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Efficient RAFT polymerization of *N*-(3-aminopropyl)methacrylamide hydrochloride using unprotected "clickable" chain transfer agents

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ABSTRACT

The reversible addition fragmentation chain transfer (RAFT) of *N*-(3-aminopropyl)methacrylamide hydrochloride (APMA) using unprotected "clickable" chain transfer agents in water/dioxane mixtures is reported. The controlled character of the polymerization was confirmed by the linear increase of the polymer molecular weight with monomer conversion, the narrow molecular weight distribution ($Đ \leqslant 1.1$) and by chain extension experiments. The alkyne-terminated PAPMA was further functionalized by "click" chemistry with an azido-functionalized coumarin derivative. The method reported here will be useful for the preparation of novel PAPMA based materials for biomedical applications using a strategy that does not require challenging protection/deprotection steps.

1. Introduction

Reversible deactivation radical chain polymerization (RDRP) [1] has been studied for the past two decades as a powerful tool for the preparation of complex polymer structures with well-defined molecular weights, molecular weight distributions, compositions, architectures, topologies and chain-end functionalities. Reversible addition fragmentation chain transfer (RAFT) [2] is one of the most versatile RDRP techniques due to its tolerance to different functionalities, the range of monomers that can be polymerized and the diversity of affordable polymer architectures [3]. Several polymers, such as poly(meth)acrylates [4,5], poly(meth)acrylamides [6–9] and polystyrene [10,11], have been successfully synthesized by RAFT polymerization and used in numerous applications. Special attention has been given to polymers with functionalized chain-ends that can be used in further post-modification reactions [12–14] to produce unique polymer structures.

One of the most popular post-polymerization reactions involving polymers prepared by RDRP is the Huisgen 1,3-dipolar cycloaddition between azide and acetylene chain-end functionalities, which is typically catalyzed by copper(I) complexes [15]. This type of "click" chemistry reaction became very attractive due to its high yield, mild reaction conditions and absence of by-products [16]. Click strategies are particularly useful for the synthesis of well-defined block copolymers [17]. Using this technique, it is possible to prepare block copolymers that combine homopolymers with very distinct natures, derived from monomers with different reactivity. Additionally, it is possible to maintain the low dispersity ($Đ$) of block copolymers because the building blocks can be synthesized separately using suitable RDRP techniques and further coupled by click reactions [18]. The combination of RDRP and click chemistry has been extensively reported in the literature [19–23], as it allows the design of high performance materials with very unique structures and properties that can be applied in relevant areas (*e.g.,* the biomedical field) [24–26].

Polymers with pendant primary amino groups, which can be used for post-polymerization modification reactions [27–29], such as Michael addition reactions, are of prime importance for the development of new materials for biomedical applications. For instance, *N*-(3-aminopropyl)methacrylamide hydrochloride (APMA) has been used in the preparation of copolymers and cross-linked micelles for gene delivery [30,31] and drug delivery [32–34]. The controlled synthesis of this polymer is usually performed by RAFT polymerization mediated by 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTP) [35] or by RAFT

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copolymerization with other (meth)acrylamide monomers [30–32,36,37] or with 2-(diisopropylamino)ethyl methacrylate [33,34]. The post-polymerization modification of controlled/"living" poly(3-aminopropyl methacrylamide hydrochloride) (PAPMA) typically involves reaction of the amino groups to either prepare shell cross-linked micelles [34] or to attach molecules of interest (*e.g.*, D-glucuronic acid sodium salt) [38] for the studied application. To the best of our knowledge, the chain-ends of controlled/"living" PAPMA have never been used in post-polymerization modification reactions.

Here, we report the successful synthesis of PAPMA by RAFT polymerization mediated by non-protected acetylene or azide functionalized chain transfer agents (CTAs) to allow further modification of the PAPMA chain-ends. An alkyne-terminated PAPMA was coupled with a biocompatible coumarin derivative *via* copper(I) catalyzed azide alkyne cycloaddition to demonstrate the usefulness of the strategy. The primary goals of this work were to develop suitable polymerization conditions and a facile procedure for the preparation of controlled chain-end functionalized PAPMA in order to allow further chemical modifications, expanding the range of applications of this polymer [39].

2. Experimental section

2.1. Materials

Glacial acetic acid (Fisher Scientific, 99.79%), acetone (Fisher Scientific, HPLC grade), APMA (Polysciences, >98%), deuterated chloroform ($CDCl_3$, Euriso-top, 99.50% D), 3-chloro-1-propanol (Aldrich, 98%), copper (II) sulfate pentahydrate (⩾98%, Aldrich), CTP (Sigma–Aldrich, >97%), dichloromethane (DCM) (Fisher Scientific, 99.99%), diethyl ether (Fisher Scientific, 99.85%), 4-dimethylaminopyridine (DMAP, Acros Organics, 99%), deuterium oxide (D_2O, Euroiso-top, 99.90% D), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Sigma–Aldrich, ⩾98.0%), ethanol (EtOH, absolute, Fisher Chemical), ethyl acetate (99.98%, Fisher Scientific), hexane (Fisher Scientific, 99.05%), propargyl alcohol (PgOH) (Aldrich, 99%), silica gel (Panreac, 63–200 μm), sodium ascorbate (crystalline, ⩾98%, Aldrich), sodium azide (Panreac, 99%), and sodium sulfate (anhydrous) were used as obtained.

