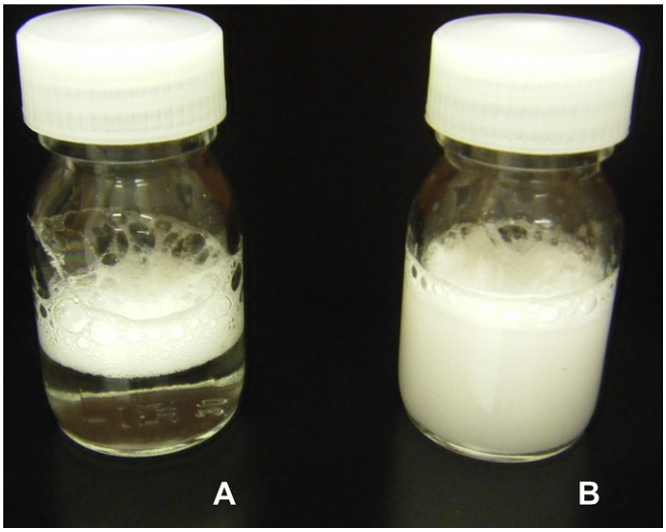


Fig. 3. A) Lipid-b-PAA **3c** in water, B) Emulsion dichloromethane/water stabilized by **3c**.

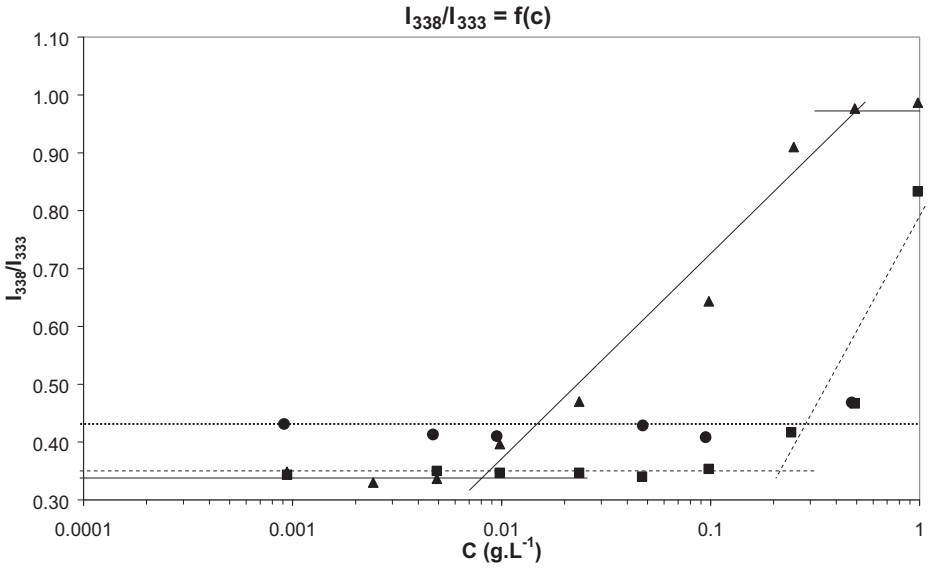


Fig. 4. I_{338}/I_{333} *vs.* concentration for copolymer **3c** at (▲) pH = 4.6, (■) pH = 7 and (●) pH = 10.

