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Temperature-Responsive Copolymers without Compositional Drift by RAFT
Copolymerization of 2-(Acryloyloxy)ethyl trimethylammonium chloride and 2-
(Diethylamino)ethyl acrylate

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Solubility tests, free radical polymerization kinetics, conversion versus time curves, evolution of feed molar composition with conversion, ¹H NMR spectra, Debye plots from static light scattering measurements (pdf).

ABSTRACT

We prepared novel temperature and pH responsive copolymers of [2-(acryloyloxy)ethyl] trimethylammonium chloride (AETMACl) and 2-(diethylamino)ethyl acrylate (DEAEA) by RAFT polymerization. Such copolymers show a volume phase transition temperature that explains the excellent performance of hydrogels of the same monomers, selected among 600 different monomer combinations, to release cultured stem cells using only a mild temperature stimulus.¹ We carried out a thorough investigation of the (co)polymerization kinetics of AETMACl and protonated DEAEA in ethanol, and proposed convenient and reliable methods to follow the conversion of these two monomers by ¹H NMR. Interestingly, for a very large range of feed compositions, the cumulative copolymer composition versus global conversion plots indicated that there was almost no compositional drift, corresponding to apparent reactivity ratios of about 1. Therefore, the copolymer has the same composition as the comonomer feed, regardless of both conversion and initial molar ratio. This rare situation enables the preparation of a variety of copolymer chains with highly homogeneous composition and microstructure, offering excellent prospects for the use of these materials as triggers in controlled release applications.

INTRODUCTION

Stimuli-responsive polymers have been studied for the last thirty years in order to mimic biological systems that continuously adapt themselves to changing conditions.^{2,3} Such polymers can undergo significant reversible changes in their chemical or physical structure in response to a small external stimulus (temperature, pH, ionic strength, light, electric or magnetic field). Among stimuli-responsive polymers, those that respond to changes in temperature are especially attractive, finding applications in many fields such as drug delivery, tissue engineering, cell culture and separation devices.^{4,5}

Most thermoresponsive polymers are homopolymers, such as poly(*N*-isopropyl acrylamide) and its various derivatives,^{6,7,8} which are characterized by a LCST (Lower Critical Solution Temperature)^{9,10} and/or an UCST (Upper Critical Solution Temperature),¹¹ above/below which the polymer chains undergo a volume phase transition with a drastic change of conformation, collapsing from a coil to a globule.⁶ The use of copolymers instead of homopolymers, offers important design advantages such as the modulation of the volume phase transition temperature (T_{VPT}) by finely tuning the molar composition.¹² Moreover, a structure based on a copolymer can be used to improve the mechanical properties of a thermoresponsive material, as demonstrated in the case of poly(*N*-isopropyl acrylamide-*co*-butyl methacrylate) hydrogels.¹³

A study by the group of Mark Bradley reported the free radical copolymerization of [2-(acryloyloxy)ethyl] trimethylammonium chloride (AETMACl) and 2-(diethylamino)ethyl acrylate (DEAEA) monomers in the presence of a cross-linker, in order to prepare thermoresponsive hydrogels adequate for stem cell culture.^{1,14} These hydrogels were selected among more than 600 different monomer combinations by a combinatorial synthesis process, for their excellent performance in releasing cultured stem cells using only a mild temperature stimulus. These results indicate that copolymers of AETMACl and DEAEA are very good candidates for other controlled release applications. However, to the best of our knowledge, there are no other studies about this comonomer pair and no copolymerization kinetics was yet reported. In addition, no study about the temperature-responsiveness of those copolymers has been carried out, except for the effect of the hydrogels on stem cell culture.

The AETMACl and DEAEA monomers (Scheme 1) have already been homopolymerized and copolymerized with other comonomers but never together, except in the hydrogels of Bradley *et. al.*. AETMACl has been copolymerized by free radical polymerization with several (meth)acrylate or acrylamide derivatives, such as acrylamide,^{15,16} acrylic acid¹⁷ (for polaroid

instant photographic films), polyethylene glycol diacrylate¹⁸ (for stationary phases in chromatography columns), and with a cationic methacrylate.¹⁹ In most cases, the free radical (co)polymerization was carried out in water. AEtMACI has also been polymerized by controlled Reversible Deactivation Radical Polymerization (RDRP), in particular by the RAFT process in aqueous solution, either to prepare homopolymers²⁰ or block copolymers with ethylene glycol acrylate.²¹ Concerning DEAEA, very few studies report its polymerization contrary to its methacrylate counterpart, diethylaminoethyl methacrylate (DEAEMA). DEAEA has been copolymerized by free radical polymerization with hydroxyethyl methacrylate (in bulk) to prepare hydrogels.²² More recently, ATRP has been used to copolymerize DEAEA with *n*-butyl acrylate (in anisole) to synthesize polymer brushes on silica nanoparticles.²³ The RAFT process has also been used to polymerize DEAEA (in toluene) in order to prepare pH-sensors based on silicon films coated with pH-responsive polymer.²⁴

In most of the studies with AEtMACI or DEAEA, very little information is given about homopolymerization kinetics, except in a very recent study where the kinetic parameters of AEtMACI free radical polymerization were determined in aqueous solution.²⁵ Moreover, there are very few studies investigating the copolymerization kinetics of AEtMACI with other comonomers including the determination of reactivity ratios.^{15,19}

Considering the high potential of thermoresponsive materials based on AEtMACI and DEAEA for controlled release applications, in particular for cell culture, and the absence of kinetic data on this comonomer pair, we carried out a thorough investigation of their RAFT homo- and copolymerization kinetics. We propose different methods to follow the conversion by ¹H NMR and suggested the determination of the molecular weights by static light scattering using a Debye plot. Finally, the temperature response of the copolymer was

determined by dynamic light scattering, revealing a broad volume phase transition temperature that explains the excellent performance of this copolymer in stem cell culture.¹

Scheme 1. Monomers (AEtMACl and DEAEA) and RAFT CTA (CPADB) used in this study.

