Characterisation of Double Hydrophilic Block Copolymers

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Masters (Honours)

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

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**Figure 49.** a) Electropherograms with UV signal and b) mobility distributions $w(\mu)$ for PAA2kPAM10k (red) and PAA10kPAM10k (blue). PAA peak present in PAA10kPAM10k between 3.7 and $4.0 \times 10^{-8}$ m² V⁻¹ s⁻¹. Injection concentration was 5 g L⁻¹ for each sample. PAA2kPAM10k was separated in an extended light path capillary with a total length of 60.6 cm (effective length 52.1 cm) while PAA10kPAM10k was separated in a standard fused silica capillary with a total length of 59.9 cm (effective length 51.4 cm).

**Figure 50.** Electropherograms of PAPTAC4kAA1k (green) and PAPTAC4kAA2k (purple) obtained by CE-CC. Dashed lines show repeat injections. Injection concentration of each individual polymer sample was 5 g L⁻¹.

**Figure 51.** Electropherograms of PAPTAC1kPNIPAM9k (purple), PAPTAC2kPNIPAM8k (blue), PAPTAC5kPNIPAM5k (red) and a blend of PAPTAC5k and PNIPAM5k (black) obtained by pressure assisted CE-CC. Dashed lines show repeat injections. Injection concentration of each individual polymer sample was 5 g L⁻¹ and 1 mM for hexaaminocobalt(III) chloride. Hexaaminocobalt(III) chloride migrates between 7 and $9 \times 10^{-8}$ m² V⁻¹ s⁻¹, PAPTAC homopolymer migrates between 5 and $6 \times 10^{-8}$ m² V⁻¹ s⁻¹.
and the insert shows the PNIPAM peak and the majority of the block copolymer peaks.

**Figure A-1.** NMR spectra of the MADIX agent Rhodixan A1. a) $^1$H NMR spectrum and b) $^{13}$C NMR spectrum.

**Figure A-2.** $^1$H NMR spectra of PAA10k in dioxane-$d_8$ using the inversion recovery pulse sequence. The different spectra correspond to different $\tau$ lengths: 0.693 s (black), 1.386 s (red) and 3.465 s (blue).

**Figure A-3.** Calibration curves of the concentration of homopolymer against the corrected peak area: a) PAA homopolymers (PAA2k, green and PAA10k, red), b) PAPTAC homopolymers (PAPTAC2k, blue and PAPTAC5k orange).

**Figure A-4.** Calibration curves of the concentration of homopolymer against the SNR. a) PAA homopolymers: PAA2k (black, $R^2 0.94$) and PAA10k (purple, $R^2 0.93$). b) PAPTAC homopolymers: PAPTAC2k (pink, $R^2 0.989$) and PAPTAC5k (dark blue, $R^2 0.998$).
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# List of Abbreviations