1,4-Dioxane (Sigma–Aldrich, 99+%) was passed through an activated alumina column before use to remove any peroxides.

4,4′-Azobis(4-cyanovaleric acid) (ACVA) (Aldrich, 75%) was recrystallized from methanol before use.

Deionized water was obtained from a Milli-Q® Millipore reverse osmosis unit (resistivity = 18.0 MΩ).

3-azido-7-diethylaminocoumarin was synthesized as reported in the literature [40].

2.2. Techniques

The polymers were analyzed by a size exclusion chromatography (SEC) system equipped with an online degasser, a refractive index (RI) detector and a set of columns comprising a Shodex OHpak SB-G guard column and OHpak SB-804HQ and OHpak SB-804HQ columns. The polymers were eluted at a flow rate of 0.5 mL/min with 0.1 M Na_2SO_4 (aq)/1 wt% acetic acid/0.02% NaN_3 at 40 °C. Before the injection (50 μL), the samples were filtered through a polytetrafluoroethylene (PTFE) membrane with 0.45 μm pores. The system was calibrated with five narrow PEG standards, and the polymer molecular weights (M_n^{SEC}) and $Đ$ (M_w/M_n) were determined by conventional calibration using Clarity software version 2.8.2.648.

400 MHz ^{1}H NMR spectra of the reaction mixture samples were recorded on a Bruker Avance III 400 MHz spectrometer with a 5-mm TIX triple resonance detection probe in D_2O. The conversion of the monomers was determined by integration of the monomer and polymer NMR signals using MestRenova software version 6.0.2-5475.

Fourier transform infrared attenuated total reflection (FTIR-ATR) spectroscopy was performed using a Jasco model 4000 UK spectrometer. The samples were analyzed with 64 scans and 4 cm^{-1} resolution between 500 and 3500 cm^{-1}.

2.3. Procedures

2.3.1. The synthesis of the alkyne-CTP

The synthesis of the alkyne-terminated chain transfer agent was adapted from a procedure described in the literature [41]. Briefly, a mixture of CTP (700 mg, 2.51 mmol), PgOH (170 mg, 3.01 mmol) and DCM (40 mL) was added to a round bottom flask equipped with a magnetic bar and a rubber stopper. The solution was cooled to 0 °C and purged with argon. A solution of EDC (720 mg, 3.76 mmol) and DMAP (50 mg, 0.38 mmol) in DCM (10 mL) was added to the flask under argon atmosphere. The mixture was allowed to react at 0 °C for 2 h and then at room temperature overnight. The reaction mixture was washed with water (100 mL, 3 times) and dried over anhydrous Na_2SO_4. The DCM was removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2 and hexane/ethyl acetate = 4/1 (v/v)). The pure product (0.60 g, 75%) was analyzed by ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 1.94 (s, 3H, (CN)C—CH_3); 2.49 (t, 1H, HC≡C); 2.5–2.8 (t(x2), 4H, —CH_2—CH_2); 4.72 (d, 2H, CH_2—O—C); 7.4–7.9 (t (x2), d, 5H, ArH).

2.3.2. The synthesis of the azido-CTP

The synthesis of the azide-terminated chain transfer agent was adapted from a procedure described in the literature [41]. First, 3-chloro-1-propanol (3 g, 31.7 mmol) and NaN_3 (3.5 g, 54.0 mmol) were dissolved in a mixture of acetone (50 mL) and water (5 mL) and refluxed overnight. The acetone was removed under reduced pressure, and 35 mL of water were added to the remaining solution. The product was extracted with diethyl ether (3 × 70 mL), the organic layer was dried over anhydrous Na_2SO_4, and the 3-azido-1-propanol was obtained as a colorless oil (1.6 g, 50%) after solvent removal under reduced pressure. The product was analyzed by FTIR: ν_{max}/cm^{-1} 3340, 2949, 2884, 2098, 1449, 1262, 1051, 955, 900. In a second step, a mixture of CTP (700 mg, 2.51 mmol), 3-zido-1-propanol (380 mg, 3.76 mmol) and DCM (40 mL) was added to a round flask equipped with a magnetic bar and a rubber stopper. The solution was cooled to 0 °C and purged with argon. A solution of EDC (720 mg, 3.76 mmol) and DMAP (50 mg, 0.38 mmol) in DCM (10 mL) was added to the flask under argon atmosphere. The mixture was allowed to react at 0 °C for 2 h and then at room temperature overnight. The reaction mixture was washed with water (100 mL, 3 times) and dried over anhydrous Na_2SO_4. The DCM was removed under reduced pressure, and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2 and hexane/ethyl acetate = 4/1 (v/v)). The pure product (0.61 g, 67%) was analyzed by ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 1.94 (s, 3H, —C(CN)CH_3); 2.04–2.18 (m, 2H, —CH_2—CH_2—N_3); 2.30–2.80 (m, 4H, (CN)C—CH_2—CH_2—C(=O)); 3.60 (t, 2H, CH_2—N_3); 4.28 (t, 2H, —CH_2—CH_2— CH_2—N_3); 7.40–7.90 (t (x2), d, 5H, ArH).

