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Purity of double hydrophilic block copolymers revealed by capillary electrophoresis in the critical conditions

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Highlights:

- separation of charged block copolymers and their parent / unintended homopolymers
- free solution capillary electrophoresis of charged block copolymers
- quantification of the purity of charged block copolymers
- pressure assisted capillary electrophoresis of cationic copolymers

Abstract:

Block copolymers enable combining properties of different polymers; double hydrophilic block copolymers are innovative examples. Size-exclusion chromatography (SEC or GPC) has a quasi-monopoly in separation-based characterization methods for polymers, including block copolymers. However, in terms of purity determination (unintended homopolymers present in the copolymers), SEC resolution proves insufficient except for the extreme compositions for which the second block is much larger than the first one. The free solution capillary electrophoresis (capillary zone electrophoresis) technique does not separate charged homopolymers by their molar mass and we thus named the corresponding method capillary electrophoresis in the critical condition (CE-CC). CE-CC provides a means to assess the purity of poly(acrylic acid-*b*-acrylamide) - P(AA-*b*-AM) - copolymers, as well as of the more challenging cationic poly(acrylamido-*N*-propyltrimethylammonium chloride-*b*-*N*-isopropylacrylamide) - P(APTAC-*b*-NIPAM). In addition it can identify that a block copolymer has been produced. It is to be noted that P(APTAC-*b*-NIPAM) block copolymers cannot be eluted in SEC due to their exceptional ability to adsorb onto surfaces, while some information is obtained from CE-CC. Both possible parent homopolymers can be detected and their quantity estimated in a single injection by CE-CC. In both cases, one of the parent homopolymers is neutral and comes with the electro-osmotic flow. If the electro-osmotic flow is weak (conditions used for the cationic copolymer) then pressure assisted CE-CC is used to detect this homopolymer.

Keywords: capillary electrophoresis; critical conditions; block copolymers; residual homopolymer quantification; purity; pressure assisted CE-CC

1. Introduction

Block copolymers provide a means of incorporating the properties of multiple polymers into the one material. Stimulus-responsive or 'smart' polymers can be included to provide, for example, the pH-responsiveness in one block and adhesion in another. For this reason they have a wide variety of potential applications including improving medical imaging [1], drug delivery [2], capacitors [3] and removable coatings [4]. Double hydrophilic block copolymers (DHBCs) are especially innovative [5], for example in the field of controlled mineralization [6,7] or quantum dots [8]. With further development, these applications may become a reality.

To continue the advance of these block copolymers more accurate means of characterizing them are required. There is no established method for the control of the purity of DHBCs. The most common tool for separating and characterizing polymers is Size Exclusion Chromatography (SEC), also known as Gel Permeation Chromatography (GPC) [9,10]. SEC separates according to hydrodynamic volume and not according to molar mass [10,11]. When the hydrodynamic volume is altered not only by molar mass, but also by copolymer composition or branching, there is an incomplete separation according to molar mass leading to errors of up to 100 % in the determined molar mass [12,13]. Moreover, if any unintended homopolymers are present in the block copolymer sample it is unlikely to be detected by routine NMR spectroscopy and SEC experiments, leading to further errors in the determined molar mass. The difference in hydrodynamic volumes of a copolymer and of the corresponding homopolymers is usually not sufficient to allow their baseline separation to occur using SEC. Furthermore, SEC of smart polymers such as polyelectrolytes can be plagued with aggregation [14] and 'ion exclusion' [15-17]. This can then lead to low accuracy of the determined molar mass values [10,18]. More particularly, interactions of the polycations with the SEC stationary phase is a challenge, which limits reproducibility and separation efficiency. These interactions can generally be suppressed with high salt eluents and co-solvents [19,20] however in some cases there is a risk of precipitation and poor recovery. Columns for aqueous SEC of cationic polymers at low salt conditions are being developed as shown in application notes [21-23]. In the case of DHBCs, these SEC conditions for polycations are however incompatible with the SEC conditions for some second (neutral) block. For example, poly(*N*-isopropylacrylamide) – PNIPAM is a very challenging polymer to properly characterize using aqueous SEC due to its thermoresponsiveness in water [24]. PNIPAM requires polar organic mobile phases such as dimethylformamide (DMF) containing LiBr [25]. Therefore, the analysis of block copolymers such as poly(acrylamido-*N*-propyltrimethylammonium chloride-*b*-NIPAM) - P(APTAC-*b*-NIPAM) - or P(APTAC-*b*-*N*-vinyl pyrrolidone) by SEC is usually unsuccessful [26-29]. Both PAPTAC and PNIPAM homopolymers can be analysed by SEC with some success but no common conditions for the related DHBCs characterisation could be found. Therefore, the development of applications of cationic DHBCs such as P(APTAC-*b*-NIPAM) has been hindered by their lack of characterization.

Liquid Chromatography under Critical Conditions (LC-CC) is a separation technique which separates polymers independently from molar mass [30]. By having one block in critical conditions the other block can be separated according to its molar mass providing the molar mass distribution of this other block. LC-CC leads to complete separation of the block copolymer from one of the corresponding homopolymers. However, determining the critical conditions is tedious, LC-CC suffers from poor recovery [31,32] and the mobile phase is usually required to be a mixture of solvents leading to preferential solvations and adsorption on the stationary phase [33]. Therefore, easier alternatives to LC-CC are highly desired. One example is Liquid Chromatography under Limiting Conditions of Desorption (LC-LCD), which is capable of separating block copolymers from their corresponding homopolymers [34-36]. LC-LCD has been used to show that a number of commercial

poly(methyl methacrylate-*b*-styrene) block copolymers contained small amounts of both poly(methyl methacrylate) and polystyrene [35,37]. Furthermore, LC-LCD has been applied to poly(methyl methacrylate-*b*-styrene) copolymers synthesized by Nitroxide Mediated Polymerization (NMP) and a significant amount of residual homopolymer was detected [38]. However, LC-LCD has not been applied to highly polar and charged polymers for which finding the correct solvent conditions would be particularly challenging.

An alternative method for quantifying the unintended homopolymers of polar and charged polymers is free solution Capillary Electrophoresis (CE), or capillary zone electrophoresis. Free solution CE can be used to separate a wide variety of compounds from sugars [39] to proteins [40]. Free solution CE separates compounds according to their charge to friction ratio. Free solution CE can separate poly(acrylic acid-*co*-diallyldimethylammonium chloride) from residual poly(diallyldimethyl ammonium chloride), PDADMAC, homopolymer and from its monomers [41]. The amount of unreacted poly(acrylic acid), PAA, in an amphiphilic block copolymer has also been previously determined by free solution CE [42,43]. DNA has similarly been separated from some of its block copolymers by free solution CE, the method being called End Labeled Free Solution Electrophoresis (ELFSE), which involved attaching a large uncharged protein or synthetic polymer (second block) to the end of DNA (first block) causing a change in the overall charge to friction ratio [44]. The additional friction resulted in separation of the free DNA (residual homopolymer) from the labeled DNA (copolymer) [45]. This is because in the case of polyelectrolytes, when the chain reaches a certain size, the electrophoretic mobility is independent of molar mass, as shown for poly(styrene sulfonate) [46], single and double stranded DNA [47,48] and PAA [49]. The absence of separation according to molar mass is due the electrostatic friction outweighing the hydrodynamic friction [50,51]. This situation of electrophoretic mobility being independent of molar mass is analogous to the 'critical conditions' observed in LC-CC. Free solution CE can separate with different modes, in the case of free solution CE of large polyelectrolytes, we refer to it as CE in the Critical Conditions (CE-CC). The nomenclature is extensively discussed and defined in a recent review [52]. In this mode, separation of polymers can occur according to structure or to end groups. Previous work has already used CE-CC to separate polyacrylates according to branching [53], statistical copolymers such as chitosan and gellan gum by their composition (degree of acetylation) and conformation [54,55] as recently reviewed [52].

Herein we present a method for the quantification of parent homopolymers and thus an assessment of the purity of complex charged block copolymers exhibiting either a weak anionic block (PAA) or a cationic block (PAPTAC). The capabilities of SEC and CE-CC were compared and assessed to quantify homopolymers in block copolymer samples. Poly(acrylic acid-*b*-acrylamide) - P(AA-*b*-AM) - samples spiked with PAA were used to develop this method since P(AA-*b*-AM) samples with minimal residual homopolymer could be synthesized. This method was then applied to cationic P(APTAC-*b*-NIPAM) to quantify the amount of parent PAPTAC and PNIPAM homopolymers.

2. Material and Methods

2.1. Materials

Acrylic acid (AA, 98 %), (3-acrylamidopropyl)trimethylammonium chloride solution (APTAC, 75 % wt in water) and *N*-isopropylacrylamide (NIPAM, 97 %) were purchased from Sigma Aldrich. Acrylamide (AM, 50 % wt in water stabilized with 100 ppm MEHQ, *p*-methoxyphenol) was obtained from SNF. *O*-ethyl-S-(1-methoxycarbonyl)ethyldithiocarbonate agent (Rhodixan A1) was obtained from Solvay. 4,4'-azobis(4-cyanopentanoic)acid (ACVA, >98 %) was obtained from Fluka. 2,2'-Azobis(2-methylpropionamidine)dihydrochloride (V-50, 98 %), ammonium persulfate (APS, 98+ %), sodium formaldehyde sulfoxylate dihydrate (NAFS, 98 %) and L(+)-ascorbic acid (AsAc, 99 %) were obtained from Acros Organics. Ethanol and deionized water were used as solvent for polymer syntheses. Diethyl ether was used to purify the diblock copolymers.