EXPERIMENTAL SECTION

Materials

2-(diethylamino)ethyl acrylate (DEAEA, 95%, Sigma-Aldrich, inhibitor: monomethyl ether hydroquinone) was purified by vacuum distillation. [2-(acryloyloxy)ethyl] trimethylammonium chloride solution (AEtMACl, 80 wt% in water, Sigma-Aldrich, inhibitor: monomethyl ether hydroquinone) was purified by passing through an inhibitor removal column (Prepacked column 306312, Sigma-Aldrich). 2,2'-azobisisobutyronitrile (AIBN, >98%, Fluka) was re-crystallized from ethanol. Ethanol (EtOH, absolute 99.9%, Fisher), used as polymerization solvent, was purified by distillation. 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPADB, >97%, Sigma-Aldrich) was used as received as chain transfer agent (CTA). Trioxane (>99%, Sigma-Aldrich), tetramethylsilane (TMS, >99.9%, Sigma-Aldrich), trimethyl silyl-3 propionic acid-d₄ (TMSP, S.d.S), chloroform-d (CDCl₃, 99.8%, 0.03% TMS, Sigma-Aldrich), deuterium oxide (D₂O, 99.9%, Sigma-Aldrich), trifluoroacetic acid (TFA, 99%, Fisher), acetone (>99%, Fisher) and diethyl ether (>99.5%, puriss, Sigma-Aldrich) were used as received. MilliQ deionized water (Millipore system) was used.

General polymerization procedure

Solubility tests

The solubility of each monomer and CTA was tested in several solvents. A few drops (or mg) of monomer (or CTA) were added to *ca.* 1 mL of solvent in a glass vial. The results (full/partial/non-soluble, presence of an emulsion or of two phases) are reported in Table S1 in ESI.

Reversible Addition-Fragmentation chain Transfer (RAFT) Polymerization

In the experiments where DEAEA was protonated (DEAEA⁺), a given molar equivalent of trifluoroacetic acid (TFA) was added to the monomer solution in ethanol, as indicated (Table 1). Monomer(s), AIBN, CPADB, ethanol (and trioxane if used) were introduced in a Schlenk tube, equipped with a magnetic stirrer (Table 1). The mixture was degassed by five freeze-evacuate-thaw cycles. Before heating the mixture in a thermostated oil bath under a slight nitrogen overpressure (0.3 bar), a sample was withdrawn as t_0 . Periodically, samples were withdrawn from the polymerization medium via a cannula for analyses.

For each experiment, $[AEtMACl]_0$, $[DEAEA]_0$, and $[CTA]_0$ were chosen such as to give the desired M_n value at 100% conversion (Eq. 1, neglecting AIBN-initiated chains)²⁶:

$$M_n = \frac{[AEtMACl]_0 M_{AEtMACl} p_{AEtMACl} + [DEAEA]_0 M_{DEAEA} p_{DEAEA}}{[CTA]_0} + M_{CTA} \quad (\text{Eq. 1})$$

where $[AEtMACl]_0$ and $M_{AEtMACl}$ (193.67 g mol⁻¹) are respectively the initial molar concentration and the molecular weight of AEtMACl; $[DEAEA]_0$ and M_{DEAEA} (285.24 g mol⁻¹) are respectively the initial molar concentration and the molecular weight of DEAEA; $p_{AEtMACl}$ and p_{DEAEA} are respectively the individual conversion of AEtMACl and DEAEA determined by ¹H NMR; $[CTA]_0$ and M_{CTA} (279.38 g mol⁻¹) are respectively the initial molar concentration and the molecular weight of the CTA.

Table 1. Experimental conditions for RAFT (co)polymerization of AEtMAcI and/or DEAEA in ethanol, in the presence of AIBN and CPADB (total monomer concentration $[M]_0 = 2$ M).

Exp	Temperature (°C)	AEtMAcI/DEAEA molar ratio	Equiv.of TFA ^a	$[M]_0/[CTA]_0$	$[CTA]_0/[AIBN]_0$
1	60	0/100	0	-	- ^b
2	70	0/100	0	-	- ^b
3	60	100/0	0	-	- ^b
4 ^c	60	100/0	0	-	- ^d
5	70	100/0	0	217	5
6	80	100/0	0	217	5
7	85	100/0	0	388	5
8 ^e	85	100/0	0	388	5
9	80	0/100	0	217	5
10	80	0/100	1	217	5
11	80	0/100	1.25	217	5
12	83	0/100	1.25	388	5
13	80	0/100	1.25	388	5
14	80	75/25	0	217	5
15	80	75/25	1.2	217	5
16	85	75/25	1.25	388	5
17 ^e	85	75/25	1.25	388	5
18	85	60/40	1.25	388	5
19	85	25/75	1.25	388	5
20 ^e	85	90/10	1.25	388	5
21 ^e	85	25/75	1.25	388	5

^aRelative to [DEAEA]. ^bConventional free radical polymerization, [AIBN]₀ = 0.02 M. ^c[M]₀ = 1 M. ^dConventional free radical polymerization, [AIBN]₀ = 0.005 M. ^ePolymerization on a larger scale (5-6 g instead of 1-2 g).