2.3.3. Typical procedure for the RAFT polymerization of APMA with $[APMA]_0/[CTP]_0/[AVCA]_0$ = 1/1/0.5 ($[APMA]_0$ = 1.87 M; H_2O:1,4-dioxane = 2:1 (v:v))

APMA (0.5 g, 2.80 mmol) was dissolved in deionized water (1 mL), CTP (7.82 mg, 0.028 mmol) was dissolved in 1,4-dioxane (0.5 mL), and both solutions were inserted into a Schlenk tube

reactor. AVCA (1.57 mg, 0.0056 mmol) was weighed and added to the reactor, which was subsequently sealed and frozen in liquid nitrogen. The Schlenk tube reactor containing the reaction mixture was deoxygenated with four freeze–vacuum–thaw cycles and purged with nitrogen. The Schlenk tube reactor was placed in an oil bath at 70 °C under stirring (700 rpm). Different reaction mixture samples were collected during the polymerization using an airtight syringe and purging the side arm of the Schlenk reactor with nitrogen. The samples were analyzed by ^{1}H NMR spectroscopy to determine the monomer conversion and by aqueous SEC to determine the molecular weights and dispersity of the polymers. The final reaction mixture was dialyzed against deionized water, and the polymer was obtained after freeze drying.

The other RAFT polymerizations were conducted employing the same procedure described but using alkyne-CTP or azido-CTP as the chain transfer agent.

2.3.4. Typical procedure for the chain extension of PAMA

A sample of alkyne-terminated PAPMA ($M_n^{SEC} = 10,400$; $M_w/M_n = 1.19$), obtained after dialysis and freeze drying, was used as the macro-CTA in a new RAFT polymerization. Briefly, the macro-CTA (43.7 mg, 0.0042 mmol) and APMA (300 mg, 1.70 mmol) were dissolved in a mixture of water (1.93 mL) and 1,4-dioxane (0.97 mL) inside a Schlenk tube reactor. Subsequently, AVCA (0.59 mg, 0.0021 mmol) was weighed and added to the reactor, which was immediately frozen in liquid nitrogen. The Schlenk tube reactor containing the reaction mixture was deoxygenated with four freeze–vacuum–thaw cycles, purged with nitrogen and placed in an oil bath at 70 °C under stirring (700 rpm). After 24 h of reaction, a sample was collected and analyzed by SEC in order to observe the movement of the SEC trace toward a higher molecular weight relative to that of the macro-CTA.

2.3.5. Click reaction between alkyne-terminated PAPMA and (3-azido-7-diethylaminocoumarin)

An alkyne-terminated PAPMA sample ($M_n^{SEC} = 8.7 \times 10^3$; $Đ = 1.08$) was purified by dialysis against deionized water, and the polymer was recovered after freeze drying. A solution of 3-azido-7-diethylaminocoumarin (0.6 mg; 2.2 μmol), alkyne-terminated PAPMA (15.6 mg; 1.79 μmol) in deionized water (100 μL)/EtOH (300 μL), and a stock solution of $CuSO_4.5H_2O$ (28 mM; 100 μL) in deionized water were placed in a round-bottom flask equipped with a magnetic stir bar and sealed with a rubber septum. The mixture was bubbled with nitrogen for 20 min to remove oxygen. Lastly, a degassed stock solution of sodium ascorbate in water (11 mM; 100 μL) was injected into the flask under nitrogen atmosphere. The reaction was allowed to proceed with stirring (700 rpm) at room temperature for 96 h. The product was purified by dialysis against water, followed by dialysis against ethanol and a second dialysis against water, then recovered by freeze drying. The functionalized polymer was analyzed by ^{1}H NMR spectroscopy.

