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Poly(*N*-*tert*-butyl acrylamide-*b*-*N*-acryloylmorpholine) amphiphilic block copolymers via RAFT polymerization: Synthesis, purification and characterization

Bertrand de Lambert, Marie-Thérèse Charreyre*, Carole Chaix, Christian Pichot

Unité Mixte CNRS/bioMérieux, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, F-69364 Lyon Cedex 07, France

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Abstract

Amphiphilic block copolymers consisting of two poly(acrylamide) derivative blocks have been synthesized via the reversible addition fragmentation chain transfer (RAFT) polymerization process with a hydrophobic block, poly(*N*-*tert*-butyl acrylamide), poly(TBAm), and a non-ionic hydrophilic one, poly(*N*-acryloylmorpholine), poly(NAM). Both polymerization orders, poly(TBAm-*b*-NAM) and poly(NAM-*b*-TBAm), were compared in terms of conversion and control over molecular weights (MW). Purification of the block copolymers was carried out via several methods in order to optimize their subsequent characterization. ^{1}H NMR analysis resulted in an accurate determination of the second block MW whereas determination of the CMC by the pendant drop method confirmed the ability of the poly(TBAm-*b*-NAM) block copolymers to self-assemble into micelles in aqueous phase.

Keywords: Amphiphilic block copolymers; Acrylamide derivatives; Reversible addition fragmentation chain transfer

1. Introduction

Synthesis of amphiphilic block copolymers has met a renewed interest during the last decade with the appearance of control radical polymerization (CRP) techniques since it is now possible to obtain block copolymers from a wide variety of monomers. CRP processes are especially relevant to synthesize amphiphilic block copolymers from monomers bearing ionic or polar side-groups, which was often difficult using living ionic polymerization techniques. For instance, to get a poly(acrylic acid) block, two steps are generally required: the synthesis of a poly(*tert*-butyl acrylate) block by living anionic polymerization followed by a hydrolysis step.

All the different monomer families (styrenes, (meth)acrylates, (meth)acrylamides) can now be polymerized by the main CRP techniques, namely NMP [1], ATRP [2,3] and RAFT [4,5]. However, the (meth)acrylamide derivatives are rarely polymerized by NMP and ATRP, even if recent articles show improved results [6–8]. Considering the RAFT process, all kind of acrylamide derivatives (hydrophilic [9–12] or hydrophobic [13]) can indeed be polymerized up to high conversions and with a very good control over MW and molecular weight distribution (MWD) even for high MW values (300 000 g mol^{-1}) [14].

Numerous block copolymers consisting of at least one block of a poly(acrylamide) derivative have been obtained by RAFT polymerization so far. Most of them are amphiphilic block copolymers, with a hydrophobic block of, for instance, poly(styrene) [15–18], poly(*tert*-butyl methacrylate) [18] or poly(*N*-acryloxysuccinimide) [19], and with a hydrophilic block of a poly(acrylamide derivative), generally neutral like poly(*N*-acryloylmorpholine) [17], poly(dimethylacrylamide) [15,16,19] or a thermosensitive block of poly(*N*-isopropylacrylamide) [18].

Alternatively, block copolymers consisting of two poly(acrylamide) blocks have also been synthesized by the RAFT

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* Corresponding author. Tel.: +33 4 72 72 83 60; fax: +33 4 72 72 85 33.
E-mail address: mtcharre@ens-lyon.fr (M.-T. Charreyre).

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process, either with two ionic blocks, poly((*ar*-vinylbenzyl) trimethylammonium chloride-*b*-*N*,*N*-dimethylvinylbenzylamine) [20], poly(sodium 2-acrylamido-2-methylpropanesulfonate-*b*-sodium 3-acrylamido-3-methylbutanoate) [21], poly(sodium 2-acrylamido-2-methylpropanesulfonate-*b*-sodium 6-acrylamidohexanoate) [22], or with a neutral block and a ionisable one, poly(dimethylacrylamide-*b*-dimethylaminoethyl methacrylamide) [23,24], poly(*N*-[3-(dimethylamino)propyl] methacrylamide-*b*-dimethylacrylamide) [25], poly(2-(acrylamido)-2-methylpropane sulfonate-*b*-*N*-isopropylacrylamide) [26], poly(3-[*N*-(3-methacrylamidopropyl)-*N*,*N*-dimethyl] ammoniopropanesulfonate-*b*-*N*-isopropylacrylamide) [27], or with two neutral blocks, poly(methacrylamide-*b*-acrylamide) [28] and poly(*N*-acryloylpyrrolidine-*b*-dimethylacrylamide) [29].

In this research work, we have synthesized by RAFT polymerization amphiphilic block copolymers where the two blocks consist of a poly(acrylamide) derivative, a hydrophobic one, poly(*N*-*tert*-butyl acrylamide), poly(TBAm), and a non-ionic hydrophilic one, poly(*N*-acryloylmorpholine), poly(NAM). These block copolymers were designed for applications in biological diagnostic tests onto microarrays (DNA chips), the hydrophilic block bearing single-strand DNA probes and the hydrophobic block ensuring a good immobilization of the block copolymer onto the surface of the microarray.

One major advantage of such block copolymers fully based on poly(acrylamide) derivatives is their ability to resist strong acidic or basic conditions, much better than block copolymers bearing a poly(acrylate) or poly(methacrylate) block. This advantage has been recently used to grow single-strand DNA probes from a poly(NAM) backbone bearing nucleotide starters, via a strategy that requires a deprotection step in strong basic conditions after each nucleotide addition [30]. The same strategy has been applied to a poly(*N*-*tert*-butyl acrylamide-*b*-(*N*-acryloylmorpholine-*co*-*N*-acryloxysuccinimide)) block copolymer synthesized in a similar way than the poly(TBAm-*b*-NAM) reported here [31].

The RAFT polymerization has been carried out in the presence of *tert*-butyl dithiobenzoate as chain transfer agent (CTA) since both *N*-acryloylmorpholine and *N*-*tert*-butyl acrylamide have been previously homopolymerized with this dithioester with high conversions (70–95%) and controlled MW [11,13]. Polymer chains that reached 120 000 g mol^{-1} could be synthesized with PDI below 1.3. A detailed study about the influence of experimental conditions (temperature, monomer concentration, CTA/initiator molar ratio) provided valuable information which resulted in an additional improvement of the control (PDI below 1.1) in the case of NAM [32].

In addition to the synthesis part, we have especially studied the purification of the block copolymers. Indeed, the purification of amphiphilic block copolymers is often very difficult to carry out and rarely described in the literature. In fact, when designing a block copolymer by RAFT using a macroCTA as first block, it is inherent in the technique to get a small amount of each of the two blocks mixed with the desired block copolymer. On the one hand, since part of the macroCTA chains are not ended by a dormant group, some residual dead chains of the first block will be present. On the other hand, since it is necessary to add some initiator to start the polymerization of the second monomer, some homopolymer chains (corresponding to the second block) will grow in parallel to the block copolymer chains. An adequate choice of the synthetic conditions of the macroCTA and of the block copolymer will allow one to reduce the amount of these parasite chains, but it will never totally eliminate them. Finally, three parameters will control the quality of the block copolymer synthesis:

- The blocking efficiency, related to the nature of the two monomers and to the order in which they are polymerized [33].
- The CTA/initiator ratio used to synthesize the macroCTA, which determines the amount of dead chains of the first block.
- The macroCTA/initiator ratio used to synthesize the block copolymer, which determines the amount of homopolymer chains of the second block.