- **μ** Electrophoretic mobility
- **μ₀** Electrophoretic mobility in the critical conditions
- °C Degrees Celsius
- 1D One Dimensional
- 2D Two Dimensional
- \([\eta]\) Intrinsic viscosity
- \(\xi\) Chemical charge density
- \(\alpha\) Rescaling factor
- \(A_0\) Amount of RAFT/MADIX agent
- **ACVA** 4,4′azobis(4-cyanopentanoic) acid
- APS Ammonium persulfate
- **AsAc** L(+)-ascorbic acid
- **ATRP** Atom Transfer Radical Polymerisation
- BGE Background Electrolyte
- \(c\) Concentration
- \(C\) Composition
- \(C_n\) Number average Composition
- \(C_w\) Weight average Composition
- CE Capillary Electrophoresis
- **CE-CC** Capillary Electrophoresis in the Critical Conditions
- COSY COrrelation SpectroscoPY
- **CRYSTAF** Crystalisation Analysis Fractionation
- **DHBC** Double Hydrophilic Block Copolymer
- \(D\) Dispersity
- **DAD** Diode Array Detector
- **DAM** Dispersity of Apparent Mobility Distribution
- **DB** Degree of Branching
- **DC** Dispersity of Composition Distribution
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>De</td>
<td>Amount of Dead chains</td>
</tr>
<tr>
<td>DEM</td>
<td>Dispersity of Electrophoretic Mobility Distribution</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarisation Transfer</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic Light Scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ε</td>
<td>Molar absorptivity coefficient</td>
</tr>
<tr>
<td>ELFSE</td>
<td>End Labelled Free Solution Electrophoresis</td>
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<tr>
<td>EOF</td>
<td>Electro-Osmotic Flow</td>
</tr>
<tr>
<td>EST</td>
<td>Estimates</td>
</tr>
<tr>
<td>ESI MS</td>
<td>Electrospray Ionisation Mass Spectrometry</td>
</tr>
<tr>
<td>ELSD</td>
<td>Evaporative Light Scattering Detector</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
</tr>
<tr>
<td>GN</td>
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<tr>
<td>GPC</td>
<td>Gel Permeation Chromatography</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>HMQC</td>
<td>Heteronuclear Multiple-Quantum Correlation spectroscopy</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HT LC</td>
<td>High Temperature Liquid Chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>I₀</td>
<td>Amount of initiator</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>L</td>
<td>Litres</td>
</tr>
<tr>
<td>LAC</td>
<td>Liquid Adsorption Chromatography</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid Chromatography</td>
</tr>
<tr>
<td>LC-CC</td>
<td>Liquid Chromatography under Critical Conditions</td>
</tr>
<tr>
<td>LC-LCD</td>
<td>Liquid Chromatography under the Limiting Conditions of Desorption</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of Detection</td>
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<tr>
<td>LS</td>
<td>Light Scattering</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>-------------</td>
</tr>
<tr>
<td>m</td>
<td>Metres</td>
</tr>
<tr>
<td>$M$</td>
<td>Molar mass</td>
</tr>
<tr>
<td>$M_n$</td>
<td>Number average Molar mass</td>
</tr>
<tr>
<td>$M_w$</td>
<td>Weight average Molar mass</td>
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<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>MALLS</td>
<td>Multiangle Laser Light Scattering</td>
</tr>
<tr>
<td>MEHQ</td>
<td>p-methoxyphenol</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>mol</td>
<td>Moles</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of polymer chains</td>
</tr>
<tr>
<td>$N_m$</td>
<td>Total number of polymer chains</td>
</tr>
<tr>
<td>$N_t$</td>
<td>Total number of monomer units in a polymer chain</td>
</tr>
<tr>
<td>$n_c$</td>
<td>Number of charged monomer units</td>
</tr>
<tr>
<td>$n_u$</td>
<td>Number of uncharged monomer units</td>
</tr>
<tr>
<td>NAFS</td>
<td>Sodium formaldehyde sulfoxylate dihydrate</td>
</tr>
<tr>
<td>NMP</td>
<td>Nitroxide Mediated Polymerisation</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser Effect</td>
</tr>
<tr>
<td>NS</td>
<td>Number of Scans</td>
</tr>
<tr>
<td>OL</td>
<td>Overlapping</td>
</tr>
<tr>
<td>pH</td>
<td>Potential Hydrogen</td>
</tr>
<tr>
<td>PAA</td>
<td>Poly(acrylic acid)</td>
</tr>
<tr>
<td>PAM</td>
<td>Polyacrylamide</td>
</tr>
<tr>
<td>PAPTAC</td>
<td>Poly(acrylamido-$N$-propyltrimethylammonium chloride-$b$-$N$-isopropylacrylamide)</td>
</tr>
<tr>
<td>PDADMAC</td>
<td>Poly(diallyldimethyl ammonium chloride)</td>
</tr>
<tr>
<td>PEO</td>
<td>Poly(ethylene oxide)</td>
</tr>
<tr>
<td>PNIPAM</td>
<td>poly($N$-isopropylacrylamide)</td>
</tr>
<tr>
<td>$R^2$</td>
<td>Regression coefficient</td>
</tr>
<tr>
<td>RAFT/MADIX</td>
<td>Reversible Addition-Fragmentation chain Transfer/Macromolecular Design via Interchange of Xanthates</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>RI</td>
<td>Refractive Index</td>
</tr>
<tr>
<td>RDRP</td>
<td>Reversible-Deactivation Radical Polymerisation</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
</tr>
<tr>
<td>s</td>
<td>Seconds</td>
</tr>
<tr>
<td>$S_{UV}$</td>
<td>UV detector Signal</td>
</tr>
<tr>
<td>SEC</td>
<td>Size Exclusion Chromatography</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to Noise Ratio</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
</tr>
<tr>
<td>$t_{EOF}$</td>
<td>Migration time of EOF marker</td>
</tr>
<tr>
<td>$t_{is}$</td>
<td>Migration time of Internal Standard</td>
</tr>
<tr>
<td>$t_{m}$</td>
<td>Migration time</td>
</tr>
<tr>
<td>$t_{ref}$</td>
<td>Migration time of Reference</td>
</tr>
<tr>
<td>TDA</td>
<td>Triple Detection Array</td>
</tr>
<tr>
<td>TGIC</td>
<td>Temperature Gradient Interaction Chromatography</td>
</tr>
<tr>
<td>ThFFF</td>
<td>Thermal Field Flow Fractionation</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>$V$</td>
<td>Voltage</td>
</tr>
<tr>
<td>$V$-50</td>
<td>2,2'-Azobis(2-methylpropionamidine)dihydrochloride</td>
</tr>
<tr>
<td>$V_h$</td>
<td>Hydrodynamic Volume</td>
</tr>
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Abstract

Block copolymers enable combining the properties of multiple polymers into a single material. A particularly innovative means of combining properties is through Double Hydrophilic Block Copolymers (DHBCs) which contain ‘smart’ polymers. DHBCs are diblock copolymers where both blocks are water soluble. Smart polymers have the ability to respond to changes in their environment, examples of them are the pH responsive poly(acrylic acid) – PAA – and the thermo-responsive poly(N-isopropylacrylamide) – PNIPAM. Therefore smart block copolymers have numerous potential applications in drug delivery, removable coatings, controlled mineralisation and many others. However, the current means of characterising DHBCs suffers from many limitations resulting in their applications being significantly hindered.

The chemical structure of block copolymers is described by a number of distributions including distributions of molar masses, of branching architectures, of compositions and of end groups. These distributions and the purity of the block copolymer influence the properties of the resulting material. Currently smart polymers are mainly characterised in terms of their molar mass using size exclusion chromatography (SEC). However, cationic DHBCs such as poly(acrylamido-N-propyltrimethylammonium chloride-b-NIPAM) – P(APTAC-b-NIPAM) – cannot be characterised by SEC due to strong adsorption onto the stationary phase. Furthermore even when SEC takes places with a quantitative recovery, the separation is by hydrodynamic volume and cannot alone yield all information regarding the chemical structure. This work involved the development of a means to assess the purity and composition of DHBCs, namely diblock copolymers, using free solution capillary electrophoresis (CE), also known as capillary zone electrophoresis, thus enabling the characterisation of DHBCs including cationic ones. Charged oligomers (<~50 monomer units) are separated by molar mass while charged homopolymers (≥~50 monomer units) are separated independently from their molar mass in free solution CE, therefore for homopolymers this is referred to as CE in the critical conditions (CE-CC). This work also aimed at characterising the smart homopolymers used to form DHBCs in terms of
their branching and molar mass using SEC, CE-CC and Nuclear Magnetic Resonance (NMR) spectroscopy.