3. Results and discussion

3.1. RAFT homopolymerization of APMA

CTP, which contains a phenyl Z group that strongly stabilizes the intermediate radical, has previously been used for the successful RAFT polymerization of a wide range of activated monomers, including both acrylamide and methacrylamide monomers [8,35,42]. To the best of our knowledge, there are only two reports that show kinetic data regarding the RAFT of APMA using CTP as a chain transfer agent [35,36]. Other publications have reported the synthesis of responsive block copolymers including PAPMA segments, mainly with the purpose to be used on the biomedical field [30–34,36,37]. In this work, the RAFT system described in the literature for the successful polymerization of PAPMA [35,36] was used as a starting point for the evaluation of the synthesized "clickable" CTAs chain transfer activity. Alkyne and azido CTP derivatives (Fig. 1) were prepared by esterification with PgOH (with no protection of the alkyne termini) and 3-azido-1-propanol, respectively, following procedures reported elsewhere [41] (see Fig. S1 in the ESI†). The chemical structure and purity of the CTAs were confirmed by ^{1}H NMR spectroscopy (see Fig. S2 in the ESI†) to ensure that all RAFT polymerizations were performed using the same conditions and were free of significant impurities, allowing data comparison. The polymerizations were conducted at 70 °C using a solvent mixture of water:1,4-dioxane = 2:1 (v:v) and ACVA as the initiator under nitrogen atmosphere. The pH of the reaction mixture was measured before the polymerization to ensure a value between 4 and 5 in order to avoid CTP hydrolysis and/or aminolysis [43]. Notably, no pH adjustment was necessary during the polymerization. The monomer conversions were determined using ^{1}H NMR spectroscopy by comparing the integrals of the vinyl protons of the monomer at 5.80–5.35 ppm and those of the methylene protons of the PAPMA backbone at 1.25–0.70 ppm.

Initially, both alkyne-CTP and azido-CTP were tested in the RAFT polymerization of APMA for different targeted degrees of polymerization (DP) using the same experimental conditions. The kinetic parameters presented in Table 1 shows that both CTAs were able to mediate successful RAFT polymerization of APMA for different chain lengths, as judged by the very low $Đ$ values. Polymerization control decreased for shorter polymer chains, as expected, but still afforded PAPMA with a narrow molecular weight distribution ($Đ \approx 1.2$).

Kinetic studies were conducted to evaluate the usefulness of the synthesized CTAs for the control of the PAPMA molecular weight during polymerization. In our hands and under the experimental conditions used in this work, the maximum monomer conversion achieved (red symbols in Fig. 2) in the RAFT of APMA mediated by CTP was lower than that reported in the literature [35]. Taking this into consideration, the reaction was repeated three times, and the same results were obtained in all cases. Nevertheless, the control over the polymer molecular weight was very good, as the $Đ$ was always lower than 1.1, with the exception of the first kinetic point (Fig. 2(b)). In addition, the SEC traces (see Fig. S3 in the ESI†) of the different samples were unimodal and symmetric with no tailing, suggesting that no termination reactions occurred during polymerization. In terms of the monomer conversion evolution, the results were consistent with the literature, showing first-order kinetics with respect to monomer conversion (Fig. 2(a)).

It has been reported that the use of unprotected alkyne-terminated CTAs can interfere with the RAFT polymerization process [41]. The synthesized unprotected alkyne-CTP was used to mediate the RAFT polymerization of APMA under the same conditions previously described for the CTP. The kinetic results presented in Fig. 2 (black symbols) show that the alkyne-CTP had higher chain transfer activity than the CTP, with high monomer conversion (≈95%) achieved after 4 h of reaction. This behavior can be attributed to the strong dependence of the efficiency on the CTA R group, considering that the two CTAs have the same Z group [3]. The polymer molecular weight was well controlled throughout polymerization

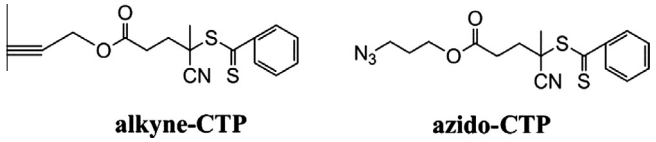

Fig. 1. Chemical structures of both alkyne-CTP and azido-CTP.

Table 1
Kinetic parameters for the RAFT polymerization of APMA using azido-CTP or alkyne-CTP in water:1,4-dioxane = 2:1 (v:v) at 70 °C. Conditions: $[APMA]_0$ = 1.87 M; $[ACVA]_0$ = 0.5 (molar ratio in comparison to the CTA number of moles).

Entry	CTA	Targeted DP	Time (h)	Conv. (%)	$M_n^{SEC} \times 10^{-3}$	Đ
≡$PAPMA_{25}$[a]	Alkyne-CTP	25	5	82	10.4	1.19
≡$PAPMA_{50}$	Alkyne-CTP	50	18	98	29.4	1.08
≡$PAPMA_{100}$	Alkyne-CTP	100	4	95	25.1	1.07
N_3PAPMA_{25}	Azido-CTP	25	4	94	16.2	1.21
N_3PAPMA_{100}	Azido-CTP	100	4	50	19.1	1.11

[a] An ACVA molar ratio of 0.2 was used in this polymerization.