Determination of monomer conversion

Monomer conversion was determined by ¹H NMR spectroscopy (Bruker spectrometer Avance 300 MHz) using four different calculation methods as described below. During each (co)polymerization, the withdrawn samples were directly analyzed by ¹H NMR without evaporation of the polymerization solvent. Typically, 500 μL of deuterated solvent (D₂O or CDCl₃) respectively containing TMSP or TMS for spectra calibration, were added to 50 μL of each sample.

Method 1 is based on the addition of an internal standard (trioxane, 5.1 ppm in CDCl₃) to the polymerization medium ²⁷ to quantify the decrease of DEAEA or AEtMACl vinyl proton resonances (Figure S1 and calculation method in ESI). This method provided the best accuracy (below 2%) and reproducibility for experiments carried out below 80°C. When temperature was higher than the solvent boiling point (78°C for ethanol under 760 mmHg), we noticed that the accuracy decreased probably because of the co-evaporation of some trioxane.

Method 2 is based on the comparison between the ¹H NMR resonances of residual AEtMACl monomer ²¹ and growing PAEtMACl homopolymer chain (backbone part) (Figure S2 and calculation method in ESI). A very good accuracy (around 2%) was obtained for conversions above 20%, with a sufficiently high signal/noise ratio for the polymer backbone resonances.

Method 3 is based on a given proton of DEAEA for which the corresponding ¹H NMR resonances in residual monomer and homopolymer are close but clearly resolved (Figure S3

and calculation method in ESI). A very good accuracy (around 2%) was obtained for conversions as low as 10%, since the considered resonance at 4.2-4.4 ppm is much narrower than the polymer backbone resonances (between 1.4 and 2.5 ppm) and thus exhibits a better signal/noise ratio. For DEAEA, Method 3 gave better reproducibility than Method 2. For AEtMACl, Method 3 could not be applied since there was no zone where monomer and polymer resonances did not overlap.

Method 4 is based on the comparison between the ^1H NMR resonances of residual monomers and growing copolymer backbone (Figure S4 and calculation method in ESI). A very good accuracy (around 2%) was obtained for conversions above 20%, with a sufficiently high signal/noise ratio for the copolymer backbone resonances.

Purification of the (co)polymers

Several tests were carried out in order to find the best non-solvents to precipitate the (co)polymers for purification (Table S2 in ESI). The PAEtMACl homopolymer as well as the various copolymers were precipitated first in acetone, centrifuged (Eppendorf centrifuge, model 5810R, 10 minutes at 7232 g), re-solubilized in ethanol and precipitated twice in diethyl ether (centrifuged for 10 min at 12,857 g). The PDEAEA⁺ homopolymer was precipitated three times in diethyl ether and centrifuged (10 min at 12,857 g). The final polymers were dried under vacuum for several days. The complete elimination of residual monomers was confirmed by ^1H NMR analysis.

Characterization of the (co)polymers

^1H NMR spectroscopy

Each purified (co)polymer was analysed by ^1H NMR spectroscopy (Bruker spectrometer Avance 300 MHz) and all the resonances were identified in order to determine the molar composition of the various copolymers.

Static Light Scattering (SLS)

Molecular weights were determined by SLS, performed on Zetasizer Nano ZS (Malvern) operating at an angle of 173° , using SUPRASIL quartz cells 10×10 mm (the same cell was used for all the measurements on each experiment). All the material used was cleaned to remove dust, using compressed air. Ethanol (filtered on PTFE filter $0.45 \mu\text{m}$) was used to wash the cell between measurements and toluene was used as scattering standard. The polymer samples were found to be hygroscopic, and thus were weighted in a glovebox. Deionized water from a Milipore Milli-Q system (with a Millipak membrane filter $0.22 \mu\text{m}$) was used for the preparation of polymer solutions with a concentration of 1 mg mL^{-1} . These solutions were left stirring until used. The dilutions (80%, 60%, 40%, 20% and 10% from original solution) of each polymer sample were prepared on the day of the measurements, using fresh Milli-Q water. The molecular weight measurement sequence requires a series of intensity measurements: first of a standard (toluene) to establish the reference scattering intensity, then of the solvent (Milli-Q water) and then of each of the prepared solutions.²⁸ Ten measurements were made for each concentration (1, 0.8, 0.6, 0.4, 0.2 and 0.1 mg mL^{-1}).

The SLS measurements were analyzed with the Rayleigh equation (Eq. 2):

$$\frac{KC}{R_\theta} = \left(\frac{1}{M_w} + 2A_2C \right) P(\theta) \quad (\text{Eq. 2})$$

where R_θ is the Rayleigh ratio, which characterizes the scattered intensity at the scattering angle θ , M_w the sample molecular weight; A_2 the 2nd virial coefficient; C the concentration. $P(\theta)$ is the form factor, which is related to the shape and size of the dispersing object and the

angle at which the scattering is determined. For measurements at very large angle (as in our case), the form factor is approximately 1, and therefore a plot of KC/R_θ versus C is expected to be linear (Debye Plot) with an intercept equal to $1/M_w$ (Eq. 3)

$$\frac{KC}{R_\theta} = \left(\frac{1}{M_w} + 2A_2C \right) \quad (\text{Eq. 3})$$

with the optical constant, K , defined as

$$K = \frac{2\pi^2}{\lambda_0^4 N_A} \left(n_0 \frac{dn}{dc} \right)^2 \quad (\text{Eq. 4})$$

where n_0 is the solvent refractive index (1.330 for water), λ_0 the laser wavelength (633 nm, in the used apparatus), N_A the Avogadro's constant and dn/dc the differential refractive index increment (depends on the polymer sample).