Water of MilliQ quality (Millipore, Bedford, MA, USA) was used in SECs and Free solution CE. Agilent fused-silica capillaries with an extended light path detection window (50 μm i.d., 360 μm o.d.) and poly(ethylene oxide) coated (named "WAX") and fluorocarbon coated (named "FC") fused-silica capillaries (50 μm i.d., 360 μm o.d.) were purchased from Pacific Laboratory Products (Australia). Sodium hydroxide (98 %) pellets were obtained from Univar (Ingleburn, NSW, Australia). Boric acid (≥ 98 %) and sodium azide (99 %) were purchased from BDH AnalaR, Merck Pty Limited. Phosphoric acid (≥ 99.0 %) was purchased from Fusions (Homebush, Australia). Hexaamminecobalt(III) chloride (≥ 99.5 %), absolute ethanol, dimethyl sulfoxide (DMSO, >99 %), disodium hydrogen phosphate (99 %), ammonium nitrate (≥ 99.0 %), acetonitrile (HPLC grade, ≥ 99.99 %), and poly(diallyldimethyl ammonium chloride) (PDADMAC, 'average M_w ' 200 000-350 000, 20 wt. % in H_2O) were supplied by Sigma Chemical company. Pullulan standards were purchased from PSS (Mainz, Germany).

2.2. Polymer samples

All polymers were synthesized by Reversible-Addition Fragmentation chain Transfer/Macromolecular Design via Interchange of Xanthates (RAFT/MADIX). PAA homopolymers were synthesized as in [5]. The synthesis of the P(AA-*b*-AM) block copolymers is shown in supporting information. PNIPAM homopolymer was synthesized as in [25], with the procedure detailed in supporting information.

PAPTAC homopolymers and P(APTAC-*b*-NIPAM) block copolymers were typically synthesized as follows: APTAC solution (10.7 g) corresponding to 7.98 g of pure APTAC (38.6 mmol), Rhodixan A1 (0.24 g, 1.15 mmol), V-50 (0.06 g, 0.19 mmol), ethanol (1.81 g) and deionized water (7.39 g) were placed in a 50 mL two neck round bottom flask. The solution was degassed by argon bubbling for 30 min before heating at 60 $^{\circ}\text{C}$ for 3 h. Conversion was monitored using ^1H NMR, on a Bruker Avance 300 taking aliquots until it reached 100 %. The mixture was evaporated under reduced pressure to remove ethanol and then the solid content in the solution was determined by gravimetry (50.7 %) in order to use this PAPTAC solution as macroMADIX agent for the synthesis of the diblock copolymer. PAPTAC solution (11.8 g, 0.80 mmol), NIPAM (9.77 g, 86.0 mmol), APS (0.20 g, 0.90 mmol) and deionized water (15.8 g) were placed in a 100 mL round bottom flask. The solution was degassed for 30 min by argon bubbling. AsAc (0.15 g, 0.86 mmol) was separately dissolved in 2.00 g of water and degassed for 30 min the same way. Then AsAc was added to the mixture to initiate the reaction that was left for 24 h until completion. The solution was evaporated under reduced pressure and the residue was dissolved in ethanol before precipitating in diethyl ether to yield a white powder.

Table 1. Homopolymers and block copolymers investigated in this study.

Sample Code	Polymer	Theoretical molar mass ($\text{g}\cdot\text{mol}^{-1}$)
PAA2k	PAA	2 000
PAA2kPAM10k	P(AA- <i>b</i> -AM)	2 000- <i>b</i> -10 000
PAA10k	PAA	10 000
PAA10kPAM10k	P(AA- <i>b</i> -AM)	10 000- <i>b</i> -10 000
PAPTAC5k	PAPTAC	5 000

PNIPAM5k	PNIPAM	5 000
PAPTAC5kPNIPAM5k	P(APTAC- <i>b</i> -NIPAM)	5 000- <i>b</i> -5 000
PAPTAC2k	PAPTAC	2 000
PAPTAC2kPNIPAM8k	P(APTAC- <i>b</i> -NIPAM)	2 000- <i>b</i> -8 000
PAPTAC2kPNIPAM3k	P(APTAC- <i>b</i> -NIPAM)	2 000- <i>b</i> -3 000
PAPTAC1k	PAPTAC	1 000
PAPTAC1kPNIPAM9k	P(APTAC- <i>b</i> -NIPAM)	1 000- <i>b</i> -9 000
PAPTAC1kPNIPAM3k	P(APTAC- <i>b</i> -NIPAM)	1 000- <i>b</i> -3 000
PAPTAC3k	PAPTAC	3 000
PAPTAC3kPNIPAM3k	P(APTAC- <i>b</i> -NIPAM)	3 000- <i>b</i> -3 000
PAPTAC6k	PAPTAC	6 000
PAPTAC6kPNIPAM3k	P(APTAC- <i>b</i> -NIPAM)	6 000- <i>b</i> -3 000

2.3. SECs

Two different SEC set ups were used. SEC of PAA homopolymers and block copolymers was conducted with a Malvern Triple Detector Array (TDA) SEC Model 305 with an online degasser, pump and a manual injector. They were eluted through one SEC SUPREMA pre-column (particle size of 5 μm) then through three SEC SUPREMA columns (two 1000 \AA , particle size of 5 μm and one 30 \AA , particle size of 5 μm) from Polymer Standards Service (PSS, Mainz) with an aqueous eluent containing 0.1 $\text{mol}\cdot\text{L}^{-1}$ Na_2HPO_4 and 200 ppm NaN_3 at 50 $^\circ\text{C}$ and 1 $\text{mL}\cdot\text{min}^{-1}$ flow rate. The TDA includes the following detectors: right-angle laser light scattering (RALLS) and 7° low angle laser light scattering (LALLS) at 670 nm, refractometer and viscometer. Data was treated using OmniSEC version 4.7.0 and was plotted using OriginPro 8.5. Injections of PAA homopolymers and block copolymers used ethylene glycol as a flow rate marker. All samples were filtered through 0.45 μm PES or PVDF membrane filter before injection. The system was calibrated using 10 pullulan standards ranging from 342 to 708 000 $\text{g}\cdot\text{mol}^{-1}$ (molar mass at the peak) with dispersity inferior to 1.27. The obtained calibration curve was fitted with a 4th order polynomial: $\log M = 142.9 - 20.76x + 1.184x^2 - 0.0301x^3 + 0.0002824x^4$ ($R^2=0.9994$), calibration curve shown in Fig. S-1.

SEC of PAPTAC homopolymers and block copolymers was conducted on an Agilent 1100 HPLC system with model 2424 refractive index and a DAWN HELEOS (Wyatt) light scattering detector. A guard column Shodex SB806-MT and two 8 mm*300 mm Shodex columns (SB 806 M HQ, 13 μm , and 802.5 HQ, 13 μm) were used with as eluent 1 M NH_4NO_3 solution of water/acetonitrile (80/20, w/w) containing 100 ppm of PDADMAC (flowrate 1 $\text{mL}\cdot\text{min}^{-1}$). All samples were filtered through 0.45 μm filter before injection. The data were recorded using Astra and plotted using OriginPro 8.5.

2.4. Capillary electrophoresis

Separations were performed on an Agilent 7100 (Agilent Technologies Waldbronn, Germany) with a Diode Array Detector (DAD) monitoring at 200 and 285 nm with 10 and 20 nm bandwidths, respectively. Buffers were sonicated for 5 min and filtered before use. All samples were injected hydrodynamically by applying 30 mbar of pressure for 5 s followed by the running buffer injected in the same manner. All separations were performed at 30 kV and 25 °C unless specified. Data was acquired using Chemstation A.10.01 and plotted, integrated and migration time was converted to electrophoretic mobility using OriginPro 8.5. Electrophoretic mobility was calculated as shown in supporting information. The peak areas were corrected by dividing them by the migration time at the relevant apex.

2.4.1 PAA based samples

PAA based samples were dissolved at 5 g L⁻¹ in 10 mM NaOH aqueous solution. 1 µL of DMSO was added to each 500 µL sample to mark the electro-osmotic flow (EOF). 110 mM sodium borate buffer (NB110, pH 9.2) was prepared as stated in reference [53]. All separations were carried out in NB110 buffer, with an extended light path fused-silica capillary with a total length of 60.6 cm (effective length 52.1 cm). The capillary was pre-treated prior to use by flushing for 10 min with 1 M NaOH, for 5 min with 0.1 M NaOH, for 5 min with water and for 5 min with NB110. Preconditioning between injections involved a 2 min flush with 1 M NaOH followed by a 5 min flush with NB110. After the last injection, the capillary was flushed for 1 min with 1 M NaOH, for 4 min with 0.1 M NaOH, for 10 min with water and for 10 min with air.