Free solution CE of PAA and PAPTAC homopolymers was capable of providing complete resolution of the short oligomers by their molar mass as well as their separation from the polymer chains. Through this separation the fraction of oligomers in the homopolymers was determined. Using one and two dimensional NMR spectroscopy a near complete assignment of the NMR signals of PAA was performed. Quantitative $^{13}$C NMR conditions for PAA were determined to occur when the repetition delay was 6 s. With the established quantitative conditions the $M_n$ determined by $^1$H and $^{13}$C NMR spectroscopy were compared which is rarely compared in the literature. Furthermore with quantitative $^{13}$C conditions it was found that two PAA samples had a Degree of Branching (DB) below 1 %. These PAA samples synthesised by Macromolecular Design via Interchange of Xanthates (MADIX) were compared to PAA samples synthesised by Nitroxide Mediated Polymerisation (NMP) and were found to have a significantly lower DB. Since CE-CC separates charged homopolymers by branching the heterogeneity of branching of the PAA samples was obtained, for the first time, by CE-CC. The samples synthesized by MADIX were found to exhibit more homogenous branching than those by NMP. After the characterisation of the homopolymers the project shifted focus towards the DHBCs which had been produced from these homopolymers.

Block copolymers can contain both parent homopolymers from their synthesis, although the latter are rarely quantified in the literature. CE-CC provides a means to assess the purity of DHBCs through the quantification of these homopolymers. Using poly(AA-$b$-acrylamide) - P(AA-$b$-AM) – copolymers the CE-CC method was developed and evaluated. A complete separation of the homopolymer from the block copolymer was shown as well as a recovery of PAA greater than 95 %. The CE-CC method was then applied to challenging cationic P(APTAC-$b$-NIPAM). Although incomplete separation of the homopolymer and block copolymer was observed, the resolution was sufficient to estimate the amount of residual homopolymer. In one sample 30 %
(mol/mol) of the polymer chains were found to be homopolymers. Using the CE-CC method both possible parent homopolymers can be detected and their quantity estimated in a single experiment. In both cases, one of the parent homopolymers is neutral and migrates with the electro-osmotic flow. When the electro-osmotic flow is weak (conditions used for the cationic copolymer) pressure-assisted CE-CC was used to detect this homopolymer. Using pressure-assisted CE-CC the formation of a P(APTAC-b-NIPAM) block copolymer was evidenced, which had not been previously achieved in the literature, despite careful selection of the stationary phase used in SEC.

Limited techniques are available to determine the distribution of compositions of a copolymer. Furthermore these techniques are often not applicable to DHBCs hence CE-CC was investigated for its potential to determine the composition distribution of a DHBC. In this work a means of converting the detector signal into a weight fraction in CE-CC is presented along with the theory for obtaining the Dispersity of Composition (DC). Through the DC the heterogeneity of the composition in a block copolymer can be assessed. While no composition distribution was obtained the broadness of the composition distribution of a number of block copolymer samples was compared through the Dispersity of Electrophoretic Mobilities (DEM).

In summary, the ability of CE-CC to characterise DHBCs including highly adsorbing samples is presented. With the CE-CC method developed in this work, the amount of both parent homopolymers and the composition of a block copolymer sample can be assessed in a single separation. With further development CE-CC could be used to study a range of adsorbing homopolymers and block copolymers which are very difficult to characterise by other separation techniques.
CHAPTER 1: BACKGROUND

1.1 Structures and Applications of Block Copolymers

Polymers are a crucial part of everyday life. From the plastic used in packaging and appliances to the rubber in footwear, polymers provide a cost effective way of producing these materials. Research in polymer science aims at further developing materials and providing a cost effective means of producing such materials for solar cells [1], coatings [2], stationary phases for liquid chromatography [3] and electrokinetic chromatography [4-6] etc. On the forefront of these developments are copolymers [7].

Copolymers are polymers made from different repeating units while homopolymers consist of the same repeating unit (Figure 1). If the different repeating units are distributed randomly throughout the polymer it is referred to as a statistical copolymer (Figure 1b). When one series of repeating units is followed by another series of repeating units in the one polymer chain, it is referred to as a block copolymer (Figure 1c).

![Figure 1](image.png)

**Figure 1.** Graphic representation of a: a) homopolymer, b) statistical copolymer, and c) block copolymer.

1.1.1 Applications of block copolymers

Block copolymers provide the means of incorporating the properties of multiple polymers into one material. Stimulus-responsive or ‘smart’ polymers can be included to provide, for example, pH-responsiveness in one block and adhesion in another. For this reason they have a wide variety of potential applications including improving medical imaging [8], drug delivery [9], capacitors [10] and removable coatings [11]. The
polymer industry has shifted its focus from homopolymers to copolymers, producing millions of tons of vinyl copolymers yearly [7]. Recent types of block copolymers include Double Hydrophilic Block Copolymers (DHBCs), which are block copolymers where both blocks are hydrophilic. DHBCs are a particularly innovative means of using ‘smart’ polymers [12,13]. DHBCs have potential applications in the field of controlled mineralisation [14,15] or quantum dots [16]. With further development, these new applications may become a reality.

To further develop block copolymers for the potential applications listed above the elucidation of their chemical structure is required. For example the molecular size of one of the blocks may be proportional to the adhesive strength of the material. Furthermore, the ratio between the numbers of monomer units in each block may impact the thermoresponsivity. In the development of these new polymer materials there are synthesis-structure and structure-property relationships that must be identified (Figure 2). Identifying this relationship involves synthesising a new compound followed by characterising its chemical structure and discovering its useful properties. Since there is no clear link between the synthesis and the properties understanding the chemical structure is crucial in having a cost effective means of manufacturing the product with the desired properties. Establishing these relationships allows for a material with ideal properties to be directly produced without using costly trial and error processes. However, with more complex chemical structures being synthesised more intricate characterisation techniques are required [17].
1.1.2 Synthesis of polymers