The specific refractive index increment (dn/dc) in acetate buffer (acetic acid 0.2 M + ammonium acetate 0.15 M, pH 4.5, 40°C) was determined for PDEAEA⁺ ($dn/dc = 0.15 \pm 0.01$), for PAEtMACl ($dn/dc = 0.19 \pm 0.03$) and for the P(AEtMACl-*co*-DEAEA⁺) copolymer with a 75/25 molar ratio ($dn/dc = 0.21 \pm 0.01$) using a manual differential refractometer (C. N. Wood RF-600) operating at 658 nm. The dn/dc of the other copolymers was determined by interpolation of the values obtained for PDEAEA⁺ and PAEtMACl.

Dynamic Light Scattering (DLS)

To determine the temperature response of the copolymers, dynamic light scattering experiments were performed using a Zetasizer Nano ZS (Malvern), for temperatures between 10 and 40 °C. The auto-correlated functions were analyzed by the CONTIN method, to determine the hydrodynamic diameter. Polymer solutions (2 mg mL⁻¹) of P(AEtMACl-*co*-DEAEA⁺) copolymer with a 75/25 molar ratio (Exp. 17) were prepared in phosphate buffer

(pH 7.5, 1 M), in Milli-Q water at pH 7 with 0.15 M NaCl, and in Milli-Q water at pH 9 (using a 0.1 M NaOH solution to adjust the pH).

RESULTS AND DISCUSSION

The excellent performance of AEtMACl/DEAEA based thermoresponsive hydrogels in cell culture¹ and the lack of information on the use of the RAFT process to produce well-controlled copolymer chains from those monomers, motivated us to perform a systematic study on the different parameters controlling their RAFT (co)polymerization. In addition to the kinetics experiments, our objective was to produce copolymer chains of at least 50000 g mol⁻¹, in order to favor a strong thermoresponsive behavior.

As chain transfer agent (CTA), we selected 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPADB, Scheme 1), successfully used with acrylate monomers.²⁹ Additionally, it has a carboxylic group that can be further used to graft the chains on the surface of solid supports.³⁰ The first experimental step was to select an appropriate polymerization solvent for both monomers and the CTA. After several tests (Table S1 in ESI), ethanol was chosen since it easily solubilizes DEAEA, AEtMACl and CPADB. Moreover, it is easy to remove in the purification step. The absence of information regarding the homopolymerization kinetics of DEAEA and AEtMACl in ethanol, led us to evaluate the behavior of each monomer first in free radical polymerization (FRP) (see ESI, Figures S5 and S6, Exp. 1 to 4, Table 1).

To study the (co)polymerization kinetics, we developed convenient ¹H NMR methods adapted to AEtMACl and DEAEA to follow monomer conversion (see experimental part and ESI); up to now, the few papers reporting kinetics studies with AEtMACl used HPLC,¹⁹ gravimetry,^{20,21} or near-infrared spectroscopy²⁵ methods.

Kinetics of AEtMACl RAFT homopolymerization

The RAFT homopolymerization of AEtMACl in ethanol was performed under similar conditions to its FRP, except for the presence of CPADB as CTA. Since temperature is a key parameter in RAFT polymerization,³¹ several experiments were carried out at different

temperatures (oil bath), 70°C (Exp. 5), 80°C (Exp. 6) and 85°C (Exp. 7) (Table 1 and Figure 1), with a CTA/initiator ratio of 5.

Figure 1. Monomer conversion versus time plots for AEtMACl RAFT polymerization in ethanol at different temperatures, 70°C (Exp. 5, ♦), 80°C (Exp. 6, ■) and 85°C (Exp. 7, ●). Exp. 8 (■) is similar to Exp. 7 but carried out at a larger scale (5.6 g of monomer instead of 0.8 g). Conversions were determined by ^1H NMR using Method 2 (except for Exp. 5 where Method 1 was used).

At 70°C (Exp. 5), the induction period (initial step with very low monomer consumption)³² was around 2 hours and the conversion plateau reached 40-50%. When temperature was increased to 80°C, the conversion plateau was significantly higher (75%) as expected, and the induction period reduced to 20 min as better evidenced by the $\ln([M]_0/[M]_t)$ versus time plots (Figure S7 in ESI). Moreover, the linear increase of these plots indicated that the concentration of radical species remained constant until rather high conversions (for instance, 67% for Exp. 6). After that (here, *ca.* 3 hours), the new radicals produced by AIBN decomposition did not compensate any more the loss of propagating radicals via irreversible termination reactions, therefore conversion reached a plateau. Finally, at 85°C (Exp. 7), the

induction period was just a few minutes (7 min) and it was possible to reach 81 % conversion in 4 hours. Taking into account the initial amounts of AEtMACl and CTA, that conversion corresponded to a calculated M_n value of 61000 g mol^{-1} , in agreement with our objective.

Kinetics of DEAEA RAFT homopolymerization

The RAFT homopolymerization of DEAEA was performed under similar conditions to those for AEtMACl. However, it exhibited an unexpected behavior. First, an experiment conducted at 70°C led to very low conversions (results not shown). An increase in temperature to 80°C (Exp. 9, Table 1 and Figure 2) did not improve significantly the conversion that reached a plateau at approximately 10%.

Figure 2. Monomer conversion versus time plots for DEAEA RAFT polymerization in ethanol at 80°C without TFA (Exp. 9, ■), with 1 eq. TFA (Exp. 10, ●) and with 1.25 eq. TFA (Exp. 11, ▲). Conversions were determined by ^1H NMR using Method 1 (Exp. 9) and Method 3 (Exp. 10 and 11).