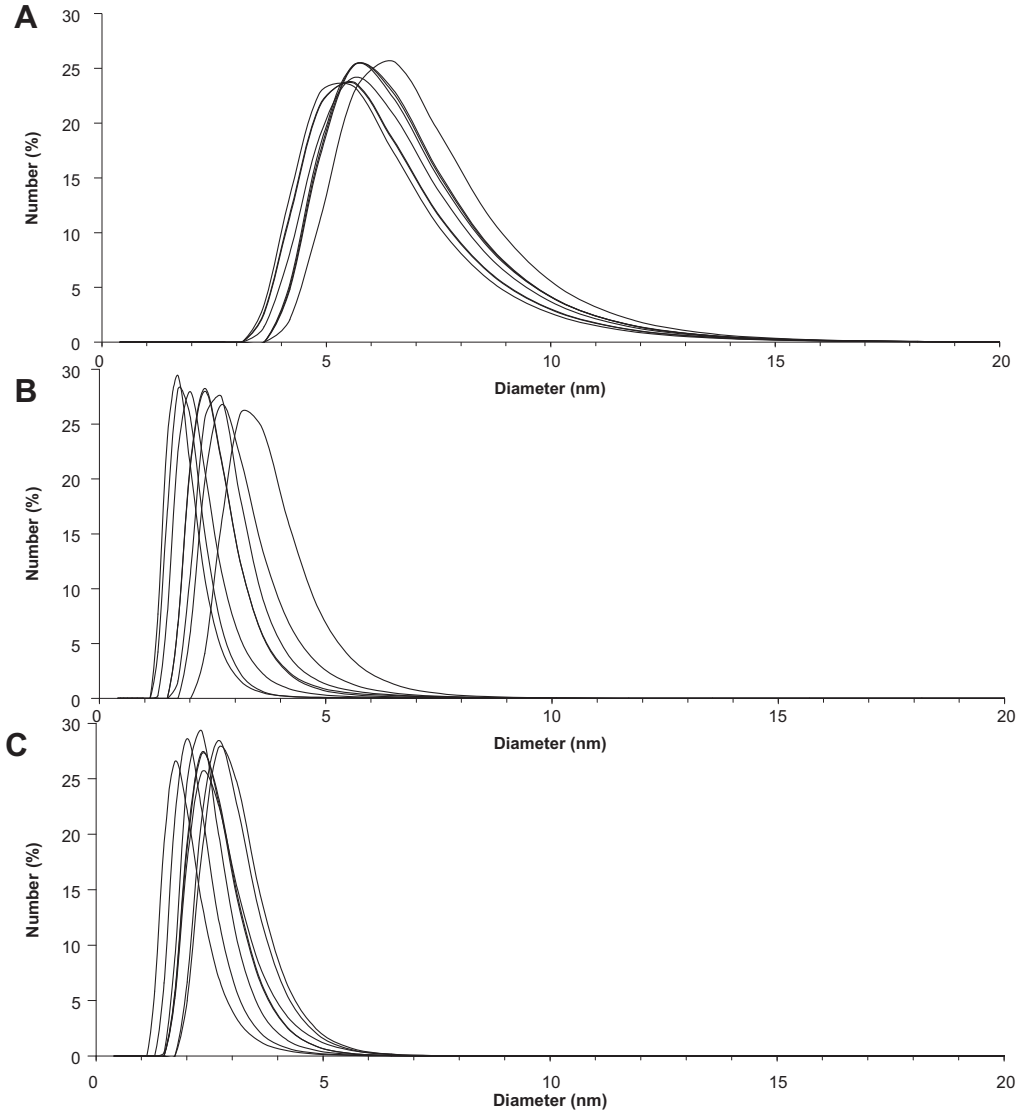


Fig. 5. QELS analysis (size distribution by number) for copolymer **3c** at A) pH = 4.6, B) pH = 7 and C) pH = 10.

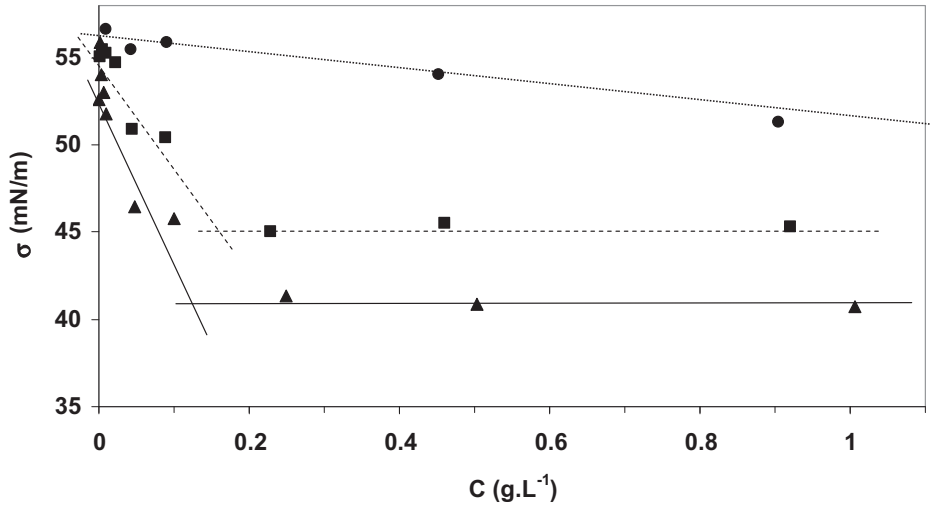


Fig. 6. Surface tension *vs.* copolymer **3c** concentration at (▲) pH = 4.6, (■) pH = 7 and (●) pH = 10.