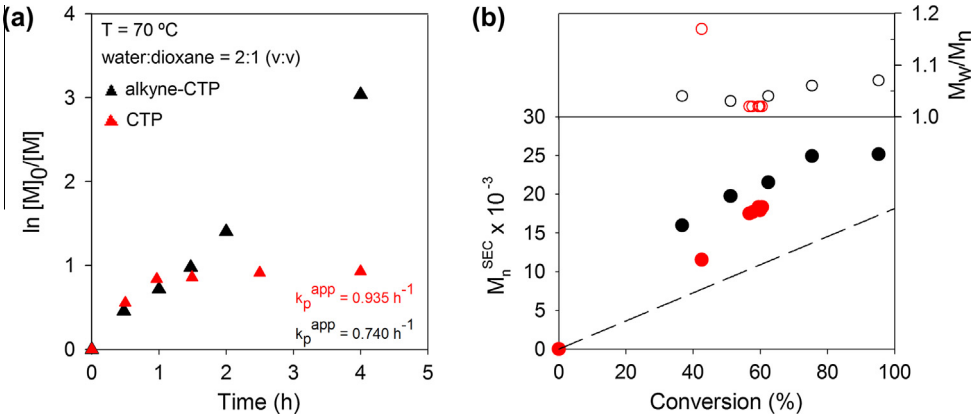

Fig. 2. (a) Kinetic plots of $\ln[M]_0/[M]$ *vs.* time and (b) plot of number–average molecular weight (M_n^{SEC}) and Đ (M_w/M_n) *vs.* monomer conversion (the dashed line represents the theoretical molecular weight at a given conversion) for the RAFT of APMA at 70 °C in a water:1,4-dioxane = 2:1 (v:v) mixture using CTP (red symbols) and alkyne-CTP (black symbols) as chain transfer agents (CTA). Reaction conditions: $[APMA]_0/[CTA]_0/[ACVA]_0$ = 100/1/0.5; $[APMA]_0$ = 1.87 M. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Đ < 1.1), and the molecular weight distribution was characterized by unimodal SEC traces (see Fig. S4 in the ESI†). Despite the low Đ values, the experimental molecular weights of PAPMA determined by SEC were not in close agreement with the corresponding theoretical ones (Fig. 2(b)). The observed differences can be explained considering that the molecular weight values were determined by conventional calibration using PEG standards, which have a different hydrodynamic volume than PAPMA. These results are extremely promising, demonstrating that alkyne-terminated PAPMA can be prepared and directly used in further click reactions without the need for deprotection steps.

Alternatively, to use the alkyne functionality, one can take advantage of azide terminated polymers for click chemistry purposes. An experimental study reported by Ladmiral and co-workers showed that azide groups can undergo 1,3-cycloaddition to the double bonds of some monomers in the absence of a catalyst, especially for long reaction times and high temperatures [44]. In the case of APMA RAFT polymerization, the results suggested that the occurrence of this type of side reaction can be neglected, as the Đ of the polymer decreased with polymerization time (Fig. 3(b)). The low reactivity of acrylamides as well as the presence of the methylene group at the double bond of APMA, which causes steric hindrance, may prevent this type of side reaction. In addition, it was possible to confirm the polymer functionality by identifying the —CH_2 protons near the N_3 group by ^{1}H NMR spectroscopy (Fig. 5(b)).

Nevertheless, the kinetic results showed that the azido-CTP did not allow the same level of control over the PAPMA molecular

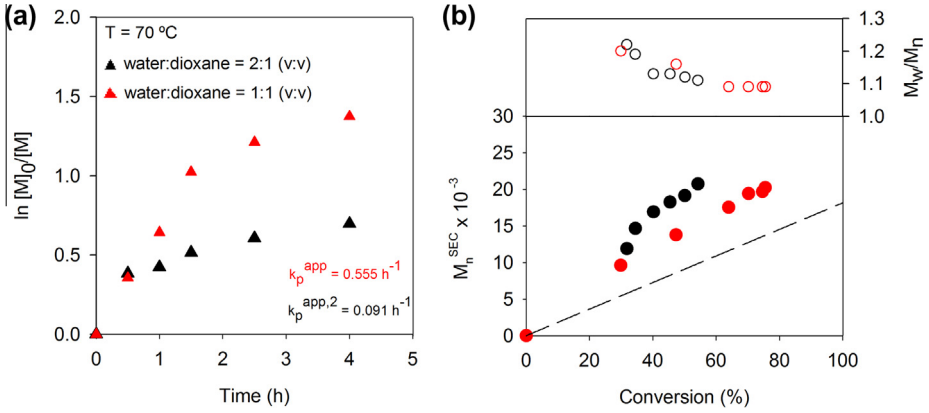

Fig. 3. (a) Kinetic plots of $\ln[M]_0/[M]$ *vs.* time and (b) plot of the number–average molecular weight (M_n^{SEC}) and Đ (M_w/M_n) *vs.* monomer conversion (the dashed line represents the theoretical molecular weight at a given conversion) for the RAFT of APMA at 70 °C in water:1,4-dioxane = 2:1 (v:v) (black symbols) and water:1,4-dioxane = 1:1 (v:v) (red symbols) mixtures. Reaction conditions: $[APMA]_0/[azido\text{-}CTP]_0/[ACVA]_0$ = 100/1/0.5; $[APMA]_0$ = 1.87 M. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