Block copolymers are often produced by synthesising a homopolymer, which is then reacted with a different type of monomer to produce another block. Conventional radical polymerisation is unable to produce a block copolymer because the propagating radical used to make the initial homopolymer undergoes irreversible termination on a shorter timescale than what is needed to react with another. In order to form a block copolymer the ‘livingness’ of the initial homopolymer must be maintained [18]. The ‘livingness’ of a polymer refers to the ability of a polymer to undergo propagation. Some methods for maintaining the ‘livingness’ of a homopolymer include living anionic polymerisation and Reversible-Deactivation Radical Polymerisation (RDRP). RDRP enables the synthesis of a homopolymer and with a transfer agent attached the polymer chain and thus can be reinitiated to form a block copolymer (Figure 3b). A range of RDRP techniques have been used for the synthesis of block copolymers including Atom Transfer Radical Polymerisation (ATRP) [19,20], Nitroxide Mediated Polymerisation (NMP) [21] and Reversible Addition-Fragmentation chain Transfer/Macromolecular Design via Interchange of Xanthates (RAFT/MADIX) [7]. Among these methods RAFT/MADIX is the method of choice for the synthesis of DHBCs [22]. That is because RAFT/MADIX is compatible with environmentally-friendly reaction conditions.
as opposed to alternative approaches like ATRP [23,24]. Therefore, a homopolymer can be converted to a block copolymer at room temperature in pure water [25].

Homopolymers bearing a transfer agent as an end group are referred to as ‘living’, while those without it are considered ‘dead’ chains (Figure 3a). ‘Dead’ chains form due to early irreversible termination of the propagating chain [26]. Consequently when making a block copolymer these ‘dead’ homopolymer chains remain in the block copolymer sample (Figure 3b). The initial homopolymer remaining in the block copolymer sample is generally referred to in the literature as unreacted or residual homopolymer. In the synthesis of the second block, if an external initiator is used as is the case for RAFT/MADIX or if there is transfer to either the monomer or solvent resulting in an initiator, these initiators can undergo propagation with the monomer units forming a different homopolymer instead of a second block attached to the first homopolymer (Figure 3b). In the literature this homopolymer and any initial unreacted homopolymer are referred to in many different terms including parent, undesired, unwanted and unintended homopolymers. The reason for this variation in terms is that there is no established means of identifying or quantifying these homopolymers.

![Figure 3](image_url)  
**Figure 3.** Graphical representation of the synthesis of a) homopolymers and b) block copolymers by RDRP, specifically RAFT/MADIX.
There are numerous types of DHBCs with different properties. Some of the DHBCs in this work were poly(acrylic acid-\textit{b}-acrylamide) – P(AA-\textit{b}-AM) – (Figure 4a) and poly(AA-\textit{b}-ethylene oxide) – P(AA-\textit{b}-EO) (Figure 4b). These DHBCs were used as model compounds to develop new characterisation techniques since they have a established synthesis and have been characterised by different methods in the literature. These particular DHBCs have potential applications as a particle stabilisers [27], flocculant, thickening agent and other aspects of water purification [28]. DHBCs which had not been well characterised yet were also of interest, these include poly(acrylamido-\textit{N}-propyltrimethylammonium chloride-\textit{b}-\textit{N}-isopropylacrylamide) – P(APTAC-\textit{b}-NIPAM) – (Figure 4c) and P(APTAC-\textit{b}-AA) (Figure 4d). These polymers combine the adhesive properties of the PAPTAC block [29] with either the thermoresponsiveness of the PNIPAM block [30] or the pH responsive properties of the PAA block [31,32]. P(APTAC-\textit{b}-NIPAM) have potential applications in thermoresponsive nanoparticles and coatings as well as modelling drug delivery [33-37]. Therefore these compounds are suitable for the development of new characterisation techniques and their characterisation will benefit their development towards new applications.

![Chemical structures](image)

**Figure 4.** Chemical structures of a) P(AA-\textit{b}-AM), b) P(AA-\textit{b}-EO), c) P(APTAC-\textit{b}-NIPAM), d) P(APTAC-\textit{b}-AA).
1.1.3 Structure of block copolymers

Characterising the chemical structure of polymers is more difficult than that of substances composed of small molecules since polymer samples contain a mixture of different chemical architectures, meaning there could be thousands, if not millions of different chemical structures in a sample (Figure 5) [38]. Block copolymers complicate the matter again since an additional monomer reagent is used. In a synthetic block copolymer sample there is a distribution of the total chain lengths and of the block lengths. Subsequently there is a distribution of chemical compositions present in each polymer sample. Chemical branching is often present in polymer materials, leading to many different chemical structures since some chains may have several branches, others a single branch (3 arm star structure) and others no branches (linear structure) [39]. The position of the branching point can also change from one polymer chain to the next and the length of each branch may be different as well. As a result the broad peaks produced from polymers in separation techniques can represent distributions of different chemical structures even when band-broadening is minimised (Figure 6). Synthetic polymers can vary by what chemical structure is present at each end of the polymer chain. Inevitably in each polymer sample there is a distribution of these chain ends. The purity of the polymer sample also impacts the properties, this is especially important for block copolymers since their synthesis involves reacting a homopolymer with a new monomer to produce another block. Not only can the monomers remain in the sample but the original homopolymer can be present as well resulting in a mixture of block copolymers and homopolymers. Completely elucidating the chemical structure of a block copolymer is thus rarely achieved especially with the characterisation techniques which are currently available for material scientists to use.
**Figure 5.** Different aspects of a block copolymer’s chemical structure. The purity involves any molecule which is not a block copolymer, including homopolymers, monomers, initiators, etc.

**Figure 6.** Example distribution of a polymer’s chemical structure. It could be for example a distribution of molar masses, of composition, branch length or position.