2.4.2 PAPTAC-based samples

PAPTAC-based samples were dissolved at 5 g L⁻¹ in 1 mM [Co(NH₃)₆]Cl₃ aqueous solution unless stated otherwise. 10 mM NaH₂PO₄ (PB10) was prepared by taking 1 M H₃PO₄ and diluting it to 0.5 M; the resulting solution was titrated to pH 2 with 1 M NaOH, and then diluted to 10 mM. Preconditioning involved a 20 min flush with 10 mM H₃PO₄ followed by a 5 min flush with ethanol and then 5 min PB10. All separations were carried out in PB10 buffer, in a 'WAX' capillary with a total length of 34.5 cm (effective length 28.0 cm) unless stated otherwise. After the last injection, the capillary was flushed for 20 min with 10 mM H₃PO₄, for 5 min with ethanol, for 20 min with water and for 10 min with air.

2.5 Pressure mobilization and pressure assisted free solution CE

All conditions were the same as those stated for PAPTAC based samples, except for the following. For pressure mobilization, samples were dissolved in PB10 and 50 mbar of pressure was applied instead of an electric field during the migration. For pressure assisted free solution CE, 50 mbar of internal pressure was applied in addition to the electric field during the separation.

3. Results and Discussion

3.1. Comparison of the separation of homopolymers and block copolymers by SEC and CE-CC

The separation of a PAA homopolymer from a P(AA-*b*-AM) block copolymer is easily achieved by CE-CC, using standard conditions in under 14 min (see Fig. 1B for PAA10kPAM10k) with a relative standard deviation (RSD) of the electrophoretic mobility at the peak maximum of PAA10k being 0.5 % (n=8). In contrast, when the same samples were analyzed by SEC, minimal separation of the homopolymer from the block copolymer was observed (Fig. 1A). Only a 0.6 % shift in elution time (calculated from the average at the peak maximum of the repeat injections) was detected for the block copolymer in SEC using established conditions for SEC of PAA and PAM [56-58]. In contrast Fig. 1B and 1C CE-CC led to a narrow PAA peak at a higher electrophoretic mobility and a broad P(AA-*b*-AM) at a lower electrophoretic mobility, which were resolved. The narrow PAA peak in CE-CC is due to the critical conditions, so the absence of separation by molar mass for PAA. The SEC chromatograms of the corresponding PAAs are broader than the pullulan standards one indicating the PAA has a molar mass distribution with a dispersity above 1.1 (Fig. S-2A). The complete separation of the block copolymer from the corresponding homopolymer in CE-CC was confirmed through spiking P(AA-*b*-AM) samples with the corresponding PAA homopolymer (Fig. 1). The narrow peak of PAA confirms that it is under critical conditions. PAA oligomers were also present in the PAA samples with small peaks around $3.5 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ (Fig. 1C and S-2 C) [49]. The lower electrophoretic mobility of P(AA-*b*-AM) compared to PAA indicates that the attached PAM neutral block is changing the overall charge to friction ratio by providing additional hydrodynamic friction but no charge [59]. In a controlled polymerization, this change in electrophoretic mobility can be used to confirm whether a homopolymer, used as a macroinitiator or macro-chain transfer agent, has been reinitiated and a block copolymer formed. The broadness of the block copolymer peak shows that there is a distribution of electrophoretic mobilities in the block copolymer sample, relating to the ratio of both block lengths in the sample. The block copolymer separation is the same as that which occurs in ELFSE where the electrophoretic mobility was linked to the ratio of the block lengths [59,60].

Insert Fig. 1

The success of a block copolymer synthesis is often probed through a shift in SEC elution time towards the higher molar masses [61-65]. The molar masses of block copolymers determined with SEC can be inaccurate due to the change of solvation properties between monomer units leading to local dispersity above 1 [10,66]. In the case of the symmetric block copolymer PAA10kPAM10k, SEC analysis fails to indicate if a block copolymer has been formed. Even if an additional method such as diffusion NMR (also named DOSY) indicated the presence of block copolymer [28], SEC does not allow the detection or quantification of any homopolymer contaminant (Fig. 1A). Therefore, it is possible to have a situation where a large amount of homopolymer is present that would be assumed to be block copolymer resulting in an impure sample with an incorrect molar mass determined. However, from CE-CC, a clear shift in migration time (Fig. 1B) and in electrophoretic mobility (Fig. 1C) is observed, providing evidence that a block copolymer was produced and whether any homopolymers are present (discussed below). Therefore, CE-CC can be used to qualitatively identify when a block copolymer is produced, as well as if any homopolymers are present, which cannot always be said for SEC, especially in the case of adsorbing cationic block copolymers. In addition, CE-CC has a lower running cost and higher throughput than chromatography based techniques [39] and does not require sample filtration[52].

3.2. Cationic copolymers: minimizing adsorption onto the capillary surface in CE-CC

SEC and CE-CC separations are now compared on more challenging cationic DHBCs. PAPTAC homopolymers did not initially elute from the column [67]. However, using the conditions developed for PDADMAC, the SEC of PAPTAC led to repeatable separations [19]. This procedure involves the addition of PDADMAC to the mobile phase, creating a positively charged coating on the stationary phase. These SEC conditions provide some indication of the size and dispersity of the PAPTAC homopolymer (Fig. S-3 and S-4). At the lowest elution volumes a signal is detected on the light scattering signal only, indicating the presence of aggregates (in minute amounts). Ion exclusion and finding appropriate standards for conventional calibration are also still a concern. In the same conditions P(APTAC-*b*-NIPAM) block copolymer yielded similar chromatograms to that of the corresponding homopolymer but with a lower signal intensity at identical sample injection concentrations (Fig. S-3 and S-4). It may seem that no block copolymer was formed; however, it is more likely that the PAPTAC homopolymer present in the block copolymer sample is eluting while the block copolymer is strongly adsorbing in the column and not eluting out of the column or only as a tail. Therefore CE-CC was used to investigate these block copolymer samples.

Cationic polymers have a tendency to adsorb onto surfaces with a negative zeta potential. PAPTAC injected in a fused-silica capillary led to a very weak signal even at pH 2 likely because of strong adsorption on the glass surface (Fig. S-5). To prevent the adsorption, capillaries with a fluorocarbon (FC) coating or a poly(ethylene oxide) (PEO) coating were used. Both coatings limited the adsorption of PAPTAC as indicated by the signal intensity being orders of magnitude higher than with uncoated fused-silica capillaries (Fig. S-5 and S-6). Joule heating was also found to not be a concern for these (thermoresponsive) polymers (supporting information). When using a FC coating the results were not repeatable likely due the adsorption attributed to hydrophobic interactions between the PNIPAM block and the hydrophobic (fluorinated) coating (Fig. S-6). The PEO coating yielded both sensitivity and repeatability with the RSD of the electrophoretic mobility of the homopolymer at the peak maximum being 0.6 % (n=7) – similar to PAA – for PAPTAC2k and 2.4 % (n=3) for PAPTAC5kPNIPAM5k.

Pressure mobilization was used to identify if the analyte interacted with the capillary surface. If no interaction is present then a Gaussian peak (or superimposed Gaussian peaks) is (are) observed with the cause of peak broadening being the diffusion of the analyte [68]. It was observed that a small fraction of PAPTAC was interacting with the capillary surface (Fig. 2). It is suspected to be due to an uneven distribution of the PEO coating, some areas of the capillary wall may have little or no coating allowing for the PAPTAC to adsorb. However, at least 75 % of the homopolymer was unaffected and the remaining homopolymer was present in peaks which returned to the baseline shortly after the main peak and thus no permanent adsorption was observed. Similar results were also observed for the PNIPAM with 46 % of it being unaffected but tailing peaks were broader and migrated further than that shown for PAPTAC (Fig. 2). The block copolymer P(APTAC-*b*-NIPAM) showed permanent adsorption with the peak tailing giving a consistent elevated baseline (Fig. 2). The adsorption appears to be mainly due to the hydrophobic interactions of the PNIPAM with the capillary surface. The improved repeatability using a PEO coating instead of a FC coating could be attributed to PEO being a more polar coating. Since the block copolymer shows the strongest adsorption both the ionic interactions of PAPTAC and the hydrophobic interactions of PNIPAM with the coating were contributing to the adsorption. The adsorption of the homopolymers would likely result in some peak tailing. To remove the adsorbed polymer the capillary was flushed with 10 mM H₃PO₄ for 20

min and ethanol for 5 min during the preconditioning of each injection. This prevented the adsorption from impacting subsequent injections.

Insert Fig. 2

The PAPTAC homopolymers in CE-CC showed well defined peaks with little effects of adsorption. (Short) oligomers were detected in the majority of the PAPTAC samples as indicated by the small peaks at lower electrophoretic mobilities (Fig. 3) [46,49]. For the lower molar mass samples such as PAPTAC1k and PAPTAC2k the oligomers made approximately 13 % (w/w) of the sample, while for all other samples it represented no more than 1 % (w/w) of the sample. This indicates that the majority of the PAPTAC samples contained mainly chain lengths longer than a few monomer units.

Although P(APTAC-*b*-NIPAM) is adsorbing, as clearly shown by the elevated baseline in Fig. 3, a prominent PAPTAC signal was noticeable in all samples. This enabled the quantification of residual PAPTAC in the P(APTAC-*b*-NIPAM) samples. Furthermore, in the case of PAPTAC1kPNIPAM9k the oligomers in the PAPTAC homopolymer were detected in the block copolymer in minute amounts (Fig. 3). These oligomers could be removed by dialysis if necessary using CE-CC to assess the success of the purification.