been performed in identical conditions (buffered solutions + 0.1M of NaCl) to investigate the object size *vs.* pH (Fig. 5). At pH = 4.6, one homogeneous population with an average diameter of 50–60 Å, consistent with the size of micelles, is observed. Although never detected by QELS analysis (size distribution in number) due to their limited numbers, the simultaneous presence of unimers in equilibrium with the micelles is highly probable [33]. At pH = 10, the average diameter is divided by 2 (20–30 Å) and could corresponds to the presence of unimers in solution. At pH = 7, the diameter distribution dispersity is a bit higher, ranging from 20 to 40 Å, and consistent with the simultaneous presence of small micelles and unimers in solution. QELS analyses then confirm the fluorescence results with a well-defined system at pH = 4.6 constituted of micelles, a transition state at pH = 7, with simultaneous presence of micelles and unimers and no micelles anymore at pH = 10.

The air/water adsorption of copolymer **3c** was also investigated through surface tension measurements (buffered solutions + 0.1M of NaCl) (Fig. 6). At pH = 4.6, the surface tension of solutions of copolymer **3c** rapidly decreases proving the fast adsorption of the copolymer at the air/water interface. The surface tension stabilizes once the interface is saturated at a low value of $\sigma = 40$ mN/m. A pH increase at 7 results in a slower copolymer adsorption and a decreased stabilizing effect ($\sigma = 45$ mN/m) after the surface saturation.

Another critical concentration can be defined from these plots, at the intersection between the two linear portions of the curve, and corresponding to the concentration of the air/water interface saturation (C_s). For molecular surfactants, when the air/water interface is saturated, further increase in concentration leads to the formation of micelles in bulk solution, C_s would thus correspond to the cmc determined by fluorescence measurements. However the behaviour of amphiphilic copolymers in aqueous solution is complex and the existence of two different critical concentrations has already been reported for amphiphilic polysaccharides by Duval-Terrié *et al.* [34]. Depending on the pH, the adsorption behaviour of copolymer **3c** at the air/water interface changes. At pH 4.6, $C_s \sim 0.12$ g L^{-1}, far above the cmc determined by fluorescence measurements (10 mg L^{-1}). This difference demonstrates that the copolymer self-associates to form micelles in solution before saturation of the air/water interface and the gap between the two concentrations (Cs and cmc) reflects the strong tendency of copolymer **3c** for self-assembly [34]. At pH 7, both values are close ($C_s \sim 0.15$ g L^{-1}, cmc = 0.2 g L^{-1}) showing a preferential adsorption of the copolymer at the air/water interface and hydrophobic clusters formation only when the surface saturation is reached, similarly to molecular surfactants. At pH 10, a really low adsorption is observed and the surface saturation is not reached at 1 g L^{-1}. This result is in coherence with a previous study were the non-surface activity of PAA at high ionization was reported [35]. As no hydrophobic cluster formation was detected either by fluorescence measurements, the copolymer hydrophilicity seems to be high enough to inhibit any self-association in bulk or interface adsorption in the range of the studied concentrations.

Finally, the influence of the hydrophilic block length on the copolymer auto-association properties was investigated. Several lipid-*b*-PAA copolymers with a hydrophilic/hydrophobic (wt/wt) balance ranging from 38/62 to 80/20 were analysed by fluorescence experiment to determine the cmc in acidic media (pH = 4.6) (Table 2).

Table 2
Characterization of a range of lipid-*b*-PAA copolymers (pH 4.6).