In the first part of this article, we have studied the influence of several of these parameters on the kinetics and the molar mass characteristics of the block copolymers. Then, a purification process has been perfected and applied to several block copolymer samples. The purified samples have been accurately characterized by NMR spectroscopy, which provided their molar composition and consequently, the molar mass of their second block. Moreover, the amphiphilic properties of these samples have been investigated using the pendant drop method with the determination of their critical micellar concentration (CMC).

2. Experimental section

2.1. Materials

N-Acryloylmorpholine (NAM) (Aldrich, 97%) was distilled under reduced pressure (120 °C, 10 mm Hg) to remove inhibitor. *N*-*tert*-Butyl acrylamide (TBAm) (Aldrich, 97%) was used without further purification. 2,2′-Azobis(isobutyronitrile) (AIBN, Fluka, 98%) was purified by recrystallization from ethanol. 1,4-Dioxane (Acros, 99%) was distilled over $LiAlH_4$ (110 °C). Trioxane (Acros, 99%) was used as received. Poly(TBAm) macroCTA synthesis (Table 1) has been described recently [13].

2.2. RAFT polymerizations

Monomer, macroCTA, AIBN, dioxane and trioxane (internal reference for ^{1}H NMR determination of monomer consumption) were introduced into a Schlenk tube equipped with a magnetic stirrer (Table 2). The mixture was degassed by four freeze–evacuate–thaw cycles and then heated under nitrogen in a thermostated oil bath.

For all experiments, the experimental conditions are described in Table 2.

Table 1
Conversion, number-average molecular weight and polydispersity index for the macroCTAs synthesized in the presence of *tert*-butyl dithiobenzoate in dioxane at 90 °C

MacroCTA	[CTA]/[AIBN]	Time (min)	Conversion (%)	$M_{n\ th.}$ (g mol^{-1})	$M_{n\ exp.}$ (SEC/LS) (g mol^{-1})	PDI
Poly(TBAm)$_{I}$	3.3	30	24	9500	14 000	1.05
Poly(TBAm)$_{II}$	10	120	41	12 500	14 000	1.05
Poly(TBAm)$_{III}$	10	210	36	2300	2650[a]	1.13[a]
Poly(NAM)	10	150	72	36 000	40 500	1.03

[a] Determined by MALDI-TOF MS.

Periodically, samples were withdrawn from polymerization media for analyses. Conversion was determined by ^{1}H NMR using a Bruker Avance 200 MHz spectrometer according to an already published method [34]. Typically, 500 μL of deuterated chloroform, $CDCl_3$, was added to 150 μL of each sample.

2.3. Purification of the amphiphilic block copolymers

Purification has been carried out in two steps. First, poly(TBAm-*b*-NAM) block copolymer samples were precipitated in diethyl ether. Typically, a known volume of polymerization mixture was poured into a larger volume (×40) of diethyl ether. Polymer powder was then recovered by filtration, washed with the same solvent and finally dried under vacuum.

In a second step, two methods have been investigated:

(i) Either performing 3–5 washes by MilliQ water at 60 °C under stirring. Typically, 2 mL of MilliQ water was added to 20 mg of dry polymer. The mixture was heated at 60 °C for 1 h, and then the water phase was withdrawn and the polymer was dried under vacuum. ^{1}H NMR analysis was carried out on the dry polymer and on the water phase to follow the purification efficiency.

(ii) Or performing a precipitation/extraction in a pentane/water 50/50:v/v mixture using a separating funnel. Typically, 20 mg of dry polymer was dissolved in dichloromethane (1 mL) and the solution was precipitated drop by drop in the upper phase (pentane, 8 mL) that became cloudy. Then, extraction was performed which allowed poly(NAM) chains to be solubilized in the lower phase (water). After removal of the water phase several additional extractions with fresh MilliQ water were carried out. After each extraction, ^{1}H NMR analyses were performed on both phases to follow the purification efficiency.

2.4. Characterization of the polymer samples

2.4.1. Poly(TBAm) homopolymer

The molecular weights of poly(TBAm) homopolymers were determined by size exclusion chromatography (SEC) coupled to a light scattering detection (LSD) using two Waters Ultrastyragel columns (HR4 and HR1). The mobile phase consisted of THF (SDS, 99%) with a flow rate of 1 mL min^{-1} using a Spectra-Physics Isochrom LC pump. On-line double detection was provided by an 18-angles DAWN light scattering photometer (Wyatt Technologies) operating at 632 nm, associated with a differential refractometer (DRI Waters 410). The specific refractive index increment (d*n*/d*c*) for poly(TBAm) in THF (0.097) has been determined with a NFT ScanRef monocolor interferometer operating at 633 nm. The molecular weight and polydispersity index were determined using the Wyatt ASTRA SEC/LS software package.

2.4.2. Amphiphilic block copolymers

Analysis of the various block copolymers was performed by both techniques:

- Size exclusion chromatography (SEC) in THF (SDS, 99%), using a Waters column (Styragel HR4E). The flow rate was maintained at 1 mL min^{-1} using a Waters 1515 isocratic HPLC pump. Analyses were performed by injection of 20 μL of polymer solution (5 mg mL^{-1}) in THF. Detection was performed using a Waters 2410 differential refractometer. The molecular weight and polydispersity data were determined using the Waters Breeze software package, according to a polystyrene calibration.
- ^{1}H NMR, using a Bruker Avance spectrometer (200 MHz).

2.5. Determination of the CMC of the amphiphilic block copolymers

For block copolymer runs 3T6 and 4T6, solutions with increasing concentration were prepared: 0.01, 0.025, 0.05, 0.1, 0.2, 0.5, 1 and 2 g L^{-1}. Typically, a known amount of polymer was first solubilized in an appropriate volume of THF and let

Table 2
Experimental conditions for poly(TBAm-*b*-NAM) and poly(NAM-*b*-TBAm) block copolymer synthesis via RAFT polymerization in dioxane at 90 °C

Run	MacroCTA	Monomer (second block)	[Monomer] (mol L^{-1})	[MacroCTA]/[AIBN]	[Monomer]/[MacroCTA]	$M_{n\ th.}$ second block (g mol^{-1})
1	Poly(TBAm)$_{I}$	NAM	1	3.3	284	40 000
2	Poly(TBAm)$_{I}$	NAM	1	3.3	142	20 000
3	Poly(TBAm)$_{II}$	NAM	1	3.3	284	40 000
4	Poly(TBAm)$_{II}$	NAM	3.75	3.3	709	100 000
5[a]	Poly(TBAm)$_{III}$	NAM	3.75	3.3	85	12 000
6	Poly(NAM)	TBAm	1	3.3	236	30 000

[a] $T = 75$ °C.

to stir for 3 h. Then, MilliQ water was added to obtain a water/THF ratio of 95/5:v/v.