weight as did both CTP and alkyne-CTP for the same experimental conditions (black symbols in Fig. 3). The $Đ$ of the polymers was slightly higher, primarily due to the presence of tailing in the molecular weight distribution curves (Fig. 4(a)). This observation could be due to radical termination reactions that occurred in the beginning of the polymerization. By comparing the area of the low molecular weight shoulder with the total area of the SEC chromatogram, it was possible to estimate that after 30 min of polymerization, there were ≈27% dead polymer chains. We anticipated that the poor control exhibited by the azido-CTP could be associated with the low solubility of this compound in the reaction solvent mixture, even at 70 °C. In comparison with both CTP and alkyne-CTP, the azido-CTP R group has a longer aliphatic chain, which decreases its solubility in water. To confirm this hypothesis, the effect of the solvent mixture composition on the control of the RAFT polymerization of PAPMA mediated by the azido-CTP was also examined. The percentage of dioxane in the solvent mixture was increased up to 50%, and the other experimental conditions were conserved. The results showed that significant improvements were achieved in terms of polymerization control, with a 64% decrease in the PAPMA dead chains (Fig. 4(b)) as well as a 39% increase in the maximum monomer conversion (red symbols in Fig. 3). In addition, better control was achieved, as evidenced by the lower $Đ$ of the PAPMA and the agreement between the experimental molecular weights and the theoretical ones (dashed line in Fig. 3(b)). These results corroborate the idea that the poorer control exhibited by the azido-CTP in this particular RAFT system was related to its solubility in the reaction solvent mixture. Some authors have suggested that the marked chain transfer activity differences between clickable CTAs derived from the same molecule

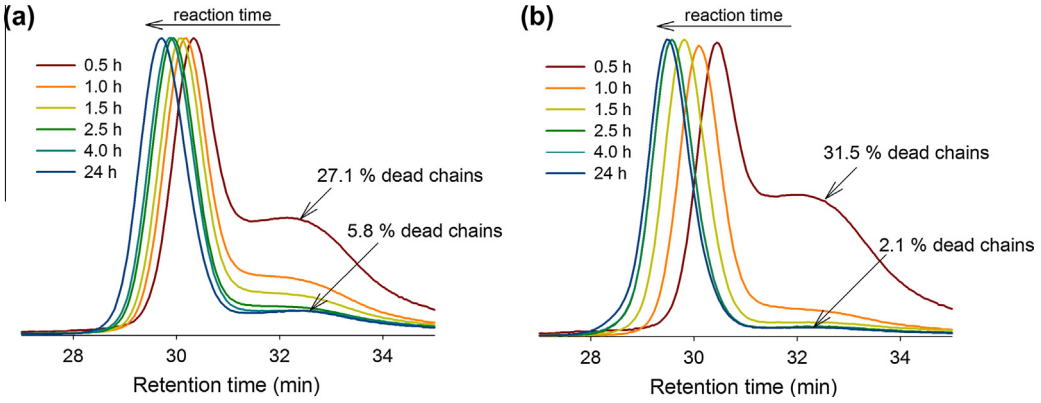

Fig. 4. SEC chromatograms of the PAPMA samples during the RAFT polymerization at 70 °C in (a) a water:1,4-dioxane = 2:1 (v:v) mixture and (b) a water:1,4-dioxane = 1:1 (v:v) mixture. Conditions: $[APMA]_0/[azido\text{-}CTP]_0/[ACVA]_0 = 100/1/0.5$; $[APMA]_0 = 1.87$ M.