A change in the distribution of the chemical structures influences the material’s possible applications. It has been previously demonstrated that the distribution of block lengths and compositions influences the self-assembly of block copolymers [40] and
that the end group impacts the thermoresponsivity of some polymers [41]. In order to properly identify the impact the chemical structure of a block copolymer has on its materials properties, the distribution of the chemical structures must be identified. Techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy [42], Dynamic Light Scattering (DLS) [34] and potentiometric titration [36] have been used in the characterisation of copolymers. However they can only provide average values such as the Weight-average Molar mass (M_w), Number-average Molar mass (M_n) or average composition [43] but no information on their distribution. To obtain a distribution for a polymer a separation technique is required.

1.2 Homopolymer Quantification

1.2.1 Role of the presence of homopolymer in the properties of a block copolymer

In the production of block copolymers if the livingness of the initial homopolymer is not maintained the homopolymer may not become a block copolymer. Alternatively a mixture of homopolymers may be produced instead of a block copolymer. In most cases this impurity may be unavoidable but minimised in the synthesis [26]. This is especially the case for block copolymers synthesised by RAFT/MADIX since an external source of radicals is needed to initiate the polymerisation of each block, so not all homopolymers may have the transfer agent present [44]. The presence of homopolymer in a block copolymer sample impacts the phase separation and mechanical properties of the material [45,46]. The phase separation in block copolymers is particularly important for their applications because of the unique self-assembly structures they form (Figure 7). When a mixture of a block copolymer and its parent homopolymer is present these phase structures are affected (Figure 8). The homopolymer present can also be useful, for example in the production of porous materials during which homopolymer is added to the block copolymer and then later removed with a solvent to create pores [47]. Therefore quantifying the amount
of homopolymer in a block copolymer is important for understanding the structure-property relationship of the block copolymer.

Figure 7. Theoretical phase diagram of a diblock copolymer, illustrating the change in phase separation with changing block lengths. Four phase structures are shown including: bcc spheres, hexagonal cylinders, cubic bicontinuous gyroid and lamellar (left to right). Figure adapted from reference [48].

Figure 8. Self-assembly structures of block copolymers in the presence of homopolymers, as shown in reference [49].
1.2.2 Current methods for homopolymer quantification

1.2.2.1 Size Exclusion Chromatography (SEC)

Residual homopolymer has previously been detected in range of block copolymer samples synthesised by anionic polymerisation [50], NMP [51] and RAFT/MADIX [45]. Although residual homopolymer is likely present in most block copolymer samples, it is rarely determined in the literature. Homopolymers and block copolymers are almost always characterised by Size Exclusion Chromatography (SEC), also known as Gel Permeation Chromatography (GPC) [17,52]. Using SEC the distribution of the molar masses of a polymer sample can be determined. Since SEC separates according to hydrodynamic volume and not according to molar mass [53], the hydrodynamic volume distributions of the homopolymers and of the block copolymers often overlap (Figure 9). The comparison of the SEC and other Liquid Chromatography (LC) methods for the separation of copolymers and homopolymers of styrene and ethylene oxide lead to the conclusion that “As expected, resolution of SEC is not high enough to separate block copolymers from their parent homopolymers” [54]. Hydrodynamic volume depends on both molar mass and composition [55]. SEC is not well suited to separate residual homopolymer from block copolymers. Furthermore, routine SEC and NMR spectroscopy experiments are unlikely to detect the presence of homopolymer leading to errors in the determined molar mass by most techniques.
Figure 9. Hydrodynamic volume ($V_h$) distribution, determined by SEC, of poly(methacrylic acid), solid line, and poly(methacrylic acid-$b$-methyl methacrylate), dashed line. At log 2 nm$^3$ is the RAFT agent and at log 3.5-4.5 nm$^3$ are the respective polymers in the sample. Figure adapted from reference [56].

SEC of charged polymers is particularly difficult, because the separation can be plagued with aggregation [57] and ‘ion exclusion’ [58-60]. This can then lead to low accuracy of the determined molar mass values [17,61]. Achieving baseline separation of homopolymers from their corresponding block copolymers is near impossible even in extreme cases where the second block is much larger than the first one.

Cationic polymers are especially difficult to analyse by SEC due to strong interactions with the stationary phase [23,34,62]. This makes it difficult to determine their molar mass or the presence of residual homopolymer. These interactions can generally be suppressed with high salt eluents and co-solvents [63,64]; however, in some cases there is a risk of precipitation and poor recovery. Columns for aqueous SEC
of cationic polymers in low salt conditions are being developed as shown in application notes \[65,66\]. In the case of DHBCs, these SEC conditions for polycations are however incompatible with the SEC conditions for some second block. For example, PNIPAM which is a very challenging polymer to properly characterise using aqueous SEC due to its thermoresponsiveness in water \[67\]. SEC of PNIPAM requires polar organic mobile phases such as \(N,N\)-dimethylformamide (DMF) containing LiBr \[28\]. Therefore, the analysis by SEC of block copolymers such as P(APTAC-\(b\)-NIPAM), or P(APTAC-\(b\)-\(N\)-vinyl pyrrolidone) shown in Figure 10, is usually unsuccessful \[23,25,34,68\]. Thus, the development of applications of cationic polyelectrolytes and DHBCs such as P(APTAC-\(b\)-NIPAM) has been hindered by their lack of characterisation.

![Chemical structure of P(APTAC-\(b\)-N-vinyl pyrrolidone)](image)

**Figure 10.** Chemical structure of P(APTAC-\(b\)-N-vinyl pyrrolidone).

### 1.2.2.2 Liquid Chromatography under Critical Conditions (LC-CC)

Liquid Chromatography under Critical Conditions (LC-CC), also known as Liquid Chromatography at the critical point of adsorption and Liquid Chromatography at the Chromatographic Critical Condition, is a separation technique which separates polymers independently from molar mass \[69\]. Critical conditions are reached by balancing the conditions used in SEC and Liquid Adsorption Chromatography (LAC) \[70\]. In SEC molecules with a larger hydrodynamic volume travel through less pores causing them to elute earlier than smaller molecules while in LAC larger molecules are retained more by the stationary phase and thus elute later than smaller molecules (Figure 11). By adjusting the mobile phase the elution order of the molecules can be a balance.
of size exclusion and adsorption, resulting the molecules eluting independent from their molar mass [71].