Second, the color of the polymerization medium turned from pink (color of the CPADB dithiobenzoate in ethanol at t_0) to pale yellow after 10 min (instead of the expected salmon color usually generated in the presence of dithiobenzoate, Figure S8 in ESI). Such

discoloration of the medium generally indicates a degradation of the CTA.³³ Third, during the polymerization reaction, we noticed some intriguing changes in the ^1H NMR spectrum (already observable at t_1 , *i.e.* less than 5% conversion), with new resonances appearing just besides the vinyl proton resonances (see for instance t_4 compared to t_0 , respectively b and a in Figure 3; the parasite resonances are marked with stars).

Figure 3. Influence of TFA on the appearance of parasite resonances (*, due to hydrolysis of DEAEA into acrylic acid and 2-diethylamino ethanol) in the vinyl region of the ^1H NMR spectrum: t_0 (a) and t_4 (b) for DEAEA homopolymerization without TFA (Exp. 9), t_0 (c) and t_7 (d) for DEAEA⁺ homopolymerization in the presence of 1.25 eq. TFA (Exp. 12).

A possible explanation is the hydrolysis of the monomer, previously observed for a polymer based on dimethylaminoethyl acrylate (DMAEA), a monomer very similar to DEAEA. PDMAEA suffers hydrolysis in aqueous solution leading to 2-dimethylamino ethanol and

poly(acrylic acid).^{34,35} Hydrolysis of DEAEA was confirmed here, with the observed new resonances assigned to acrylic acid vinyl protons. Those observations were related to the basicity of the medium (DEAEA solution in ethanol corresponded to pH≈10) that could explain both hydrolysis of the monomer and rapid degradation of the CTA (thiocarbonylthio compounds are sensitive to basic conditions).³⁶

To avoid such hydrolysis, DEAEA was protonated with trifluoroacetic acid (TFA). To investigate the influence of the protonation on the RAFT polymerization kinetics, two experiments (Exp. 10 and 11, Table 1 and Figure 2) were carried out in the same conditions as Exp. 9, but with 1 and 1.25 molar equivalent of TFA, respectively (with respect to monomer). In Exp. 10 (1 eq. TFA), conversion reached almost 50% in 4 hours compared to 10% in the absence of TFA. Moreover, the color of the polymerization medium did not change to yellow after 10 min but to the usual salmon color (stable afterwards). A slight excess of TFA (Exp. 11, 1.25 eq. TFA) appeared favorable since conversion reached around 80% in 4 hours. A further excess (1.5 eq.) did not lead to better results (data not shown). On the ¹H NMR spectra, we noticed that in the case of 1 eq. TFA (pH≈7-8), the acrylic acid resonances related to monomer hydrolysis still appeared but with a lower intensity. However, with 1.25 eq. TFA (pH≈5-6), no parasite resonance appeared during the whole polymerization course (see for instance t_7 compared to t_0 , respectively d and c in Figure 3). These results clearly show that polymerization kinetics was drastically influenced by monomer protonation. In the presence of 1.25 eq. TFA, monomer hydrolysis was fully avoided and DEAEA⁺ conversion reached 80% in a few hours.

Taking into account the initial amounts of DEAEA⁺ and CTA, the conversion plateau of Exp. 11 corresponded to a calculated M_n value of 30000 g mol⁻¹. In order to produce longer chains (of about 50000 g mol⁻¹), the [DEAEA⁺]₀/[CTA]₀ initial molar ratio was increased from 217 (Exp. 11, calculated M_n = 37400 g mol⁻¹ at 100% conversion) to 388 (Exp. 13,

Table 1, calculated $M_n = 66700 \text{ g mol}^{-1}$ at 100% conversion), keeping the $[\text{CTA}]_0/[\text{AIBN}]_0$ ratio equal to 5 (Figure S9 in ESI). However, the conversion plateau decreased from 80 to 65%, a behavior different from that observed with the *N*-acryloylmorpholine/*tert*-butyldithiobenzoate system, where the kinetics remained identical whatever the targeted chain length provided that the CTA/AIBN ratio was kept constant.³¹ Therefore, to achieve higher conversion and the desired longer chains, a temperature of 85°C was preferred in the following experiments.

Kinetics of RAFT copolymerization of the AEtMACI/DEAEA comonomer pair

Similarly to DEAEA homopolymerization, the AEtMACI/DEAEA copolymerization kinetics was also strongly influenced by the protonation of DEAEA. For example, for a 75/25 AEtMACI/DEAEA initial molar ratio, two RAFT copolymerization experiments were compared, without and with TFA (1.2 eq. with respect to DEAEA, Exp. 14 and 15 respectively, Table 1). In the presence of TFA, the conversion plateau reached 75% (instead of 25-30% without TFA, Figure S10 in ESI) and there was no degradation of CTA and no hydrolysis of DEAEA, unlike what was observed without TFA. Consequently, the following copolymerization experiments were all conducted in the presence of TFA.

In order to get polymer chains of at least 50000 g mol^{-1} while keeping a high final conversion, two parameters were simultaneously modified taking into account the above results for DEAEA⁺ homopolymerization: the $[M]_0/[\text{CTA}]_0$ molar ratio was increased from 217 to 388 and the temperature from 80 to 85 °C (Exp. 16, Table 1, calculated $M_n = 73200 \text{ g mol}^{-1}$ at 100% conversion). Comparison with Exp. 15 (Figure 4), indicated a shorter induction period at 85°C (8 min) than at 80°C (39 min) and a similar conversion plateau (75%), corresponding to a calculated M_n value of 54900 g mol^{-1} in agreement with our objective. To obtain larger amounts of that copolymer sample, the total amount of comonomers was

increased (from 1.35 to 6.7 g, Exp. 17, Table 1). This scale-up was a success and exhibited a similar copolymerization behavior (Exp. 17 compared to Exp. 16, Figure 4).