Ref.	$\overline{X_{n,\text{theo}}}$[a]	$\overline{M_{n,\text{theo}}}$[b] (g mol^{-1})	Hydrophilic/ hydrophobic balance (wt/wt)	Cmc[c] (mg L^{-1})	Cmc[d] (μmol L^{-1})
3a	4	698	41/58	8.4	12.0
3b	14	1418	72/28	7.6	5.4
3c	17	1634	75/25	7.4	4.5
3d	22	1994	79/21	6	3.0

[a] $\overline{X_{n,\text{theo}}} = [\text{monomer}]/[\text{initiator}] \times \text{conversion}$.
[b] $\overline{M_{n,\text{theo}}} = M_{\text{lipoinitiator1}} + \overline{X_{n,\text{theo}}} \times M_{AA}$
[c] Determined by fluorescence measurement using pyrene.
[d] $cmc = \frac{cmc_{\text{mass}}}{\overline{M_{n,\text{theo}}}}$.

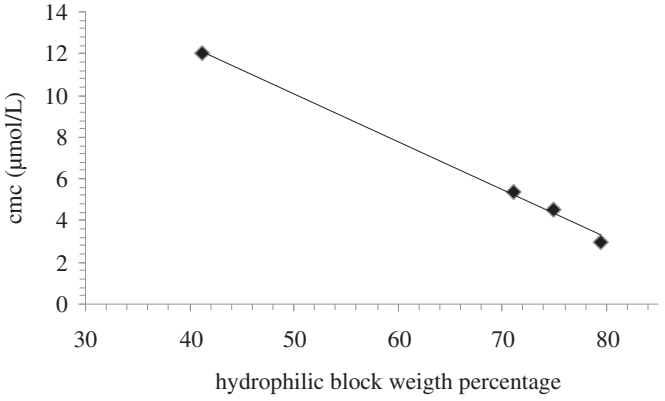


Fig. 7. Evolution of the cmc with the hydrophilic/hydrophobic balance.

A linear dependence between the cmc and the hydrophilic/hydrophobic balance was observed with a decrease in the cmc when the hydrophilic block length increases (Fig. 7).

The cmc evolution differs from the typical ionic and non-ionic surfactants behaviour. The weak hydrophilicity of protonated PAA probably results in a small effect of the block length on the copolymer solubility as already described for PMMA-b-PDMAEMA systems [36]. Simultaneously, longer PAA blocks will occupy more space on the micelle surface decreasing the number of macromolecules required to form the micelle. Finally, increasing the PAA block length leads to a predominance of the steric effect over the solubility effect resulting in a cmc decrease. The synthetic pathway, through a controlled polymerization technique, is thus of great interest to accurately control the hydrophilic block length and consequently adjust the auto-association properties.

4. Conclusion

To our best knowledge, this paper reports the first example of ATRP initiator synthesized from naturally occurring vegetable oil. The oil functionalization was conducted in two simple steps, triglycerides reduction followed by esterification of the resulting fatty alcohol to introduce the ATRP initiating site. Our original lipoinitiator leads to a well-controlled polymerization by ATRP and a range of lipid-*b*-P*t*BA copolymers was synthesized with different lengths of the synthetic block. Acidolysis of the P*t*BA block leads to amphiphilic lipid-*b*-PAA copolymers. A copolymer with an hydrophilic/hydrophobic ratio of 75/25 (wt/wt) was fully characterized and interesting properties were detected. In acidic media, micelles form at a low cmc of 7.6 mg L^{-1} (*i.e.* 5.3×10^{-6} mol L^{-1}) and a fast air/water adsorption is observed leading to a good stabilization of the interface. When the pH is raised, the cmc is highly increased ($\sim$ 0.2 g L^{-1}, *i.e.* 1.2×10^{-4} mol L^{-1}) due to a modification of the copolymer HLB and the air/water adsorption is slowed down. At pH 10, no self-association was detected anymore and the surface adsorption was inhibited. Our original lipid-*b*-PAA copolymers form well-defined and pH-sensitive micelles that start to dissociate when the pH is raised above 7, which could be a stimuli to trigger a drug release.

Finally it was demonstrated that the copolymer cmc can be adjusted through modification of the hydrophilic/hydrophobic balance further proving the interest of using a highly controlled polymerization technique in the synthesis.

This study demonstrates that well-defined micelles can be synthesized from a heterogeneous natural product. Their ability to encapsulate drugs as well as extension of this work to other stimuli-responsive polymers are currently under investigation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.polymer.2012.07.041.

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