**Figure 11.** Trends in the elution profile of macromolecules in relation to molar mass for different chromatographic modes.

By having one block in critical conditions the other block can be separated according to its size providing the means to determine the molar mass distribution of this other block, or at least the apparent molar mass distribution of this block [72]. LC-CC leads to complete separation of one homopolymer from the block copolymer [73]. However, determining the critical conditions is highly tedious and LC-CC suffers from poor recovery [74-76], the mobile phase is usually required to be a mixture of solvents leading to preferential solvations and adsorption on the stationary phase [77]. Furthermore, LC-CC is unable to separate all unintended homopolymers from the block copolymer in the sample in a single injection. For these reasons, easier alternatives to LC-CC are highly desired.

1.2.2.3 Liquid Chromatography under Limiting Conditions of Desorption (LC-LCD)

Liquid Chromatography under Limiting Conditions of Desorption (LC-LCD) is a newer method and an alternative to LC-CC capable of separating block copolymers from parent homopolymer [54,78-80]. This method involves using a combination of solvents. The mobile phase promotes desorption of all the polymers, while another
solvent is injected which promotes adsorption of one of the blocks, creating a barrier which slows homopolymers and copolymers containing that block, thereby separating homopolymers from block copolymers. LC-LCD has been used to show that a number of commercial poly(methyl methacrylate-\textit{b}-styrene) block copolymers synthesised by anionic polymerisation contained small amounts of both poly(methyl methacrylate) and polystyrene [79]. Furthermore, LC-LCD has been applied to poly(methyl methacrylate-\textit{b}-styrene) copolymers synthesised by Nitrooxide Mediated Polymerisation (NMP) and a significant amount of a residual homopolymer was detected [81]. However, LC-LCD and LC-CC have not been applied to highly polar and charged polymers such as DHBCs, as finding the correct solvent conditions would be extremely difficult.

1.2.2.4 Capillary Electrophoresis (CE)

1.2.2.4.1 Free Solution CE

An alternative method for quantifying parent homopolymers of polar and charged polymers, such as DHBCs, is free solution Capillary Electrophoresis (CE), also known as capillary zone electrophoresis. Free solution CE involves molecules migrating in an electric field while in a fluid medium, such as a buffer within a glass capillary. This is unlike gel electrophoresis which involves using a gel medium for the ions to travel through or electrokinetic chromatography which has a surfactant medium. In the gel electrophoresis the gel acts as a sieving matrix providing a size based separation [82]. Free solution CE allows for more efficient separation than gel electrophoresis because higher voltages can be used and the medium provides less friction allowing for a faster separation. The medium involved in free solution CE helps to maintain a constant pH and provides a medium for the charged species to migrate through. The molecular species move through the capillary under the influence of an electric field. The velocity at which ions move in the electric field is directly proportional to the electric field strength \(E\), the proportionality constant between these quantities is the electrophoretic mobility \(\mu\), as shown in Equation (1):

\[
Velocity = \mu E
\]
therefore ions are separated by their electrophoretic mobility [83]. The electrophoretic mobility of a charged species is dependent on the ratio of its charge to its friction as shown in Equation (2) [84].

\[ \mu \propto \frac{\text{Charge}}{\text{Friction}} \]  

(2)

The friction of the charged species in free solution CE includes both its hydrodynamic and electrostatic interactions, unlike SEC and gel electrophoresis where the friction involved is mainly hydrodynamic [85]. Hence the separation mechanism of free solution CE is different to that of chromatography techniques and gel electrophoresis.

In free solution CE there is separation of positively charged, negatively charged and neutral species. Cations are attracted to the negatively charged cathode while anions are attracted to the positively charged anode (Figure 12). In free solution CE, the Electroosmotic Flow (EOF) is the flow generated by the electric field and the charged capillary surface. The fused silica capillary usually has a negatively charged surface resulting in an electric double layer of the cations from the background electrolyte. The first layer of cations is immobile while the secondary layer undergoes a constant migration towards the negatively charged cathode forming the EOF [86]. Neutral molecules are not attracted to either the anode or cathode, thus their migration is purely a result of the EOF. Hence neutral molecules are used to measure the EOF. The force provided by the EOF is usually stronger than the charged species attraction to the anode and cathode resulting in a net flow of molecules towards the cathode (Figure 12). The force of the EOF is related to the magnitude of the surface charge.
Free solution CE can be used to separate a wide variety of compounds from sugars [87,88] to proteins [89,90]. Previous work has already shown that free solution CE can separate poly(AA-co-diallyldimethylammonium chloride) from residual poly(diallyldimethyl ammonium chloride), PDADMAC, homopolymer and its monomers [91]. The amount of unreacted poly(acrylic acid), PAA, homopolymer in a block copolymer has also been previously determined by free solution CE [45,92]. In addition DNA has been separated from some of its block copolymers by free solution CE, with a method called End Labelled Free Solution Electrophoresis (ELFSE), which involved attaching a large uncharged protein or polymer to the end of DNA causing a change in the overall charge to friction ratio [84]. The additional friction resulted in separation of the free DNA (residual homopolymer) from the labelled DNA (copolymer) [93].
1.2.2.4.2 CE in the Critical Conditions (CE-CC)