Insert Fig. 3

To further combat the adsorption of the block copolymer, pressure assisted CE-CC was used. This technique uses the same conditions as regular CE but with the addition of internal pressure during the separation so that the species migrate due to both the electric field and pressure (the separation still being due only to the electric field). Pressure assisted CE has previously been used to aid in the separation of polyelectrolytes [69]. Pressure assisted CE-CC helps migrate the adsorbed block copolymer along the capillary enabling its detection. Furthermore with a PEO coating at pH 2 there is no measureable EOF, meaning the detection of neutral species is very difficult using free solution CE, but with the aid of pressure the presence of neutral species in the sample can be more easily detected. The pressure assisted CE-CC of a mixture of PAPTAC and PNIPAM homopolymers produced well separated and relatively narrow peaks (Fig. 4 and Fig. S-7). The pressure assisted CE-CC of the P(APTAC-*b*-NIPAM) samples detected some species migrating at the same speed as the PAPTAC homopolymer, demonstrating the presence of residual PAPTAC in the samples. This was then followed by a broad peak which mainly migrated slower than the PAPTAC homopolymer but mainly faster than the neutral species showing that a block copolymer was produced and makes up the majority of the sample (Fig. 4). In the P(APTAC-*b*-NIPAM) samples, a shoulder was observed which migrates at the same time as the PNIPAM homopolymer showing that some PNIPAM homopolymer is also present in the sample. Therefore in a single run, both parent homopolymers and the block copolymer can be detected. The identification of both homopolymers has been shown for block copolymers produced by anionic polymerization [35] and RAFT/MADIX [43]. In the case of RAFT/MADIX block copolymers this is likely due to the need of an external source of radicals for initiating the polymerization. Therefore, the formation of a secondary homopolymer would be less likely with living anionic polymerization or NMP which do not require an external initiator for the second block[38]. The presence of one or two parent homopolymers in the sample would influence the properties of the final product [37,42,70].

Insert Fig. 4

354

355 3.3. Homopolymer quantification by CE-CC

356 Assessing how accurately a separation method can quantify a polymer is rarely demonstrated in the
357 literature. The group of Cottet however proved CE a reliable method to determine the cationic
358 PDADMAC homopolymer within PAA-PDADMAC statistical copolymers [41]. To accurately quantify
359 the amount of the PAA homopolymer in this work, a calibration curve between the homopolymer
360 peak area and the concentration was established (Table 2). The alternative would be to compare the
361 area of homopolymer and the block copolymer, however this is less accurate due to the different
362 absorptivity of the different monomer units. UV detection in CE-CC showed sufficient linearity with
363 all samples having a regression coefficient (R^2) greater than 0.979. The end group was shown to
364 affect the calibration curve since the same homopolymers with different molar masses produced
365 different calibration curves. Therefore the precursor homopolymers were used to quantify
366 homopolymer in the corresponding copolymers to prevent bias from the end group.

367

Insert Table 2

368 CE-CC has the advantage of using a DAD unlike LC-CC and LC-LCD which usually require an
369 Evaporative Light Scattering Detector (ELSD) due to the use of organic solvent and gradients.
370 Although the linearity of the UV detector response for the PAA homopolymers is not as high as
371 wanted, establishing linearity with an ELSD detector can be equally as difficult and in some cases
372 there is no linear response between the detector signal and concentration of the polymer [71,72], as
373 the molar mass of the polymer also influences the ELSD response. The imperfect linearity of the
374 detector response for the PAA homopolymers is likely due to variations in injection volumes
375 between injections since the samples were injected hydrodynamically and the pressure may vary
376 from injection to injection. At higher concentrations the PAA homopolymers also change the
377 solution viscosity which can influence the injection volume. To account for these differences
378 between injections the internal standard, hexaaminecobalt(III) chloride, was used for the PAPTAC
379 based polymers, which resulted in higher linearity than for the PAA homopolymers. The internal
380 standard migrated before the PAPTAC homopolymer, had strong molar absorptivity and showed no
381 sign of interaction with capillary coating or polymers as shown previously with chitosan [73]. In the
382 case of polymer lacking chromophore, indirect UV detection and conductivity detection both were
383 proved successful at quantifying PDADMAC with even lower LOD than the direct UV detection used
384 in this work [41].

385 The recovery of LC based techniques has been shown to be a problem [36,72,74]. The recovery of
386 CE-CC is quantitative in the case of PAA (Table 2). Recoveries of 113 % and 95 % are considered
387 quantitative within experimental error. The LOD of the PAPTAC homopolymers was found to be
388 significantly lower than that of the PAA homopolymers likely due to the higher UV absorption
389 coefficient of the amide in the APTAC unit compared to the carboxylate in the AA unit. Minute
390 amounts of PAPTAC homopolymer can be detected in block copolymer samples.

391

392 3.4. Quantification of parent homopolymers in block copolymer samples

Even when trying to prevent the presence of homopolymers in a block copolymer sample, there is inevitably the formation of 'dead chains' which form residual homopolymer in the block copolymer sample [38,75]. Using the equation determined above (Table 2), 2 % (w/w) of PAA homopolymer was found in PAA10kPAM10k while none was detected in PAA2kPAM10k (Table 3). Assuming ideal polymerization kinetics and that all the initiator and MADIX agent reacted, the ratio of initiator to MADIX agent reflects the maximum possible fraction of 'dead' PAA homopolymer chains, 7.5 (mol/mol)% in this work for PAA10kPAM10k. Using the value of the theoretical molar mass, the fraction of dead PAA chains was estimated by CE-CC of PAA10kPAM10k to be 4 (mol/mol)%, thus 96 % of the macro-chain transfer agent was converted to a block copolymer. The discrepancy may be due to the difference between values of the actual and theoretical molar masses (the non-ideal kinetics of polymerization of acrylic acid [76] leads to branching [77]) or to incomplete decomposition of the initiator. In the case of PAA2kPAM10k, given the theoretical maximal number of 'dead chains' the w/w % of PAA homopolymer would be below the LOD at the given injection concentration. Detection of PAM homopolymer is also possible with CE-CC as long as no EOF marker is used since as a neutral species it would migrate with the EOF.

Insert Table 3

Although there was incomplete separation of the PAPTAC homopolymer from the P(APTAC-*b*-NIPAM) block copolymer, it can be quantified with a RSD below 5 % (Table 3). A significant amount of PAPTAC homopolymer was found in all P(APTAC-*b*-NIPAM) samples although the amount was found to vary greatly between samples (Table 3). For PAPTAC5kPNIPAM5k it was found that around 30 % of the homopolymer chains did not reinitiate. Such information about the amount of homopolymer chains present in the crude block copolymer sample and can help to indicate the reactivity of the end group and whether the synthetic conditions used favor the formation of a block copolymer. The end group shows absorbance at 285 nm independent of the monomer units. However, the sensitivity of the detection of the xanthate end group was insufficient to determine whether the residual homopolymer had the end group present (i.e., "livingness" could not be assessed in this work).

The amount of PNIPAM present in the P(APTAC-*b*-NIPAM) samples was estimated by adjusting the maximum of the PNIPAM peak to the height of the shoulder. It was then estimated that the P(APTAC-*b*-NIPAM) samples likely contained between 1 and 3 % (w/w) of PNIPAM homopolymer (Fig. 4). Therefore, the P(APTAC-*b*-NIPAM) samples contain a majority of block copolymer but also significant amounts of both homopolymers.

4. Conclusions

Determining the purity of block copolymers is important for having a complete characterization of the sample. CE-CC provides a fast and cost effective means of determining the amount of homopolymers present in a block copolymer in a single injection as well as qualitatively identifying the formation of a block copolymer. The purity of the block copolymer (and of the materials they are incorporated in) as well as blocking efficiency can be assessed by CE-CC, which is not possible with SEC and NMR. In the case of a P(AA-*b*-AM) block copolymer, CE-CC was shown to easily identify the formation of a block copolymer and measure the amount of residual PAA. SEC may be able to assess

the distribution of molar masses in the initial homopolymer, however, SEC was unable to identify the presence of any homopolymers in the block copolymer and in this case it was difficult to show that a block copolymer had formed.

CE-CC uses a background electrolyte with a single component (no mixed-solvent) contrary to what is usually used in LC-CC (temperature can also be varied in LC to reach the CC) and adsorption events are limited in CE-CC since it is based on free solution CE (no stationary phase). This makes CE-CC an excellent method to characterize highly adsorbing polymers such as P(APTAC-*b*-NIPAM) and other cationic DHBCs. CE-CC was found to have linearity and recovery equal if not greater than that of LC-CC for homopolymers. SEC of P(APTAC-*b*-NIPAM) block copolymers was found not to be possible, while CE-CC and pressure assisted CE-CC were able to show that the majority of the sample was a block copolymer but significant amounts of both parent homopolymers were present in the sample. With a means to quantify homopolymers in a block copolymer a relationship between the purity and the properties can be established. Furthermore, kinetic and 'livingness' studies of the polymerization are possible since the quantity of both the block copolymer and homopolymers can be determined. The effectiveness of purification techniques such as dialysis and precipitation can also be assessed by the use of CE-CC.