Surface tension measurements were obtained using the pendant drop method. The apparatus consists of a Kruss Contact Angle Measuring System G10 and a Drop Shape Analysis 1.51 software. The drop was formed at the tip of the syringe by pressing solution out by means of a set screw. The drop shape analysis was performed as follows: first, a drop profile was extracted from the drop image (obtained with a video camera); then, a curve fitting program compared the experimental drop profile with a theoretical one and gave the corresponding surface tension value (function of the drop geometry). For each measurement, 3–5 photographs of the drop were recorded and the average value of the surface tension was determined. The reproducibility of the surface tension measurement was +/−0.3 mN m^{-1}. For each concentration of the block copolymer solution, the evolution of the drop surface tension was followed over 30 min.

3. Results and discussion

3.1. Synthesis of amphiphilic poly(TBAm-b-NAM) from a poly(TBAm) macroCTA

Amphiphilic block copolymers were synthesized from two acrylamide derivatives, *tert*-butyl acrylamide and *N*-acryloylmorpholine, using a two-step strategy: polymerization of the first monomer and use of this block as macroCTA to polymerize the second monomer.

Synthesis of poly(TBAm-*b*-NAM) block copolymers was performed from various samples of poly(TBAm) dormant chains previously synthesized by RAFT polymerization [13] (Table 1). Two samples, poly(TBAm)$_{\text{I}}$ and poly(TBAm)$_{\text{II}}$ were of the same M_n and PDI (14 000 g mol^{-1} and 1.05), but were expected to contain a different number of dead chains since they had been synthesized with a different [CTA]/[AIBN] molar ratio (3.3 and 10, respectively). Hence, poly(TBAm)$_{\text{II}}$ should contain less dead chains. Another sample, poly(TBAm)$_{\text{III}}$, was of a lower M_n, 2650 g mol^{-1} (PDI of 1.13), and was dedicated to prepare small-sized block copolymers.

In the first two runs, NAM was polymerized in dioxane at 90 °C using poly(TBAm)$_{\text{I}}$ as macroCTA and AIBN as initiator. The [NAM]/[macroCTA] ratio was chosen such that the calculated M_n should be 40 000 and 20 000 g mol^{-1} at 100% conversion (runs 1 and 2, respectively, Table 2). A third run was carried out in the same conditions as run 1 except for the use of poly(TBAm)$_{\text{II}}$ (run 3, Table 2). Another run was performed in the presence of poly(TBAm)$_{\text{III}}$, in order to prepare a short poly(NAM) block (M_n of 12 000 g mol^{-1} at 100% conversion, run 5, Table 2). In this last run, experimental conditions were changed to enable withdrawing of samples at low conversion (temperature of 75 °C instead of 90 °C) without slowing down too much the polymerization rate (monomer concentration of 3.75 mol L^{-1} instead of 1 mol L^{-1}) [32].

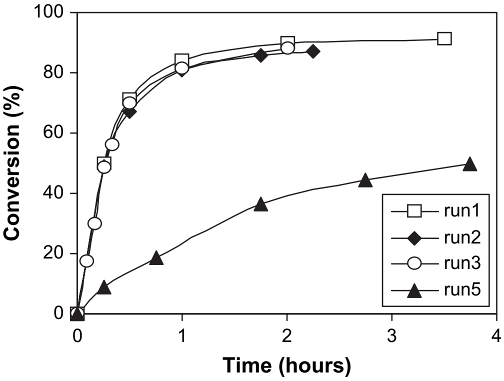

Fig. 1. Monomer conversion vs time plots for NAM polymerization in the presence of macroCTA poly(TBAm)$_{\text{I}}$, poly(TBAm)$_{\text{II}}$ and poly(TBAm)$_{\text{III}}$.

The comparison of the block copolymerization kinetics indicated that NAM polymerized in a very similar way for the various [NAM]/[macroCTA] ratios while keeping constant the [macroCTA]/[AIBN] ratio (Fig. 1), which confirmed what was already reported when using a CTA instead of a macroCTA [32]. Conversion (90%) was reached in 2 h except for run 5, slower as a consequence of the low temperature.

In order to follow the evolution of molecular weights with conversion, block copolymer samples were analyzed by SEC (THF) using a refractometer as detector. We are aware that such analyses should be taken with caution, first since polystyrene standards were used (poor estimation of poly(NAM)) and second, since in the case of amphiphilic block copolymers the hydrodynamic volume of each block is very different. Consequently, the obtained MW values are relative and only the evolution of MW with conversion within a same polymerization assay or between similar assays has been taken into consideration. The determination of the real MW values has been performed by NMR as reported later in this article.

According to Fig. 2, the evolution of the relative molecular weights was linear until 60–70% conversion that indicated a controlled synthesis of the second block. The lower dead

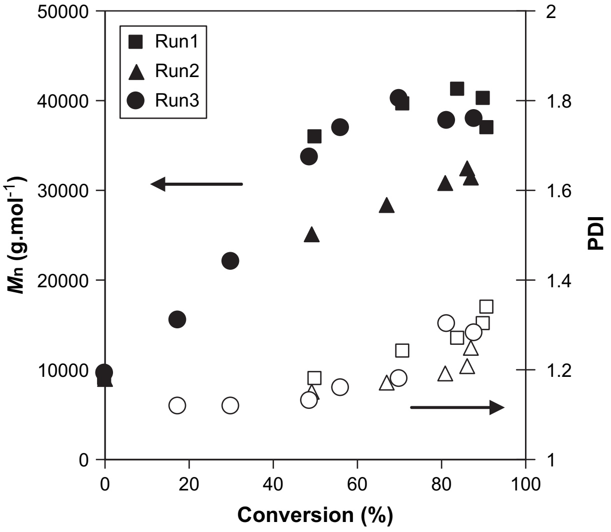

Fig. 2. Number-average molecular weight M_n and polydispersity index PDI vs monomer conversion plots for NAM polymerization in the presence of macroCTA poly(TBAm)$_{\text{I}}$ and poly(TBAm)$_{\text{II}}$; $[\text{NAM}]_0 = 1$ mol L^{-1}; $[\text{macroCTA}]_0/[\text{AIBN}]_0 = 3.3$; $[\text{NAM}]_0/[\text{macroCTA}]_0 = 284$ and 142.

chain number in poly(TBAm)$_{\text{II}}$ did not seem to have a significant influence on the control of the polymerization. In fact, the theoretical amount of dead chains for poly(TBAm)$_{\text{I}}$ and poly(TBAm)$_{\text{II}}$ samples was estimated to be around 15% and 9%, respectively, taking into account the [CTA]$_0$/[AIBN]$_0$ initial ratio and the corresponding time, according to the following equation. As these values were not so different, it could explain a similar control over polymerization.

$$\%_{\text{dead chains}} = \frac{2f[\text{AIBN}]_0\times(1-\mathrm{e}^{-k_{\mathrm{d}}\times t})}{[\text{CTA}]_0+2f[\text{AIBN}]_0\times(1-\mathrm{e}^{-k_{\mathrm{d}}\times t})}$$

with k_{d} and f, the initiator decomposition rate coefficient (at 90 °C, $k_{\mathrm{d\ AIBN}} = 7\times10^{-4}\ \mathrm{s}^{-1}$) and efficiency factor ($f = 0.5$), respectively.