Free solution CE provides an effective means of separating homopolymers from copolymers. This is because in the case of polyelectrolytes (charged polymers), when the chain reaches a certain length its electrophoretic mobility is independent of molar mass (Figure 13) [94,95]. This situation of electrophoretic mobility being independent of molar mass is analogous to the ‘critical conditions’ observed in LC-CC. Thus, the migration of polyelectrolytes in free solution CE is referred as CE in the Critical Conditions (CE-CC) [96]. These critical conditions arise in CE due to interactions of the counter ion with the polymer chain [97]. The polymer likely adopts a coiled conformation so that the electrostatic friction outweighs the hydrodynamic friction [98]. With the addition of each monomer unit the addition of the charge is offset by the screening of the counter ion adding to the electrostatic friction [99]. In this situation, separation of polyelectrolytes can occur according to structure or end groups. Previous work has already used CE-CC to separate polyacrylates according to branching [100], statistical copolymers such as chitosan and gellan gum by their composition (degree of acetylation) and conformation [43,101]; this was recently reviewed in [96]. In comparison to the critical conditions obtained in LC-CC, the critical conditions in CE-CC are much easier to obtain since it only requires the sample to have a sufficiently large molar mass. CE-CC does not require solvent mixtures with precise purities and compositions, thus making CE-CC significantly easier to conduct than LC-CC.
Figure 13. General trend for the evolution of the electrophoretic mobility with the number of monomer units in a polyelectrolyte.

For the analysis of polymers CE-CC offers a number of advantages over its chromatography based alternatives. CE-CC separates charged polymers which are either difficult or are incapable of being analysed by SEC, LC-CC, LAC and LC-LCD. However, uncharged polymers cannot be separated by CE-CC, these polymers are typically hydrophobic and so a number of solvents are available to provide sufficient conditions for SEC, LC-CC, LAC and LC-LCD. CE-CC does not require a filtered sample because of the lack of a stationary phase [96] even with a complex matrix, such as breakfast cereal [102]. Normally solid particles are trapped by the pores of a stationary phase while in CE-CC the capillary is typically below 100 µm diameter preventing most solid particles entering. Any solid particles that enter the capillary are normally passed through the capillary. Filtering polymer samples can result in sample loss, degradation and changes in colloid properties thus presenting a clear advantage for CE-CC [28,103]. CE-CC can also be applied to samples with poor dissolution such as polysaccharides as the non-dissolved component is not as likely to affect the analysis. Each CE-CC experiment consumes nL of sample which is order of magnitude less than its chromatography alternatives. However due to the low sample consumption preparative procedures are not possible as they are in chromatography. Furthermore the low sample amounts can lead to low sensitivity in CE-CC although sensitivity can be
improved using stacking method which place more sample in the capillary [104]. CE-CC is capable of performing separations in less than 5 min however the separation time varies between sample and the resolution required. CE-CC consumes considerably less solvent and sample than chromatography based techniques; the cost of capillaries in comparison to chromatography columns differs by orders of magnitude. The running cost of free solution CE, liquid chromatography and ion chromatography were compared for carbohydrate analysis and it was found that free solution CE was <1 % of the cost of the chromatography methods [87,88].

Herein a CE-CC method for the quantification of parent homopolymers and thus an assessment of the purity of complex charged DHBCs containing either a weak anionic block (PAA) or a cationic block (PAPTAC) is presented. Furthermore the capabilities of SEC and CE-CC were compared and assessed to quantify homopolymers in block copolymer samples. P(AA-b-AM) samples spiked with PAA were used to develop this method since P(AA-b-AM) samples with minimal residual PAA homopolymer could be synthesised. This method was then applied to cationic P(APTAC-b-NIPAM) to quantify the amount of parent PAPTAC and PNIPAM homopolymers.

1.3 Branching in Polymers

1.3.1 Transfer to polymer and branching

As a polymer chain propagates a number of side reactions are possible, leading to possible unwanted products. Branching is one such example, which is caused by a mid-chain radical forming from these side reactions during a radical polymerisation [39,105]. Two of the possible side reactions are intramolecular transfer to polymer and intermolecular chain transfer to polymer. Intramolecular transfer consists in the propagating radical abstracting a hydrogen from the polymer backbone producing a mid-chain radical on the backbone in the same polymer chain (Figure 14a). Intramolecular transfer to polymer is generally referred to as ‘backbiting’, which leads
to short chain branching. Intermolecular chain transfer to polymer involves the propagating radical abstracting a hydrogen from the backbone of another polymer chain (Figure 14b). These mid-chain radicals can then propagate resulting in a branch, which could be short or long in length [106]. In the case of polyacrylates the most common type of branching is short chain but long chain branches are usually also present significantly impacting on the polyacrylates chain dynamics [106,107]. The concentration of the mid-chain radical can be decreased by the presence of a transfer agent which reacts with the mid-chain radical, however, transfer agent also limit the molar mass which can be achieved [108].

These transfer reactions have been noted in many polymers such as polyethylene [109] and a variety of polyacrylates [106,110]. The formation of this mid-chain radical is possible in many polymers but it is not always investigated. For example little research has been undertaken in the formation of branches in polyacrylamides [111,112]. Obtaining clear information about branching adds to the knowledge and to the understanding of the polymerisation and polymer’s properties.

Alternatively controlled branched structures can be produced intentionally for a certain application. Star polymers have been synthesised as a potential drug carrier using multifunctional initiators [19,21,113]. Depending on the intended application different extents of branching may be desired. For example as the extent of branching increases the flexibility of the material increases although the tensile strength is lowered [114]. Not only does polymer branching affect the properties of the material it also impacts our ability to analyse them.
Figure 14. Formation of mid-chain radicals by a) intramolecular transfer and b) intermolecular chain transfer to polymer, where n = y + 1 + z.

1.3.2 Quantification of the degree of branching using NMR spectroscopy

Branching is present in many synthetic polymers made by radical polymerisation. For vinyl polymers when branching is present in the chain a quaternary carbon is produced in the aliphatic backbone where the mid-chain radical occurred (Figure 15). $^{13}$C NMR spectroscopy can then be used to detect the quaternary carbon [100,106,115,116] and estimate the Degree of Branching ($DB$). The degree of branching is defined by Equation (3):

$$DB \, (\%) = \frac{\text{number of branched points}}{\text{total number of monomer units}} \times 100$$  

(3)

this equation relates to the frequency of the occurrence of branches in a sample and not to the length of the branches or to how they are distributed amongst the polymer chains. However it allows for a comparison between highly branched polymer samples and those with minimal branching.
Figure 15. Branching point in a vinyl polymer forming a quaternary carbon (Cₜ).