Figure 4. Global monomer conversion versus time plots for AEtMACI/DEAEA⁺ 75/25 copolymerization experiments, at 80°C with $[M]_0/[CTA]_0 = 217$ (Exp. 15, ●); at 85°C with $[M]_0/[CTA]_0 = 388$ (Exp. 16, ■) and a scale-up experiment in the same conditions (Exp. 17, ●). Conversions were determined by ¹H NMR using Method 4.

In order to obtain copolymers with different compositions, we explored the RAFT copolymerization kinetics for different initial AEtMACI/DEAEA⁺ molar ratios: 60/40, 25/75 and 90/10 (Exp. 18, 19 and 20, respectively, Table 1), in the same optimized conditions as Exp. 17 (75/25, $[M]_0/[CTA]_0 = 388$, $[CTA]_0/[AIBN]_0 = 5$, 85°C). Curiously, whatever the initial molar ratio, the global conversion versus time curves were very similar (Figure 5), which is quite unusual.

Figure 5. Global monomer conversion versus time plots for AEtMACl/DEAEA⁺ copolymerizations at different initial molar ratios: 75/25 (Exp. 17, ■), 60/40 (Exp. 18, ●), 25/75 (Exp. 19, ◆) and 90/10 (Exp. 20, ▲).

To go deeper in the copolymerization kinetics, we tried to access the individual monomer conversions, since they can usually yield the apparent reactivity ratios as well as the instantaneous and cumulative copolymer molar composition.^{26,27} However, in the case of the AEtMACl/DEAEA⁺ pair, ¹H NMR determination of the individual monomer conversions was complicated by the presence of TFA. Indeed, the vinyl proton resonances of DEAEA⁺ were shifted to lower field than those of the non-protonated monomer (Figure 3, see Exp. 12 with TFA compared to Exp. 9 without TFA), overlapping with the vinyl proton resonances of AEtMACl (Figures S11-S14 in ESI). Nevertheless, it was possible to integrate the vinyl proton doublet at 6.35-6.55 ppm using a deconvolution method (Figure S15 in ESI), applicable to all the experiments except Exp. 20 (90/10 molar ratio) where the vinyl proton resonances fully overlapped.

The AEtMACl and DEAEA⁺ individual conversion values were very close (often equal) not only for all polymerization times but also for all initial molar ratios (Figure 6, left), which is rarely observed in copolymerization. Generally, one monomer is consumed faster than the

other and consequently, the molar composition of the generated copolymer varies with global conversion (known as compositional drift).³⁷ For a given comonomer pair, it may exist a particular initial molar ratio (the so-called azeotropic composition) for which the two comonomers copolymerize at the same rate, leading to a copolymer without compositional drift.^{26,27,37} However, in such a case, it usually takes place at a specific feed molar composition value or for a narrow composition range around that value.³⁸ Here, for the AEtMACl/DEAEA⁺ comonomer pair, an azeotropic behavior is observed for a wide range of initial molar ratios (at least from 75/25 to 25/75).

Figure 6. (left) Individual conversions of AEtMACl (●), DEAEA⁺ (●) and global conversion (●) versus time; (right) cumulative copolymer composition expressed as molar fractions,

$\overline{F_{\text{AEtMACl}}}$ ($\blacktriangle$) and $\overline{F_{\text{DEAEA}}}$ ($\blacktriangle$), versus global conversion. AEtMACl (- -) and DEAEA⁺ (- -) initial feed molar composition for AEtMACl/DEAEA⁺ copolymerization at different molar ratios: (A) 75/25 (Exp.17); (B) 60/40 (Exp. 18); and (C) 25/75 (Exp. 19).

From the individual conversion values, p_{AEtMACl} and p_{DEAEA} , the cumulative copolymer composition (expressed as molar fractions, $\overline{F_{\text{AEtMACl}}}$ and $\overline{F_{\text{DEAEA}}}$, respectively, calculated from Eq. 5 and 6) was plotted versus global conversion (Eq. 7) for the different copolymerization experiments (Figure 6, right). It is clearly visible that there is almost no compositional drift whatever the initial molar ratio, the cumulative copolymer composition being very close to the feed composition.

$$\overline{F_{\text{AEtMACl}}} = \frac{\Delta[\text{AEtMACl}]}{\Delta[\text{AEtMACl}] + \Delta[\text{DEAEA}]} = \frac{[\text{AEtMACl}]_0 \times p_{\text{AEtMACl}}}{[\text{AEtMACl}]_0 \times p_{\text{AEtMACl}} + [\text{DEAEA}]_0 \times p_{\text{DEAEA}}} \quad (\text{Eq. 5})$$

$$\overline{F_{\text{DEAEA}}} = \frac{\Delta[\text{DEAEA}]}{\Delta[\text{AEtMACl}] + \Delta[\text{DEAEA}]} = \frac{[\text{DEAEA}]_0 \times p_{\text{AEtMACl}}}{[\text{AEtMACl}]_0 \times p_{\text{AEtMACl}} + [\text{DEAEA}]_0 \times p_{\text{DEAEA}}} \quad (\text{Eq. 6})$$

$$\text{Global conv. (t)} = \frac{[\text{AEtMACl}]_0 \times p_{\text{AEtMACl}} + [\text{DEAEA}]_0 \times p_{\text{DEAEA}}}{[\text{AEtMACl}]_0 + [\text{DEAEA}]_0} \quad (\text{Eq. 7})$$

It was also possible to follow the evolution of the feed molar composition versus global conversion (Figure S16 in ESI). From these plots, the values of the apparent reactivity ratios can be usually calculated using the Meyer-Lowry model.^{37,39} However, in this case where the copolymerization proceeds with almost no changes in the feed molar composition, it was not possible to apply that model.