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672 Figure captions:

673 Fig. 1. Separation of PAA10k homopolymer (blue) from PAA10kPAM10k copolymer (red) using (A)
674 SEC and (B, C) CE-CC. Dashed lines in A show repeat chromatograms. The black line in B and C is the
675 electropherogram of PAA10kPAM10k spiked with PAA10k. CE-CC electropherograms are shown as a
676 function of migration time (B) and of electrophoretic mobility (C). The injection concentrations were
677 2 g L^{-1} each for SEC and 5 g L^{-1} for CE-CC (except for the mixture of PAA10k and PAA10kPAM10k, for
678 which 5 g L^{-1} of each component was injected).

679 Fig. 2. Pressure mobilization of PAPTAC5k (black solid line), PNIPAM5k (red dashed line) and
680 PAPTAC5kPNIPAM5k (blue dashed-dotted line) through a 74.8 cm PEO coated capillary. Injection
681 concentration of each polymer was 5 g L^{-1} .

682 Fig. 3. CE-CC electropherograms of PAPTAC1k homopolymer (red) and PAPTAC1kPNIPAM9k block
683 copolymer (black). The homopolymer and the internal standard (labeled as PH and IS respectively)
684 have mobilities between 4 and $5 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$, and between 6 and $9 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$, respectively.
685 Oligomer peaks (labeled as PO) occur around $2 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ as shown in the insert. The injection
686 concentrations were 0.625 g L^{-1} each for PAPTAC1k and 5 g L^{-1} for PAPTAC1kPNIPAM9k.

687 Fig. 4. Pressure assisted CE-CC of a mixture of PAPTAC5k and PNIPAM5k homopolymers (red) and of
688 PAPTAC2kPNIPAM8k block copolymer (black, with repeat shown as dashed line). The mixture of
689 homopolymers (red) indicate the migration time of the individual homopolymers in the critical
690 conditions. The separation of another copolymer PATA5kPNIPAM5k is presented on Fig. S-7.
691 Injection concentration of each individual polymer was 5 g L^{-1} . Internal standard occurs at 0.6 min.