Moreover, at a given conversion, experimental M_{n} values were indeed higher when longer chains were targeted (runs 1 and 3 compared to run 2). However, after 70% conversion, M_{n} values seemed to level off (or even decrease) whereas PDI values increased, indicating the presence of additional short chains. This phenomenon, that appeared magnified for longer targeted chains, had already been observed for homopolymer chains of either poly(TBAm) [13] or poly(NAM) [32,35], and was tentatively explained by transfer to the polymer followed by chain fragmentation at this high temperature [35].

The corresponding SEC traces (run 3, Fig. 3) are regularly shifted towards higher molecular weights with conversion (far away from the poly(TBAm)$_{\text{II}}$ first block). The presence of a tail was visible on the low molecular weight side of the peaks. It could not be due to poly(TBAm) dead chains (since they remain soluble during the precipitation step in diethyl ether) but it might correspond to poly(NAM) chains synthesized in parallel to the block copolymer chains (poly(NAM) and poly(TBAm-*b*-NAM) do precipitate in diethyl ether).

In the previous runs, poly(TBAm-*b*-NAM) block copolymer chains were obtained with relative M_{n} values between 15 000 and 40 000 g mol^{-1}. It was also possible to get short size block copolymers with a very good control of the polymerization (Fig. 4, run 5, relative M_{n} values between 2500 and 7000 g mol^{-1}).

Alternatively, long poly(TBAm-*b*-NAM) block copolymers (targeted MW of 100 000 g mol^{-1}) were synthesized from poly(TBAm)$_{\text{II}}$ (run 4, Table 2) using a modified process [14].

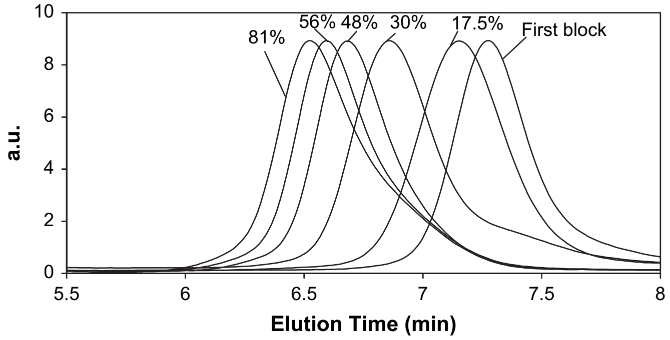

Fig. 3. SEC chromatograms of poly(TBAm-*b*-NAM) block copolymer samples corresponding to run 3 in the presence of poly(TBAm)$_{\text{II}}$ macroCTA.

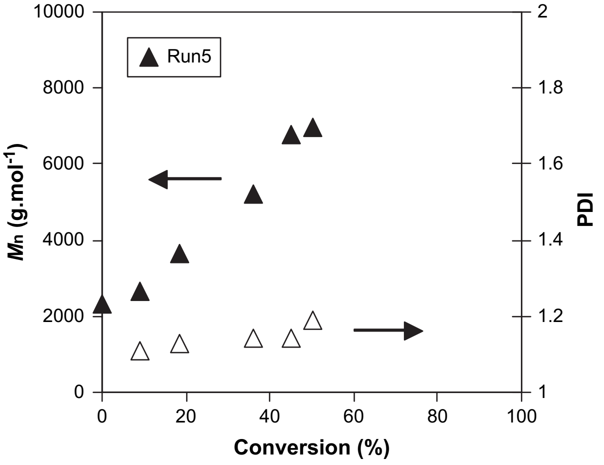

Fig. 4. Number-average molecular weight M_{n} and polydispersity index PDI vs monomer conversion plots for NAM polymerization at 75 °C in the presence of macroCTA poly(TBAm)$_{\text{III}}$; [NAM]$_0$ = 3.75 mol L^{-1}; [NAM]$_0$/[macroCTA]$_0$ = 85; [macroCTA]$_0$/[AIBN]$_0$ = 3.3.

Experimental M_{n} values linearly increased on the whole conversion range (up to 90%) avoiding the stagnation phenomenon observed for runs 1, 2 and 3 (Fig. 5). The apparent critical value of 40 000 g mol^{-1} (runs 1 and 3) was surpassed since chains of 70 000 g mol^{-1} were obtained. As that assay was performed at a higher monomer concentration (3.75 mol L^{-1} instead of 1 mol L^{-1}) and as longer chains were targeted (100 000 g mol^{-1} instead of 40 000 g mol^{-1}), the medium was very viscous that could explain the increase in polydispersity with conversion.

The corresponding SEC chromatograms exhibited a very significant shift towards higher molecular weights already at low conversion (22%, Fig. 6). However, a second population could be observed above 50% conversion due to parasite poly(NAM) chains formed in parallel to the block copolymer chains. Then, a more efficient purification method seemed

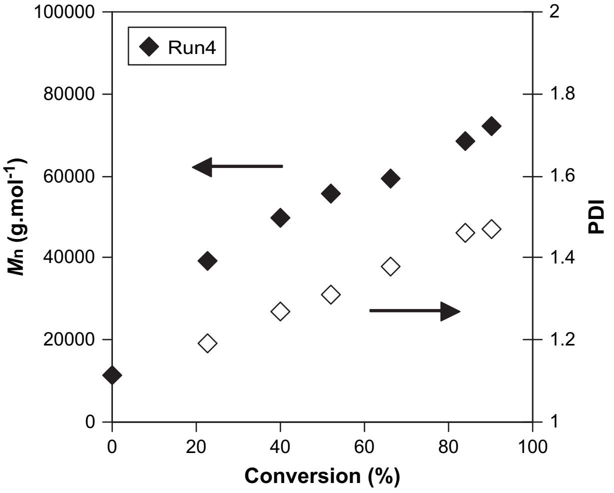

Fig. 5. Number-average molecular weight M_{n} and polydispersity index PDI vs monomer conversion plots for NAM polymerization in the presence of macroCTA poly(TBAm)$_{\text{II}}$; [NAM]$_0$ = 3.75 mol L^{-1}; [NAM]$_0$/[macroCTA]$_0$ = 709; [macroCTA]$_0$/[AIBN]$_0$ = 3.3.