This kind of copolymerization, where the copolymer composition is equal to the comonomer feed composition for a wide range of initial molar ratios, follows a Bernouillian behavior³⁷ and corresponds to the case with both apparent reactivity ratios, r_{AEtMACl} and r_{DEAEA}^+ , of about 1. Consequently, whatever the initial AEtMACl/DEAEA⁺ molar ratio, the formed copolymer

chains exhibit the same composition as the feed regardless of the conversion. Therefore, it is possible to prepare P(AEtMACI-*co*-DEAEA⁺) copolymers with high composition and microstructure homogeneity for a large choice of molar ratios.

Characterization of the purified copolymers by ¹H NMR

After thorough purification of the (co)polymers by precipitation, the ¹H NMR spectra of PAEtMACI and PDEAEA⁺ homopolymers and different P(AEtMACI-*co*-DEAEA⁺) copolymers were recorded and assigned (Figure 7). They clearly show the variations of the resonance relative intensities according to the initial comonomer molar ratio (90/10, 75/25, 60/40 and 25/75), especially visible for the protons c and g (-C(O)-O-CH₂ moiety) and for the protons b and f (-CH₂-N⁺ moiety) (Figure S17 in ESI).

For each purified copolymer, the average molar composition was quantified thanks to chosen zones of the ¹H NMR spectrum (shown for Exp. 17 as an example, Figure 7) and expressed as a molar fraction, $P_{\text{DEAEA}}/(P_{\text{DEAEA}} + P_{\text{AEtMACI}})$, where P_{DEAEA} and P_{AEtMACI} are the number of DEAEA⁺ and AEtMACI units, respectively, in the copolymer. It could be determined either from zones A (1.25 - 1.5 ppm) and C (2.9 – 4 ppm) or from zones A and B (1.5 - 2.9 ppm), according to Eq. 8 or 9, respectively. These average copolymer composition values were compared to those calculated from the corresponding individual monomer conversions according to Eq. 6 (Table 3).

Figure 7. ^1H NMR spectrum in D_2O of $\text{P}(\text{AEtMACI-co-DEAEA}^+)$ copolymer (Exp. 17, 75/25 AEtMACI/DEAEA $^+$ initial molar ratio) showing the different zones (A, B, C, D) chosen for determination of the average molar composition.

$$\begin{cases} A \propto 6P_{\text{DEAEA}} \\ B \propto 3P_{\text{DEAEA}} + 3P_{\text{AEtMACI}} \\ C \propto 9P_{\text{AEtMACI}} + 4P_{\text{DEAEA}} + 2P_{\text{DEAEA}} + 2P_{\text{AEtMACI}} \\ D \propto 2P_{\text{AEtMACI}} + 2P_{\text{DEAEA}} \end{cases} \Rightarrow$$

$$\frac{P_{\text{DEAEA}}}{(P_{\text{DEAEA}} + P_{\text{AEtMACI}})} = \frac{A/6}{A/6 - A/11 + C/11} \quad (\text{Eq. 8})$$

$$\frac{P_{\text{DEAEA}}}{(P_{\text{DEAEA}} + P_{\text{AEtMACI}})} = \frac{A}{2B} \quad (\text{Eq. 9})$$

Table 3. Average molar composition of the various P(AEtMACl-*co*-DEAEA⁺) copolymers (expressed as AEtMACl/DEAEA⁺ molar ratio).

P(AEtMACl- <i>co</i> -DEAEA ⁺) copolymers	Initial feed composition	Average copolymer composition		
		purified copolymers (¹ H NMR)		calculated from kinetics (individual
		(Eq. 8)	(Eq. 9)	conversions)
Exp. 20 _{t10}	90/10	90/10	89/11	nd
Exp. 17 _{t7}	75/25	75/25	75/25	75/25
Exp. 18 _{t10}	60/40	63/37	57/43	60/40
Exp. 19 _{t10}	25/75	35/65	27/73	25/75

nd= not determined

The results from Eqs. 8 and 9 are very similar except for Exp. 19, probably because of the poor resolution between A, B and C areas in the corresponding ¹H NMR spectrum (Figure S17 D in ESI). Moreover, these average composition values determined on the purified final copolymer samples are in good agreement with the ones calculated from the individual monomer conversions, which validate the methods used to follow the kinetics. In addition, since these values are very close to the initial feed compositions (for a global conversion around 75%), they confirm the absence of compositional drift during AEtMACl/DEAEA⁺ RAFT copolymerization for a large range of initial molar ratios.

Determination of the purified (co)polymer molecular weights by SLS

Analysis of the homopolymer and copolymer samples by Size Exclusion Chromatography (SEC) evidenced an unimodal molecular weight distribution (Fig S18 in ESI for P(AEtMACl-

co-DEAEA⁺) copolymer, Exp. 16). However, determination of the molecular weight values was not possible since the polymers showed some affinity with the stationary phase of the different tested columns (see SEC section in ESI). More specifically, a trail was observed on the SEC trace on the low molecular weight side of the polymer peak. As an alternative, the molecular weights were determined by Static Light Scattering (SLS), using a Debye plot (Eq. 3) (Figure S19 in ESI). For each polymer sample, the obtained value was consistent with the one calculated from the monomer conversion data (Table 4), which is an expected result for a well-controlled RAFT polymerization.