Figure 1

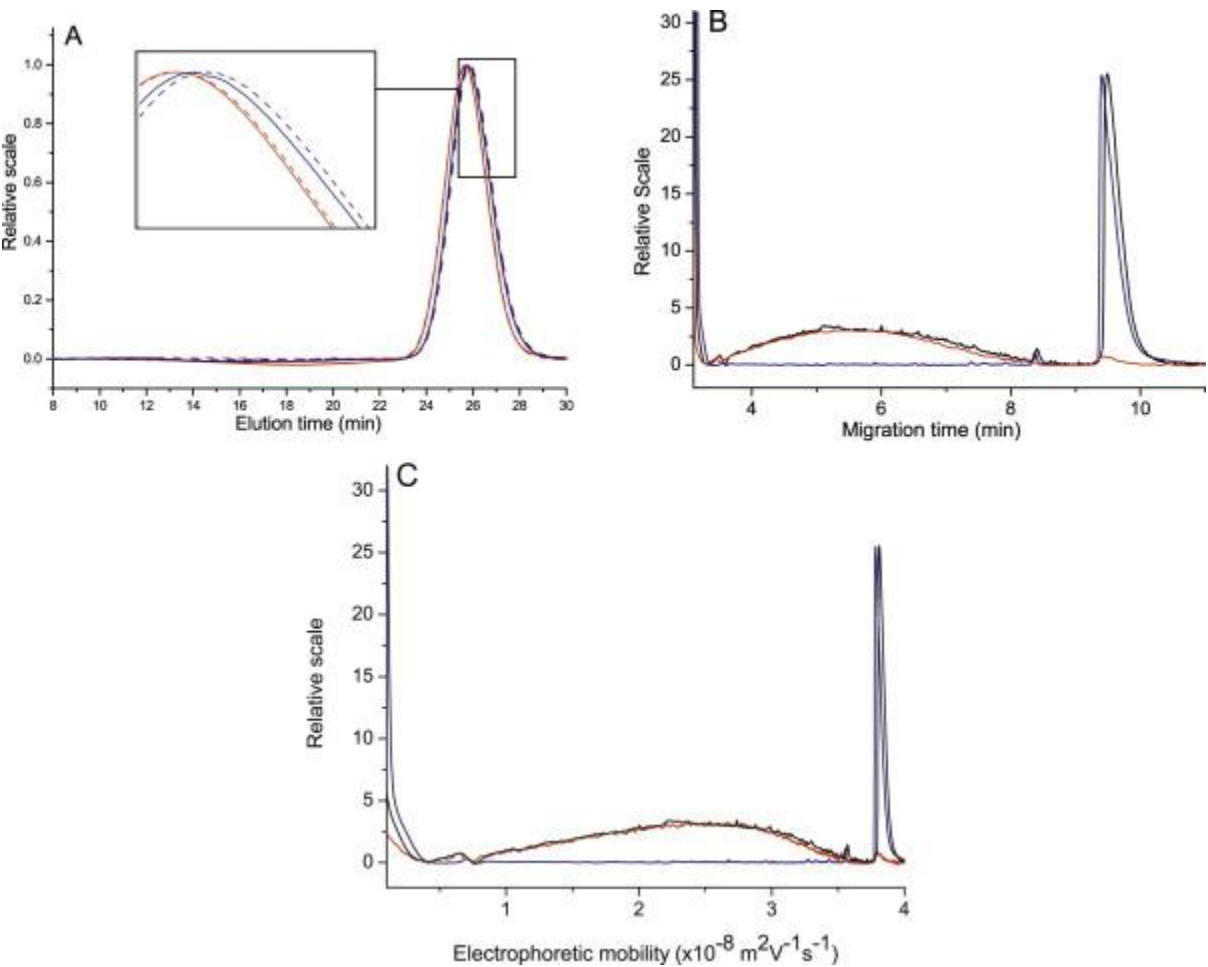


Figure 2

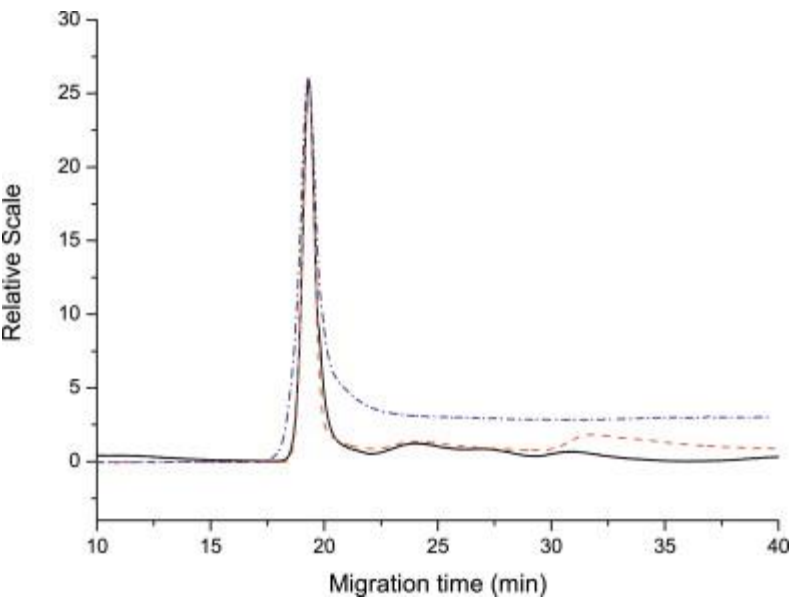


Figure 3

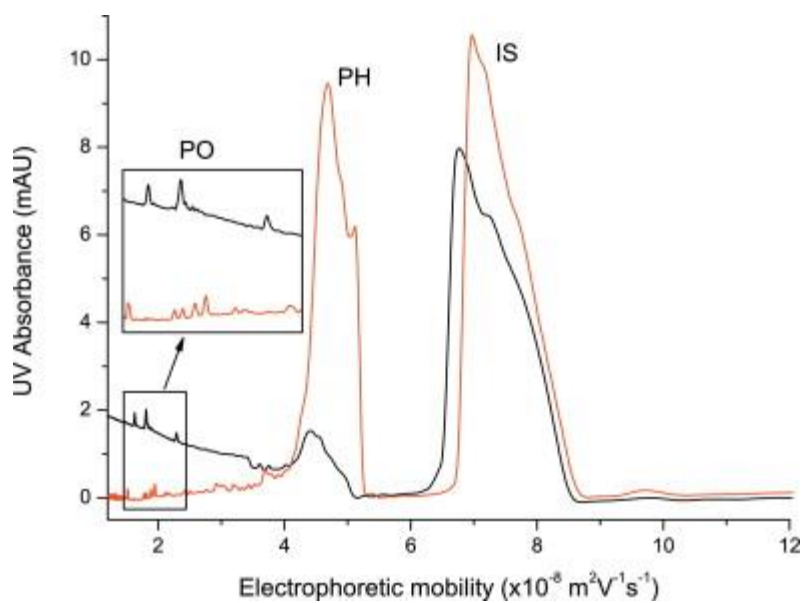
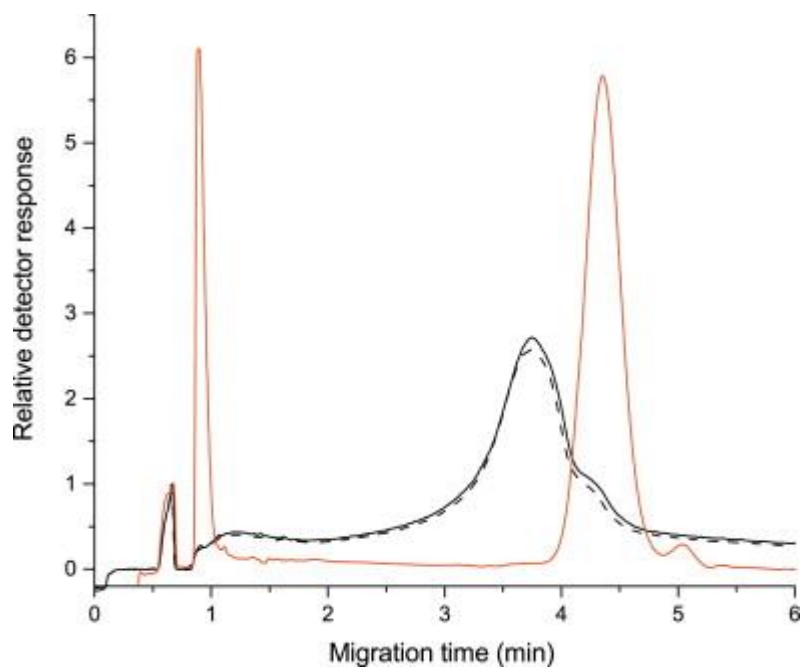


Figure 4



Purity of double hydrophilic block copolymers revealed by capillary electrophoresis in the critical conditions

SUPPORTING INFORMATION

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Polymer synthesis

Synthesis of PAA, procedure for PAA2k:

In a Schlenk flask, a solution of AA (7.00 g, 97.0 mmol), Rhodixan A1 (0.81 g, 3.90 mmol), ACVA (45.5 mg, 0.16 mmol), ethanol (1.92 g), and distilled water (8.00 g) was degassed by bubbling argon for 30 min before heating at 60 °C for 4.5 h. Conversion was monitored by ¹H NMR spectroscopy of aliquots until the completion (>99 %) of the reaction. Ethanol was evaporated under reduced pressure. Finally the aqueous solution of PAA was freeze dried to yield a white powder.

*Synthesis of the P(AA-*b*-AM) diblock copolymer, procedure for PAA2kPAM10k:*

In a Schlenk flask, a solution of PAA2k (0.81 g, 0.40 mmol), AM (8.00 g, 56.0 mmol) and water (6.50 g) was degassed by bubbling argon for 30 min. Then 2 mL of each solution (0.06 % w/w) of APS and NAFS were added simultaneously to the mixture at 10 °C. After 24 h, the reaction was complete and the solution was freeze dried to yield a white polymer powder.

Synthesis of PNIPAM, procedure for PNIPAM5k:

NIPAM (2.19 g, 19.3 mmol), APS (39.6 mg, 0.17 mmol), distilled water (3.97 g) and PDMA-XA1 (0.55 g, 0.49 mmol) macroinitiator whose synthesis is described in [E. Read, A. Guinaudeau, D. James Wilson, A. Cadix, F. Violleau, M. Destarac, Polymer Chemistry, 5 (2014) 2202-2207], were placed in a two-neck round-bottom flask and thermostated at 25°C. The solution was degassed for 30 min by argon bubbling. AsAc (35.6 mg, 0.17 mmol) was separately dissolved in 2.00 g of water and degassed for 30 min in the same manner. Then AsAc was added to the mixture under a stream of argon to initiate the reaction that was left for 24 h.

SEC

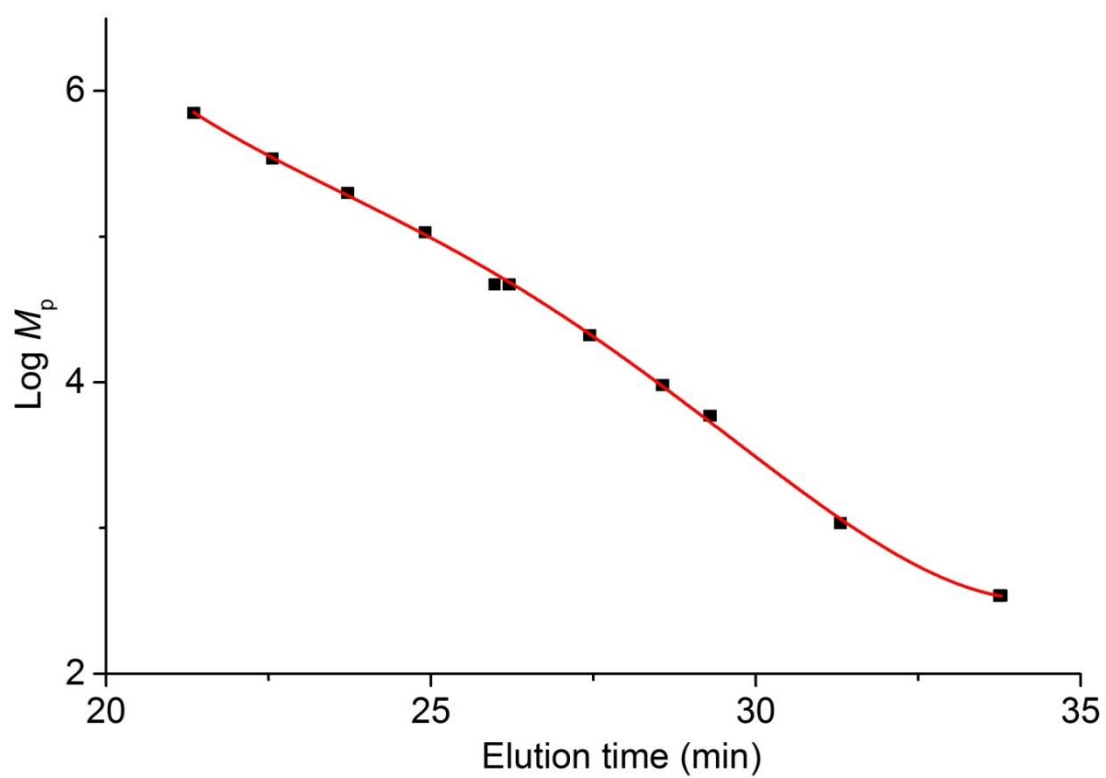


Fig. S-1. Calibration curve of the pullulan standards ranging from 342 to 708000 $\text{g}\cdot\text{mol}^{-1}$ (molar mass at the peak) fitted with a 4th order polynomial: $\log M = 142.9 - 20.76x + 1.184x^2 - 0.0301x^3 + 0.0002824x^4$ ($R^2=0.9994$).

Capillary electrophoresis

The electrophoretic mobility (μ) was determined using Equation S-1:

$$\mu = \frac{l_d l_t}{V} \left(\frac{1}{t_m} - \frac{1}{t_{\text{EOF}}} \right) \quad (\text{S-1})$$

where l_d is the length to the detection window (effective length), l_t is the total length of the capillary, V is the applied voltage, t_m is the migration time of the analyte and t_{EOF} is the migration time of the electro-osmotic flow (EOF) marker [P. Castignolles, M. Gaborieau, E.F. Hilder, E. Sprong, C.J. Ferguson, R.G. Gilbert, *Macromolecular Rapid Communications*, 27 (2006) 42-46].

In the case of the cationic polymers which were separated at pH 2, in a PEO coated capillary there is no measurable EOF within 60 min so the t_{EOF} term is negligible in the determination of electrophoretic mobility. The internal standard hexaminecobalt(III) chloride was used to correct for injection to injection variations to give a relative electrophoretic mobility. Therefore Equation S-2 was used to calculate the μ of the cationic polymers:

$$\mu = \frac{l_d l_t}{V} \left(\frac{1}{t_m} \cdot \frac{t_{\text{ref}}}{t_{\text{is}}} \right) \quad (\text{S-2})$$

Where t_{ref} is the average migration time of the peak maximum of the internal standard and t_{is} is the migration time at the peak maximum of the internal standard in the electropherogram. When no internal standard was used $t_{\text{ref}}/t_{\text{is}}$ was equal to 1.

The increase in temperature due to Joule heating inside the PEO coated capillaries was found to be 0.2 °C using the equations in [C.J. Evenhuis, R.M. Guijt, M. Macka, P.J. Marriott, P.R. Haddad, *Analytical Chemistry*, 78 (2006) 2684-2693]. Therefore there is little to no effect of Joule heating in the separation.