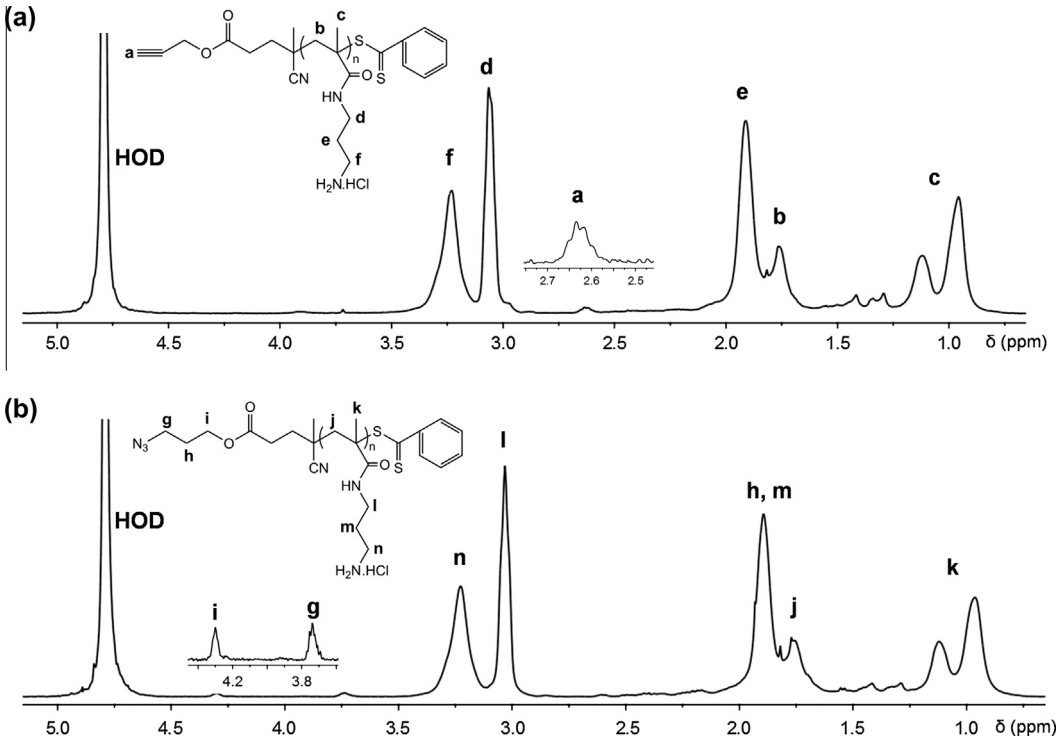

Fig. 5. 400 MHz ^1H NMR spectra in D_2O of (a) the alkyne-terminated PAPMA obtained by RAFT polymerization using $[APMA]_0/[alkyne\text{-}CTP]_0/[ACVA]_0 = 25/1/0.2$ (molar ratios) in water:1,4-dioxane = 2:1 (v:v) at 70 °C for 5 h. Conv. = 82%; $M_n^{th} = 3.7 \times 10^3$; $M_n^{SEC} 10.4 \times 10^3$; $Đ = 1.19$; (b) azide-terminated PAPMA obtained by RAFT polymerization using $[APMA]_0/[azido\text{-}CTP]_0/[ACVA]_0 = 25/1/0.5$ (molar ratios) in water:1,4-dioxane = 2:1 (v:v) at 70 °C for 4 h. Conv. = 94%; $M_n^{th} = 4.5 \times 10^3$; $M_n^{SEC} = 16.3 \times 10^3$; $Đ = 1.21$.

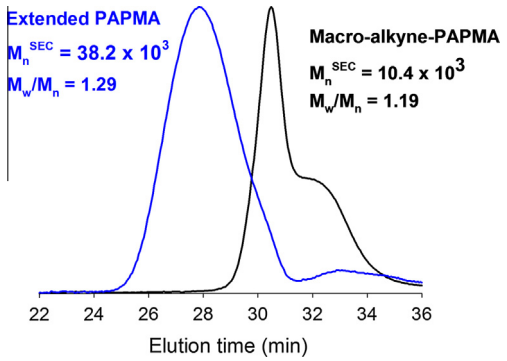

Fig. 6. SEC traces of the macro-PAPMA (alkyne-terminated) and extended PAPMA obtained by RAFT polymerization at 70 °C in water:1,4-dioxane = 2:1 (v:v).

could be related to both the leaving ability and the re-initiating ability of each R group [9]. However, this work shows that it is also important to consider other reaction parameters, namely, the type of solvent, and make small adjustments in order to improve the CTA efficacy.

3.2. ^{1}H NMR analysis and chain extension reaction

The chemical structure of low molecular weight clickable polymers prepared by RAFT polymerization was confirmed by ^{1}H NMR spectroscopy. Fig. 5(a) presents the ^{1}H NMR spectrum of a purified alkyne-terminated PAPMA sample. The characteristic signals of the PAPMA structure at 3.23 ppm (f) (2H, —CH_2—C**H$_2$**—NH_2), 3.06 ppm (d) (2H, —NH—C**H$_2$**—CH_2—), 1.91 ppm (e) (2H, —CH_2—C**H$_2$**—NH_2), 1.76 ppm (b) (2H, C—CH_3—CN—C**H$_2$**—C—) and 1.25–0.70 ppm (c) (3H, —CC**H$_3$**S—) were in agreement with the data reported in the literature [35,36]. It was also possible to identify the alkyne chain-end (a) (1H, C**H**≡C, 2.63 ppm) [45] from the alkyne-CTP, which confirms the retention of the functionality. The same analysis was conducted for the N_3-terminated PAPMA (Fig. 5(b)), which proved to have the methylene protons (i and g) associated with the azido-CTP R group (4H, N_3—C**H$_2$**—CH_2—C**H$_2$**—), while the signal of the methylene protons (h) (2H, N_3—CH_2—C**H$_2$**—CH_2—) was overlapped with signals (m) and (j). It was not possible to determine neither the percentage of functionality nor the average-number molecular weight of the PAPMA (M_n^{NMR}) by ^{1}H NMR spectroscopy because the protons of the aromatic ring (Z group) were partially overlapped with the –NH protons of the polymers.