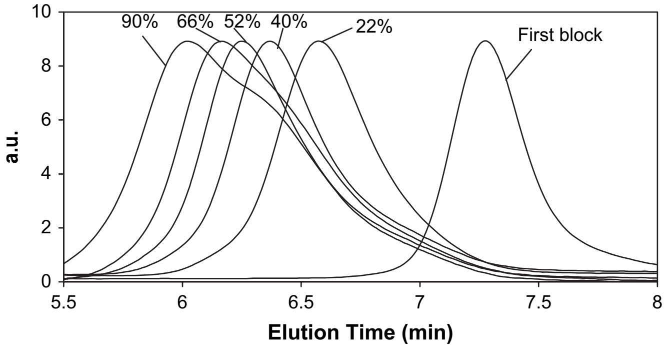

Fig. 6. SEC chromatograms of poly(TBAm-*b*-NAM) block copolymer samples corresponding to run 4 in the presence of poly(TBAm)$_{II}$ macroCTA.

necessary (described later in this article) to fully isolate the block copolymer chains.

3.2. Synthesis of amphiphilic poly(NAM-b-TBAm) from a poly(NAM) macroCTA

As TBAm and NAM monomers belong to the same family, it is in principle possible to carry out their block copolymerization in either way [33]. Then, the opposite blocking order was investigated via synthesis of poly(NAM-*b*-TBAm) block copolymers from a poly(NAM) macroCTA in order to compare the kinetics and the characteristics of the resulting block copolymers with the previous ones.

A poly(NAM) sample of $M_n = 40\,500$ g mol^{-1} (PDI = 1.03) was chosen as macroCTA (Table 1) [36] to polymerize TBAm (run 6, Table 2). Conversion reached 65% in 2 h at 90 °C (Fig. 7(a)), a similar behavior to RAFT polymerization of TBAm in the presence of *tert*-butyl dithiobenzoate [13]. Note that the polymerization of TBAm from a poly(NAM) macroCTA was much slower than the polymerization of NAM from a poly(TBAm) macroCTA (comparison of run 6 with runs 1, 2 and 3, performed in the same conditions). This is mainly due to the lower polymerization rate of TBAm compared to that of NAM (58% of conversion for TBAm instead of 77% for NAM, after 1 h of polymerization in the same conditions) [36]. Concerning the poly(NAM-*b*-TBAm) molecular weight evolution, a linear increase of M_n with conversion was observed until 60%, with PDI values comparable to those obtained for poly(TBAm-*b*-NAM) copolymers (Fig. 7(b)).

Finally, the possibility to synthesize well controlled poly(NAM-*b*-TBAm) block copolymers was demonstrated, although the monomer used for the first block is a faster polymerizing monomer than the one used for the second block (i.e. the radical corresponding to NAM is slightly less stabilized than that corresponding to TBAm). As a consequence, the fragmentation of the intermediate radical formed at the junction of the two blocks was slightly disfavoured towards release of the poly(NAM) macroradical and hence towards the formation of the block copolymer chains whereas the growth of the parallel poly(TBAm) homopolymer chains was slightly favoured.

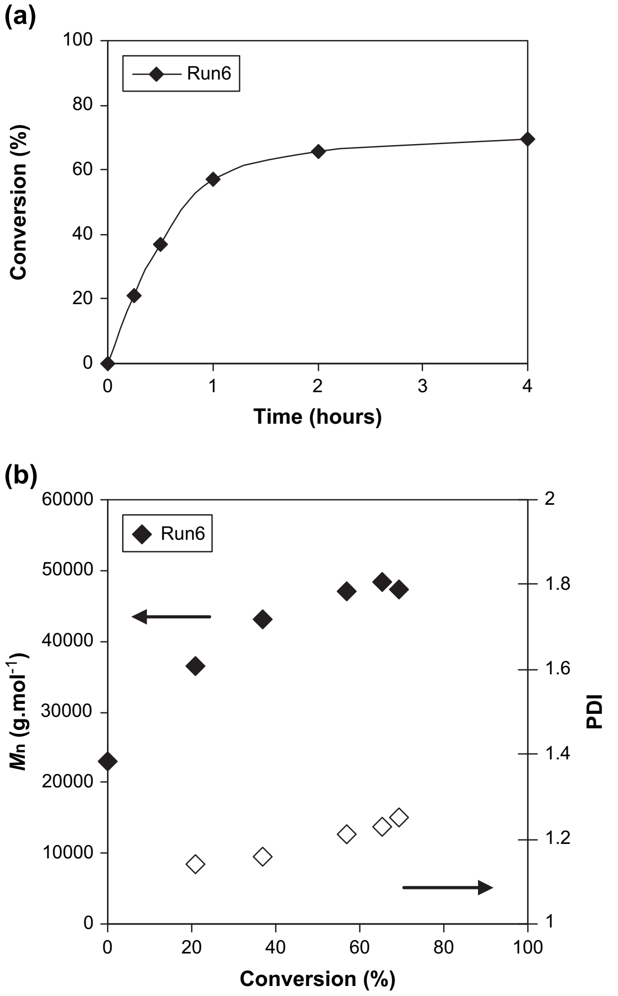

Fig. 7. Monomer conversion vs time plot (a) and number-average molecular weight M_n and polydispersity index PDI vs monomer conversion plots (b) for TBAm polymerization in the presence of macroCTA poly(NAM); $[\text{TBAm}]_0 = 1$ mol L^{-1}; $[\text{TBAm}]_0/[\text{macroCTA}]_0 = 236$; $[\text{macroCTA}]_0/[\text{AIBN}]_0 = 3.3$.

3.3. Purification of the amphiphilic poly(TBAm-b-NAM) block copolymers

In order to perform an accurate characterization of the block copolymer samples, first they have to be purified since they contain a mixture of chains, i.e. homopolymer of each block and block copolymer, due to the two-step synthesis strategy and the mechanism of the RAFT process (mixture of poly(TBAm-*b*-NAM) block copolymer chains with residual NAM monomer, poly(TBAm) homopolymer (dead chains from the first block) and poly(NAM) homopolymer (dormant chains grown in parallel to the block copolymer chains)). Considering the macroCTA/initiator molar ratio used to synthesize the block copolymers (3.3, chosen to ensure fast enough kinetics), all samples contained a significant amount of poly(NAM).

As, in the literature, very few purification methods are reported for amphiphilic block copolymers and as we found optimized conditions for poly(TBAm-*b*-NAM) samples, it is worth describing the purification method that was used.

The purification was carried out in two steps. In the first step, the samples were precipitated in diethyl ether since poly(NAM) nicely precipitates in this solvent [32] whereas poly(TBAm) does not (very few solvents are able to induce the precipitation of poly(TBAm)) [13]. Poly(TBAm-*b*-NAM) block copolymer chains mixed with poly(NAM) chains were isolated. The complete elimination of residual NAM monomer was confirmed by ^{1}H NMR and the disappearance of poly(TBAm) was confirmed by SEC analysis (decrease of the tail on the low MW side of the peak).