Results

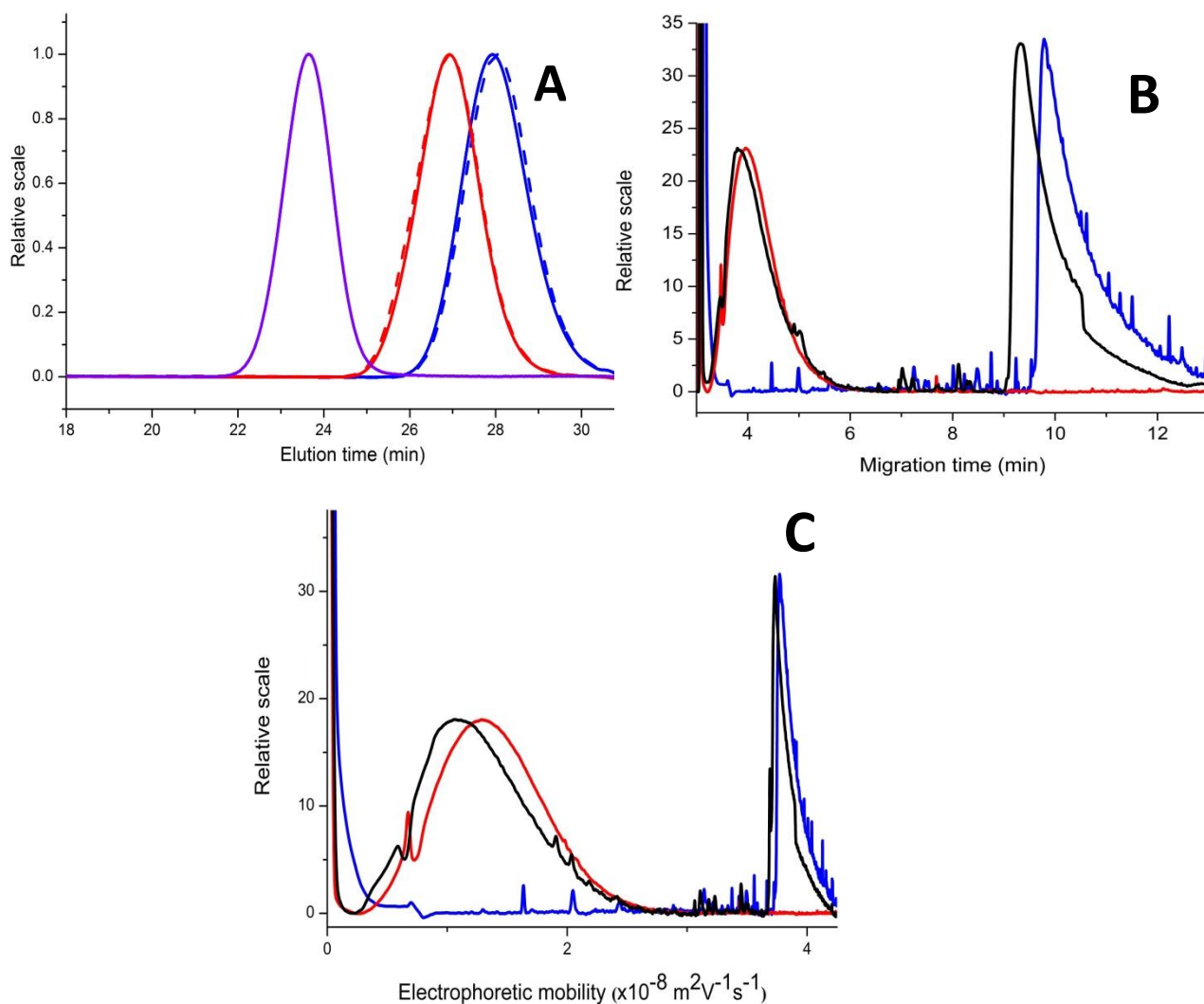


Fig. S-2. Separation of PAA2k homopolymer (blue) from PAA2kPAM10k copolymer (red) using (A) SEC and (B, C) CE-CC. Dashed lines in A show repeat chromatograms and the purple line shows the chromatogram of a pullulan standard with 200 000 g mol⁻¹ molar mass. The black line in B and C is the electropherogram of PAA2kPAM10k spiked with PAA2k. CE-CC electropherograms are shown as a function of migration time (B) and of electrophoretic mobility (C). The injection concentrations were 8 and 5 g L⁻¹ respectively for SEC and 5 g L⁻¹ for CE (except for the mixture of PAA2k and PAA2kPAM10k, for which 5 g L⁻¹ of each component was injected).

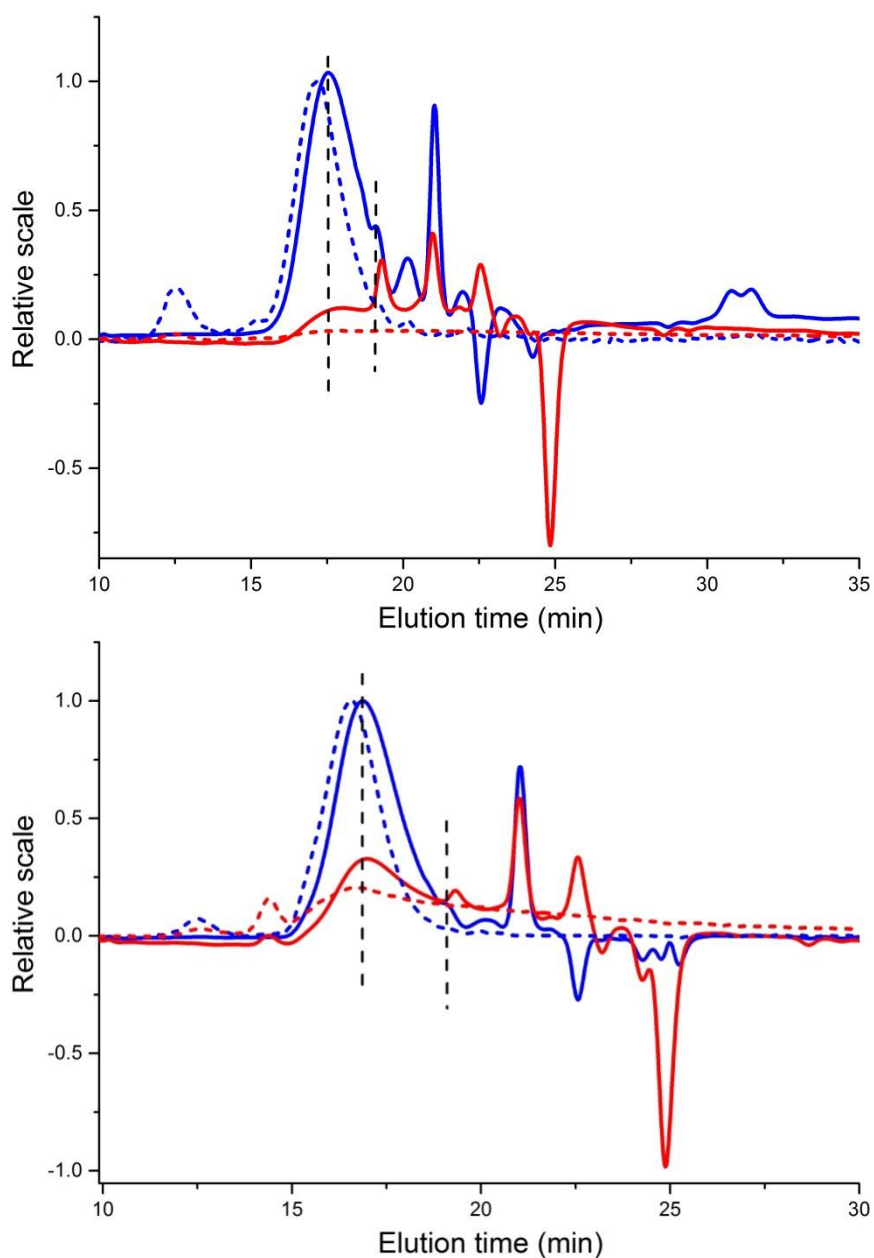


Fig. S-3. SEC chromatograms of PAPTAC homopolymers (blue) and P(APTAC-*b*-NIPAM) block copolymers (red). Figure S-3a (top) shows PAPTAC1k and PAPTAC1kPNIPAM3k while Figure S-3b (bottom) shows PAPTAC2k and PAPTAC2kPNIPAM3k. Solid and dashed lines represent signals obtained by refractive index and light scattering detectors respectively.