One of the most effective ways to demonstrate the "livingness" of polymers is to perform a chain extension reaction, in which the polymerization is re-initiated from a macro-CTA. A sample of alkyne-terminated PAPMA ($M_n^{SEC} = 10.4 \times 10^3$; $Đ = 1.19$) prepared by RAFT polymerization was isolated by extensive dialysis against deionized water (pH ≈ 5). This polymer was then used as the macro-CTA in a self-blocking experiment with a targeted DP of 400. Fig. 6 shows the clear shift of the molecular weight distribution curve of the macro-PAPMA towards higher molecular weight values, confirming the living character of the polymer. It is also important to note that the resultant extended PAPMA presented some low molecular weight tailing as well as a higher $Đ$ than the macro-CTA. This result can be due to the occurrence of some radical termination reactions or due to partial macro-CTA hydrolysis during the purification process by dialysis, which may have decreased the macro-CTA efficiency, as reported by other authors for similar polymers [42]. The same experiment was performed using an azide-terminated PAPMA sample, and the results obtained were similar to those of the alkyne-terminated PAPMA chain extension (see Fig. S5 in the ESI†).

3.3. Functionalization of alkyne-PAPMA by click chemistry

To prove the reactivity of the click moieties introduced in the PAPMA structure, an alkyne-terminated PAPMA sample was functionalized with a biocompatible azide-terminated coumarin derivative, 3-azido-7-diethylaminocoumarin, *via* a copper(I) catalyzed azide alkyne cycloaddition. The success of the click reaction was confirmed by the appearance of the triazole ring proton signal (g) at 8.47 ppm [46] in the ^{1}H NMR spectrum of the functionalized polymer (Fig. 7). In addition to the functionalization through click chemistry, the PAPMA structure allows further modification through other strategies involving the reaction of the amino groups, such as Michael addition reactions [37], to expand the range of structures/applications of this polymer. Due to the biorelevance of coumarin [47] as well as the possibility of the double functionalization of alkyne-functionalized PAPMA, we anticipate that the strategy reported here will allow the preparation of complex polymeric structures useful for biomedical applications.

4. Conclusions

We reported the synthesis of clickable poly(*N*-(3-aminopropyl)methacrylamide) (PAPMA) by RAFT polymerization using azido-CTP or alkyne-CTP as chain transfer agents without any protection/deprotection steps. Both clickable CTAs exhibited similar or higher chain transfer activity compared to the commercial analogue CTP under the experimental conditions used. The control over the PAPMA molecular weight was very good, as the polymer

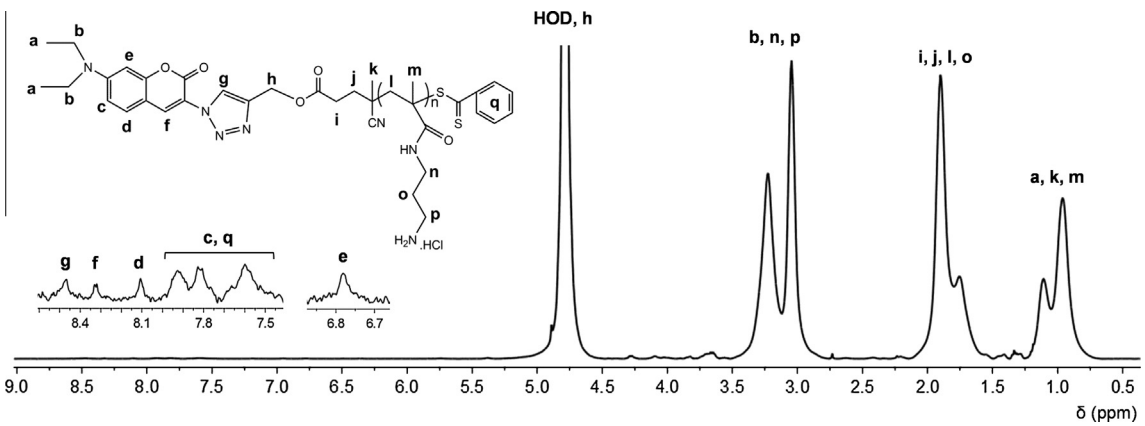

Fig. 7. 400 MHz ^{1}H NMR spectrum in D_2O of the purified coumarin-functionalized PAPMA obtained by a click reaction.

dispersity was lower than 1.1 during the polymerization. Chain extension experiments demonstrated the livingness of the synthesized PAPMA. The retention of the clickable chain-end functionality was confirmed by ^{1}H NMR spectroscopy. In addition, the alkyne-terminated PAPMA was functionalized with a biocompatible coumarin molecule by click chemistry, resulting in a material that could be applied in the biomedical field.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.reactfunctpolym.2014.04.001.

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