Precipitation efficiency was dependent on the block copolymer composition with a prevailing influence of the poly(TBAm) block. For instance, a poly(TBAm-*b*-NAM) with a 50/50 TBAm/NAM weight ratio (10 000/10 000 g mol^{-1}) did not precipitate in diethyl ether. On the contrary, if $M_{\text{n poly(NAM)}} > 20\,000$ g mol^{-1}, a complete precipitation of the block copolymer was observed. In-between (10 000 g mol^{-1} < $M_{\text{n poly(NAM)}} < 20\,000$ g mol^{-1}) fractionation of the block copolymer chains in the two phases was observed.

In the second step, it was necessary to separate the poly(NAM) chains from the poly(TBAm-*b*-NAM) chains. As poly(NAM) is soluble in water and poly(TBAm) homopolymer precipitates, we first thought for a simple precipitation in water. However, poly(TBAm-*b*-NAM) block copolymers did not precipitate in water. Then, two methods have been compared: (i) washes with water and (ii) precipitation/extraction in a pentane/water mixture.

(i) Washes (3–5 times) of the precipitate were carried out using warm water (60 °C) for 1 h under stirring. The apparition of poly(NAM) in the water phase was confirmed by ^{1}H NMR. On the contrary, not any characteristic peak of poly(TBAm) could be identified on the spectrum, indicating that precipitated poly(TBAm-*b*-NAM) chains were not solubilized by water even in the case of long poly(NAM) blocks. Successive SEC analyses (THF) of the washed precipitate indicated the progressive disappearance of chains on the low MW side of the peak. A significant decrease of PDI was observed, confirming the efficiency of this method.

(ii) Precipitation/extraction in a pentane/water mixture (50/50:v/v): The mixture of poly(TBAm-*b*-NAM) and poly(NAM) chains was solubilized in a small amount of CH_2Cl_2 and dropped into the pentane/water mixture. The pentane phase got a strong milk-like appearance. Then, extraction was performed inducing poly(NAM) chains to diffuse into the water phase. After each extraction, ^{1}H NMR analyses were performed on both phases: the water phase was only containing poly(NAM) chains whereas the pentane phase was containing poly(TBAm-*b*-NAM) plus poly(NAM) chains (with a decreasing proportion of poly(NAM)). After several extractions, no more poly(NAM) chains were recovered in the water phase and the poly(NAM)/poly(TBAm) molar ratio was no more decreasing in the pentane phase. We then considered that the block copolymer was fully purified.

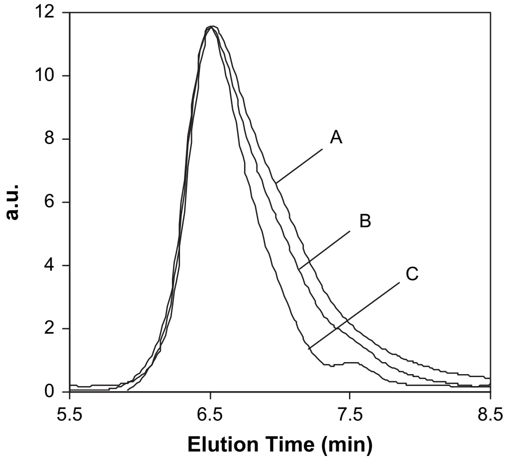

Fig. 8. Purification efficiency for poly(TBAm-*b*-NAM) block copolymer sample run 3T6 (from run 3). (A) Precipitated in diethyl ether; (B) precipitated in diethyl ether and purified via pentane/water extraction once; (C) precipitated in diethyl ether and purified via pentane/water extraction three times.

Another evidence of the purification came from the comparison of the SEC chromatograms of poly(TBAm-*b*-NAM) samples after the first purification step (precipitation in diethyl ether) and the second one (precipitation/extraction in the pentane/water mixture) (Fig. 8). The decrease of the tail, already observed after one precipitation/extraction due to the elimination of water-soluble poly(NAM) chains, was amplified after three extractions. The two methods, 'wash' and 'precipitation/extraction', led to similar PDI values for average size block copolymer chains, showing the good efficiency of the simple 'wash' method.

In the particular case of poly(TBAm-*b*-NAM) block copolymers with a long hydrophilic block (for instance, 14 000/90 000 g mol^{-1}), the "wash" method was not appropriate since long poly(NAM) chains could not be extracted from the mixture by simple washes (entanglement between long poly(TBAm-*b*-NAM) and long poly(NAM) chains). However, the second method led to a fractionation phenomenon: the presence of characteristic poly(TBAm) peaks was noticed on the ^{1}H NMR spectrum of the water phase, that indicated the diffusion of some long block copolymer chains in water. Moreover, the SEC traces before and after the purification step were very different. The whole peak moved towards lower molecular weights, confirming the disappearance of longer chains.

In conclusion, the two-step purification method (precipitation in diethyl ether followed by precipitation/extraction in a pentane/water mixture), is extremely efficient to fully purify poly(TBAm-*b*-NAM) block copolymers having a low to average poly(NAM) block (until 30 000–40 000 g mol^{-1}) considering a poly(TBAm) block of 14 000 g mol^{-1}. It is worthy to note that this empirical purification method was not trivial. In fact, both poly(NAM) and poly(TBAm) homopolymers precipitate in pentane (as a powder), and only poly(NAM) is soluble in water. Then, it was not obvious that poly(TBAm-*b*-NAM) block copolymer chains would remain in the pentane phase during the pentane/water extraction. This strange behavior (as well as the milk-like aspect of the pentane phase) may