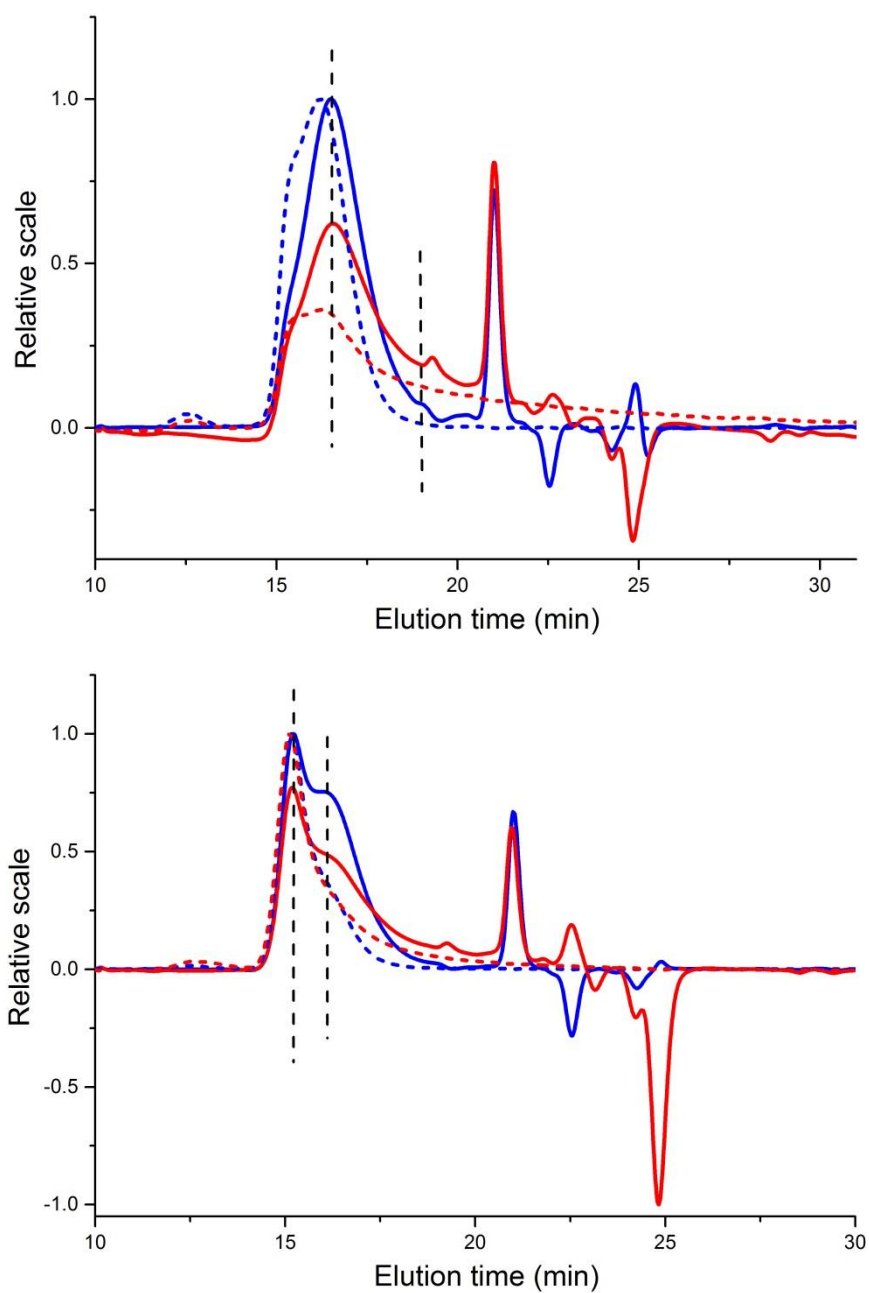


Fig. S-4 .SEC chromatograms of PAPTAC homopolymers (blue) and P(APTAC-*b*-NIPAM) block copolymers (red). Figure S-4a (top) shows PAPTAC3k and PAPTAC3kPNIPAM3k while Figure S-4b (bottom) shows PAPTAC6k and PAPTAC6kPNIPAM3k. Solid and dashed lines represent signals obtained by refractive index and light scattering detectors respectively.

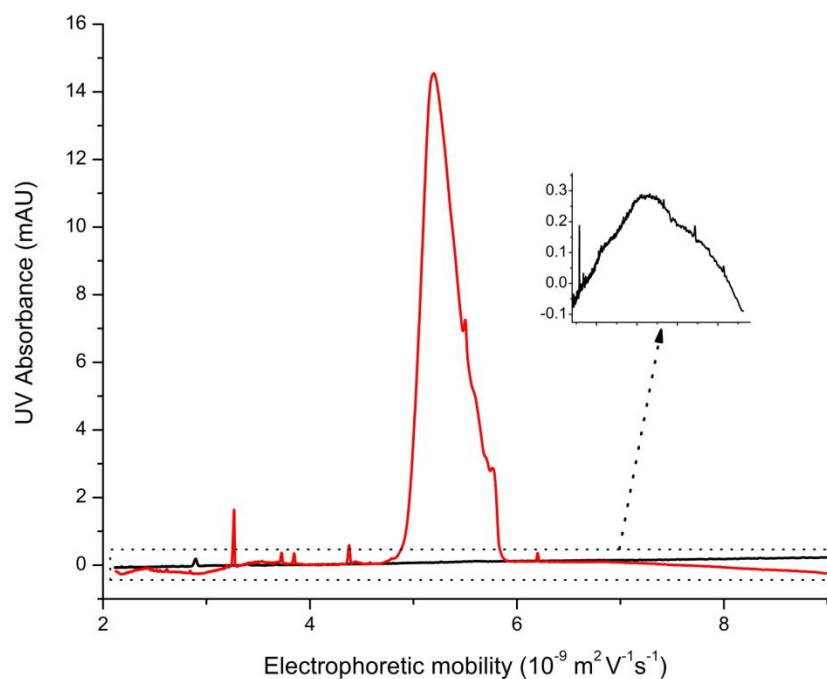


Fig. S-5. Separation of PAPTAC6k in an uncoated fused silica capillary (black) with 58.6 cm total length and a 83.8 cm PEO coated capillary (red). Both separations occurred at 13 °C with the samples dissolved in water to 8 mg mL⁻¹.

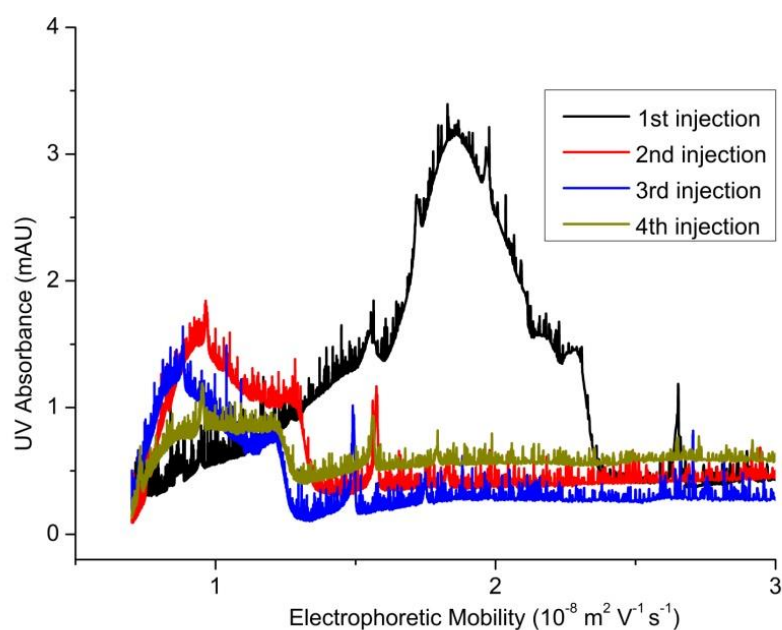


Fig. S-6. Repeatability of PAPTAC3kPNIPAM3k injected using an 83.8 cm FC coated capillary at 13°C with the samples dissolved in water to 7 mg mL⁻¹.

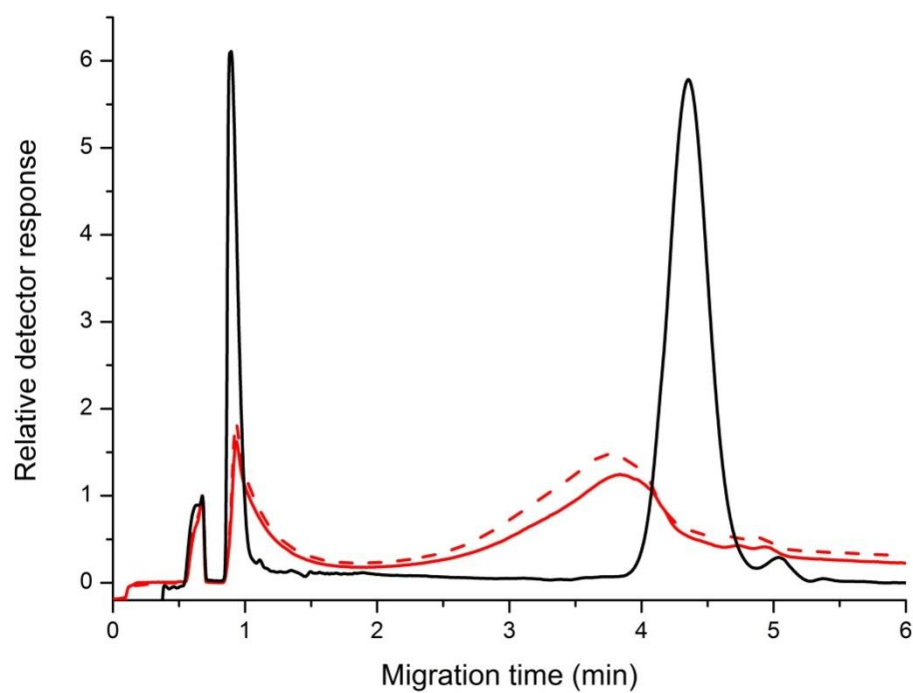


Fig. S-7. Pressure assisted CE-CC of a mixture of PAPTAC5k and PNIPAM5k homopolymers (black) and PAPTAC5kPNIPAM5k block copolymer (red). Dashed line is a repeat electropherogram. Injection concentration of each individual polymer was 5 g L⁻¹.