Table 4. Molecular weights of PAEtMACl and PDEAEA⁺ homopolymers and P(AEtMACl-*co*-DEAEA⁺) copolymers determined by Static Light Scattering (SLS), with the used refractive index increment (dn/dc) values.

Polymer Sample	Global conversion ¹ H NMR ^a	Calculated M_n ^a (g mol ⁻¹)	M_w SLS (g mol ⁻¹)	dn/dc (mL g ⁻¹)
PAEtMACl (Exp. 8 _{t7})	56%	42×10^3	$(43 \pm 18) \times 10^3$	0.19 ± 0.03
PDEAEA ⁺ (Exp. 12 _{t10})	60%	40×10^3	$(41 \pm 6) \times 10^3$	0.15 ± 0.01
COPO 90/10 (Exp. 20 _{t8})	76% ^b	56×10^3 ^b	$(57 \pm 10) \times 10^3$	0.22 ± 0.01
COPO 75/25 (Exp. 17 _{t7})	72%	65×10^3	$(55 \pm 8) \times 10^3$	0.21 ± 0.01
COPO 60/40 (Exp. 18 _{t10})	74%	57×10^3	$(49 \pm 8) \times 10^3$	0.20 ± 0.01
COPO 25/75 (Exp. 21 _{t11})	63%	49×10^3	$(44 \pm 4) \times 10^3$	0.17 ± 0.01

^aGlobal conversion and M_n calculated from the corresponding individual monomer conversions, according to Eqs. 7 and 1, respectively. ^bFor Exp. 20, global conversion was

calculated using ^1H NMR Method 4 (Eq. S4) and M_n was calculated from Eq. 1 assuming that both individual conversions are equal to global conversion.

Characterization of the responsive behavior of the copolymers

As a preliminary test of the temperature response of the new copolymers, we measured the hydrodynamic diameter of the P(AEtMACI-*co*-DEAEA⁺) copolymer in solution (75/25 molar ratio, Exp. 17) by Dynamic Light Scattering. A copolymer concentration of 2 mg mL⁻¹ was used, either in phosphate buffer at pH 7.5 (1 M) (**A**), or in Milli-Q water at pH 7 with 0.15 M NaCl (**B**), or in Milli-Q water at pH 9 (**C**) (Figure 8).

The most striking characteristic of these results is that the copolymer exhibits a broad volume phase transition below 30°C, over which the chains collapse to *ca.* 50% of their low temperature (< 20 °C) hydrodynamic diameter. At pH 7.5 (**A**), close to the pK_a of the tertiary amine of DEAEA,⁴⁰ some fluctuations were observed. Indeed, this proximity can lead to small changes in the protonation state of the amine when the temperature is changed, that interfere with the temperature response of the polymer. At pH 7 with 0.15 M NaCl (**B**), the lower pH favors DEAEA protonation, with the resulting repulsion being attenuated by the electrostatic screening due to the salt. At pH 9 (**C**), there is deprotonation of the tertiary amine of DEAEA, leading to a smaller hydrodynamic size of the expanded polymer chains at low temperature, compared to the other conditions. For (**C**), up to *ca.* 37 °C, there is also a steady decrease of the chain hydrodynamic diameter, followed by a slight increase that might indicate the onset of chain aggregation following the collapse into a globule conformation. Further studies of the copolymer response to temperature, pH and ionic strength are underway.

Even though the volume phase transition temperature is not sharp, the difference in the hydrodynamic radii of the polymer chains between 37 °C and lower temperatures (below 20

°C), indicates a corresponding increase in their hydrophilic character that justifies a decrease in cell adhesion when thermoresponsive hydrogels of these copolymers are used as substrate to culture stem cells. This change enabled a very efficient release of the cells using only a mild temperature stimulus.¹

Figure 8. Dynamic Light Scattering measurements of hydrodynamic radius, R_H , versus temperature for P(AEtMACI-co-DEAEA⁺) copolymer (Exp. 17, 75/25 initial molar ratio) in

various aqueous solutions (concentration of 2 mg mL⁻¹): (A) phosphate buffer (pH 7.5, 1 M), (B) Milli-Q water at pH 7 in the presence of NaCl (0.15 M) and (C) Milli-Q water at pH 9.

CONCLUSIONS

Remarkably, the new thermoresponsive copolymers based on [2-(acryloyloxy)ethyl] trimethylammonium chloride (AEtMACl) and protonated 2-(diethylamino)ethyl acrylate (DEAEA⁺) feature RAFT copolymerization kinetics with both apparent reactivity ratios of about 1. Consequently, whatever the initial AEtMACl/DEAEA⁺ molar ratio, the copolymer chains have the same composition as the feed, regardless of the conversion. This allows us to prepare P(AEtMACl-*co*-DEAEA⁺) copolymers exhibiting not only controlled molecular weights but also highly homogeneous composition and microstructure.

The LCST-type temperature response of the copolymer corresponds to a broad volume phase transition below 30°C, over which the chains collapse to *ca.* 50% of their low temperature hydrodynamic diameter. This response is related to the excellent performance in releasing stem cells cultured on hydrogels based on a 75/25 AEtMACl/DEAEA molar ratio. A hydrogel of this particular copolymer has been previously selected among over 600 different monomer combinations for its ability to release the cultured cells by using only a mild temperature stimulus. The copolymer also responds to pH through protonation/deprotonation of the DEAEA units. The combination of temperature/pH stimuli, in conjugation with the control over molecular weight and composition, opens very exciting opportunities for application in many fields, from controlled delivery to tissue engineering, cell culture, separation, etc.

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Conflicts of interest

There are no conflicts to declare.

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