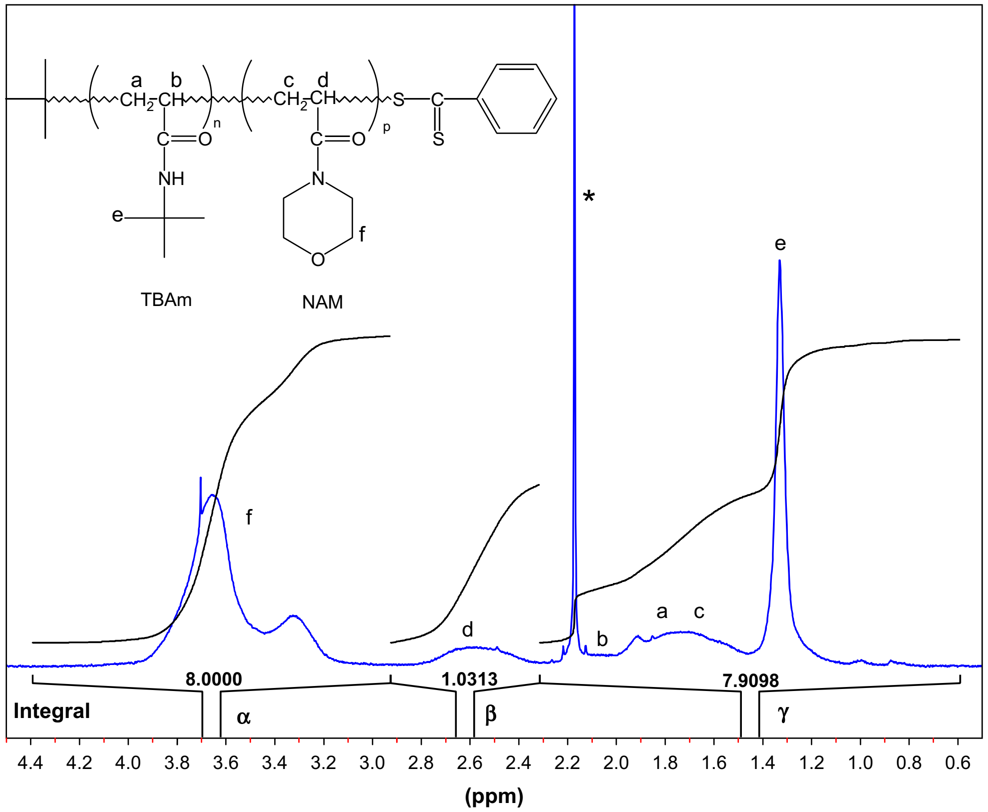

Fig. 9. ^{1}H NMR spectrum of poly(TBAm-*b*-NAM) block copolymer sample run 3T6 in $CDCl_3$ (200 MHz) (*: residual acetone).

suggest that in the pentane phase poly(TBAm-*b*-NAM) chains form pre-micellar aggregates where the chains remain individualized (no entanglement in the core of the micelles), so that block copolymer chains with a long hydrophilic block could further diffuse in the water phase. Moreover, after removal of the water phase, the pentane phase got a translucent gel-like consistency with time, suggesting organization of the chains in the pentane phase.

3.4. Characterization of the poly(TBAm-b-NAM) amphiphilic block copolymers

3.4.1. Determination of the polymerization degree by ^{1}H NMR

When dealing with amphiphilic block copolymers, the determination of the molecular weight by size exclusion chromatography does not generally lead to real values (even when using suitable standards for one block) since the hydrodynamic ratio of each block is very different. On the contrary, ^{1}H NMR is a very accurate method to determine the average copolymer MW [37a,b] provided that several criteria are fulfilled:

- The block copolymer must be fully purified from residual monomers and possible homopolymer chains.
- The shape of the spectrum must contain several areas (at least two) to allow one to determine the block copolymer molar composition.
- The real molecular weight of the first block must be known.

Concerning poly(TBAm-*b*-NAM) block copolymer samples, the corresponding ^{1}H NMR spectrum (Fig. 9) is appropriate to determine the copolymer molar composition. Then, using the real molecular weight of the first block, one can calculate the real molecular weight of the second block, and consequently of the block copolymer. Number-average molecular weight of poly(NAM) block, $M_{\mathrm{n\ poly(NAM)}}$, can be calculated as follows:

$$M_{\mathrm{n\ poly(NAM)}} = (n_{\mathrm{NAM}}/n_{\mathrm{TBAm}}) \times (M_{\mathrm{NAM}}/M_{\mathrm{TBAm}}) \times M_{\mathrm{n\ poly(TBAm)}}$$

where $M_{\mathrm{n\ poly(TBAm)}}$ is the value determined by SEC/LS for the poly(TBAm) block (Table 1), M_{NAM} and M_{TBAm} are the NAM and TBAm molecular weights, respectively, and ($n_{\mathrm{NAM}}/n_{\mathrm{TBAm}}$) is the NAM/TBAm molar composition of the copolymer obtained from the ^{1}H NMR spectrum.

The spectrum can be divided into three areas, α, β and γ, respectively, corresponding to the morpholino cycle (8 protons), the –CH– of poly(NAM) main chain (1 proton), and the $-CH_2-$ of poly(NAM) main chain plus all the poly(TBAm) protons (2 + 12 protons). Then, copolymer molar composition can be determined from the corresponding integrals, $n_{\mathrm{NAM}}/n_{\mathrm{TBAm}} = 6I_\alpha/(4I_\gamma - I_\alpha)$. Moreover, the accurate integration of the spectrum is confirmed by the relation between α and β peaks ($I_\alpha = 8I_\beta$).

Table 3
Molecular weight of various poly(TBAm-*b*-NAM) block copolymer samples determined by ^{1}H NMR (run 3T4 stands for the 4th sample withdrawn from run 3)

Poly(TBAm-*b*-NAM)	$M_{\text{n th.}}$ (g mol^{-1}) (molar composition)	$M_{\text{n exp }^1\text{H NMR}}$ (g mol^{-1}) (molar composition)	CMC value (g L^{-1}) (mol L^{-1})
Run 3T4	14 000[a]/22 500 (40.8/59.2)	14 000[a]/25 500 (37.9/62.1)	–
Run 3T6	14 000[a]/32 500 (32.4/67.6)	14 000[a]/29 000 (34.9/65.1)	(0.16)[d] (3.7×10^{-6})
Run 4T3	14 000[a]/52 000 (23.0/77.0)	14 000[a]/47 000 (24.9/75.1)	–
Run 4T6	14 000[a]/90 000 (14.7/85.3)	14 000[a]/53 000[c] (22.7/77.3)	(0.31)[e] (4.6×10^{-6})
Run 5T5	2650[b]/6300 (31.8/68.2)	2650[b]/5300 (35.7/64.3)	–

[a] Determined by SEC/LS.

[b] Determined by MALDI-TOF MS.

[c] Fractionation during purification.

[d] Value before purification: 0.20 g L^{-1}.

[e] Value before purification: 1 g L^{-1}.

The results are given in Table 3 for five different block copolymer samples. Comparison between experimental and theoretical M_n values for the poly(NAM) block indicates a good agreement. The difference never exceeds 10%, except for block copolymer sample run 4T6 due to a fractionation phenomenon during the purification step. These results confirm the controlled character of the RAFT polymerization during poly(TBAm-*b*-NAM) block copolymer synthesis as well as the high blocking efficiency.

3.4.2. Determination of the CMC by the pendant drop method

One interest of amphiphilic block copolymers is their ability to self-assemble in aqueous medium to yield macromolecular micelles. Then, the determination of the critical micellar concentration (CMC) is an essential characterization and allows one to compare various block copolymer samples within a same family or from various families. The CMC can be obtained by different techniques, such as light scattering, fluorescence or surface tension. Here, we describe surface tension measurements using the pendant drop method [38].

Considering a pendant drop, diffusion of amphiphilic block copolymer chains to the air/water interface induces the decrease of the surface tension (determined from the geometry of the drop) until saturation of the drop interface for a bulk concentration corresponding to the CMC. For higher concentrations, the excess of block copolymer chains yields micellar aggregate formation.

As these poly(TBAm-*b*-NAM) block copolymers could not be directly solubilized in water, they were first dissolved in THF and the solution was diluted with MilliQ water (water/THF ratio of 95/5:v/v). The surface tension of each solution (of increasing concentration) was then followed over time (30 min) since, contrary to their molecular counterparts, macromolecular amphiphiles do not diffuse instantaneously to the interface.

Kinetics of surface tension was first performed on a reference assay without polymer, to compare the water/THF solution to pure water. Concerning pure water, a constant surface tension of 73 mN m^{-1} was obtained (similar to literature value), followed by a slight decrease after 20 min due to a variation of the drop shape (evaporation of water). Concerning the water/THF solution, an initial value of 58 mN m^{-1} was determined, due to the presence of 5% of THF. Immediately, surface tension increased to reach 72 mN m^{-1} after 3 min indicating a fast evaporation of THF. Then, the surface tension evolution was similar to that of pure water (data not shown).

Considering block copolymer solutions, the initial surface tension was identical whatever the concentration (57 mN m^{-1}) (sample run 3T6, Fig. 10(a)). For the lower concentrations (0.01, 0.025 and 0.05 g L^{-1}), surface tension increased during the first 3 min before decreasing (decrease more pronounced than for MilliQ water) that indicated the diffusion of block copolymer chains to the interface. For the concentration of 0.1 g L^{-1}, surface tension only increased during the first minute, and then decreased (significantly more than the precedent ones). For the higher concentrations (0.2, 0.5 and 1 g L^{-1}), surface tension immediately decreased with identical kinetics for the three concentrations suggesting that they were above the CMC.

Kinetics was also determined for another poly(TBAm-*b*-NAM) block copolymer sample run 4T6, which led to similar profiles. From the kinetics results, surface tension was plotted vs block copolymer concentration, at three different times after THF evaporation, 5, 10 and 15 min (Fig. 10(b)). A linear decrease of surface tension with concentration was observed followed by a plateau, with an intersection not as sharp as for molecular amphiphiles as usually observed in the literature for block copolymers [38]. The stagnation of the surface tension occurred at the same concentration whatever the reported time and the intersection of the two parts gave the CMC value.

The CMC values for the two block copolymer runs 3T6 and 4T6, having an identical hydrophobic block of 14 000 g mol^{-1} and a different hydrophilic block (29 000 and 53 000 g mol^{-1}), are, respectively, 0.16 and 0.31 g L^{-1} (Table 3). The higher value for sample run 4T6 is consistent with its longer hydrophilic block favouring water solubility. Similar CMC values have been determined by fluorescence techniques using two kinds of probes [39].

Note that these CMC values correspond to the fully purified copolymers. The values before full purification (only after removal of residual monomer and first block dead chains) were, respectively, of 0.20 and 1.0 g L^{-1}. The lower CMC values after purification confirm the elimination of parasite hydrophilic chains of the second block during the second step of the purification. In addition, the fractionation that has occurred in the case of copolymer run 4T6 is also evidenced by the significant

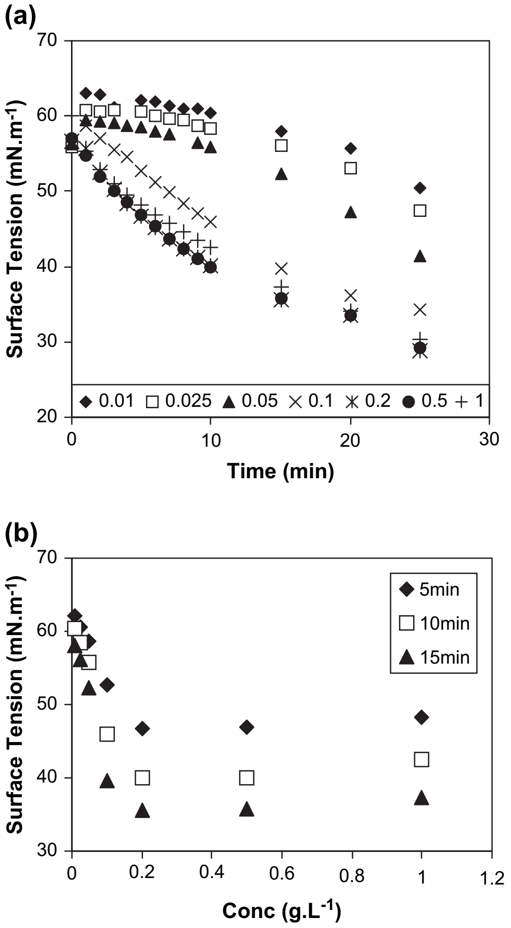

Fig. 10. (a) Surface tension kinetics for increasing concentrations (g L^{-1}) of poly(TBAm-*b*-NAM) block copolymer sample run 3T6; (b) surface tension vs concentration of poly(TBAm-*b*-NAM) block copolymer sample run 3T6 at given times 5, 10 and 15 min.

difference between the CMC values before and after purification (1.0 and 0.31 g L^{-1}, respectively).

4. Conclusion

Amphiphilic block copolymers of acrylamide derivatives were synthesized via the RAFT process from two non-ionic monomers, the *N*-*tert*-butyl acrylamide (TBAm) and the *N*-acryloylmorpholine (NAM). It was demonstrated that, using the macroCTA method, both polymerization orders were possible with a slight preference for poly(TBAm-*b*-NAM) compared to poly(NAM-*b*-TBAm). Conversion reached 90% with a linear increase of the MW up to 60–70% conversion. Poly(NAM) chains of 10 000–90 000 g mol^{-1} could be grown from a poly(TBAm) first block of 14 000 g mol^{-1}.

The purification of the poly(TBAm-*b*-NAM) block copolymers was carefully studied. A two-step method appeared to be very efficient, including precipitation in diethyl ether followed by precipitation/extraction in a pentane/water biphasic medium. The fully purified block copolymers were analyzed by ^{1}H NMR which resulted in an accurate determination of the second block MW. In addition, determination of the CMC by the pendant drop method confirmed the ability of the poly(TBAm-*b*-NAM) block copolymers to self-assemble in water.

As an application, a block copolymer bearing numerous reactive side-groups (activated ester) along the hydrophilic block has been synthesized using the same strategy as for poly(TBAm-*b*-NAM). The activated ester groups have been introduced via an acrylate derivative, *N*-acryloxysuccinimide (NAS), copolymerized with *N*-acryloylmorpholine using a 60/40 NAM/NAS molar ratio corresponding to the azeotropic composition [34]. This activated ester monomer had already been successfully polymerized by RAFT, either to give a random copolymer with *tert*-butyl methacrylate [41], dimethylacrylamide [19] and *N*-acryloylmorpholine [40], or a block copolymer with dimethylacrylamide [19].

The resulting poly(TBAm-*b*-(NAM-*co*-NAS)) amphiphilic block copolymers have been further used to bind nucleotide starters in order to grow nucleic acid sequences from the block copolymer backbone (17–100 oligonucleotides per polymer chain) [31]. The resulting bioconjugates have been evaluated in DNA hybridization assays on a diagnostic microarray [31,42]. Such reactive amphiphilic block copolymers can be used to bind a great variety of amino-derivatives for applications in many fields.

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