Swollen, solid, melt and solution state NMR techniques have been compared in the literature to determine the DB of a number of poly(n-butyl acrylate)s and other polyacrylates [106]. The DB of poly(n-butyl acrylates) has reported to be between 1 and 2.5 % [106,116] or around 3.5 % [117] when synthesised by conventional radical polymerisation or with different polymerisation temperatures to be between 1 and 5 % [118] as well as 2 and 5 % [108]. The DB was found to be less than 2 % for polyacrylates synthesised by ATRP [116,117], around 1 % when synthesised by RAFT [116] and between 1 and 4 % when synthesised by NMP [116]. For PAA only two publications have estimated the DB, and they have estimated the branching to be around 2 % when synthesised by RAFT [119] and between 4 and 6 % for NMP [115]. Therefore each sample has a different DB as changes in synthetic parameters including solvent, temperature and control agent all affect the branching [107,116]. However, with only a few publications determining the DB it is difficult to establish an accurate link between the synthesis and the frequency of branches as well as the structure-property relationship.

It is uncommon to measure the DB due to the demands of quantitative NMR. First to ensure a NMR measurement is quantitative the repetition delay between scans must be long enough to ensure all the nuclei of interest have fully relaxed, so that the relative area of the signals is not biased due to incomplete relaxation. The minimum amount of time required is five times the longitudinal relaxation time $T_1$ [120]. The value for $T_1$ can be estimated using the inversion recovery pulse sequence (Figure 16). Using this sequence if the time, $\tau$, is long enough for the nuclei to relax then a positive signal would be produced, allowing for an over estimate of $T_1$. Since a significant
amount of time is needed between scans, experiments can take days to obtain sufficient sensitivity and reasonable signal to noise ratio (SNR) [106].

![Diagram](image)

**Figure 16.** One-dimensional $T_1$ inversion recovery pulse sequence used to estimate $T_1$ for $^1$H NMR. The corresponding pulse sequence for $^{13}$C also contains a decoupling of the hydrogen nuclei [121].

The SNR of the quaternary carbon peak relates to the error in the DB as shown in empirical Equation (4) [106]:

$$Relative \ Standard \ Deviation \ (%) = \frac{283}{SNR^{1.28}}$$

since there is typically a small fraction of branching points in a polymer sample its signal in the NMR spectrum is also small, decreasing the SNR thus giving high error. With low SNR the precision of the measurement is significantly impacted making it difficult to identify the presence of the branching peak. However, the SNR of an NMR signal can be improved by increasing the Number of Scans (NS). The SNR is proportional to $\sqrt{NS}$, so in order to have twice the SNR four times the scans are needed [120]. To measure the DB when the DB is below 1 % can require days of measuring time. It is also possible to have branching in a sample but in a small fraction which cannot be precisely identified because of an insufficient number of scans. For example to ensure a linear PAA sample was obtained by anionic polymerisation, over 28 000 scans were recorded to ensure no small quaternary carbon signal was present [100].

It should be noted that the DB obtained by NMR is an average value of branching. NMR is unable to distinguish between long and short chain branching, the position of the branch along the chain or the number of branches per chain. When branching is present in a polymer sample there is a distribution of branching
architectures leading to a heterogeneity of branching in the polymer sample. To help identify the heterogeneity of branching in a polymer sample separation according to the branching architectures is required.

### 1.3.3 Heterogeneity of branching

Since SEC separates according to hydrodynamic volume when branching is present in the polymer there is an incomplete separation according to molar mass, since linear and branched chains of the same molar mass have different hydrodynamic volumes. Due to the incomplete separation errors of up to 100% can easily be obtained on the determined molar mass [122]. Assuming no effect from branching can prevent relating the value of molar mass to the properties of the final product. To separate polymers by branching alternative techniques to SEC are required.

Although branching influences the separation of many methods, as described for SEC, it is rarely the main parameter affecting the separation. Temperature Gradient Interaction Chromatography (TGIC) [123] has been one such method capable of separating polymers by their branching architecture [124,125]. TGIC has been applied to polyisoprene combs made by coupling polymer chains by anionic polymerisation, where it was able to separate the comb structures from the coupled linear chains [126]. However, TGIC primarily separates by molar mass with a higher selectivity by branching architecture making it difficult to identify the branching present. Therefore the heterogeneity of branching cannot be accurately determined due to the influence of molar mass [127]. The influence of molar mass can be overcome by using two dimensional TGIC and SEC which can be used to assess the heterogeneity of branching and molar mass in the sample [128]. However, the extreme tediousness of TGIC, in comparison to SEC, has been previous described and it is unclear how effective this technique would be for a polyelectrolyte [127].

As mentioned in section 1.2.2.2 LC-CC separates compounds independently from their molar mass allowing for separation by structural properties such as
branching. LC-CC has previously been used to separate linear polymers from branched polymers [129], star polymers based on number of arms [130] and by DB [131,132]. However, the difficulties of LC-CC mentioned in section 1.2.2.2 still occur, since finding the appropriate solvent conditions for hydrophilic polymers to assess their branching would be a highly difficult task and has not been achieved in the literature. A separation independent from molar mass is also possible with CE-CC, allowing separation of charged homopolymers by their branching. It has previously been shown that poly(sodium acrylate) of linear, 3 arm star and hyperbranched architectures can be separated by CE-CC [100]. Thus the broadness of the peak of a charged homopolymer in CE-CC can be related to the heterogeneity of branching in the polymer sample.

Identifying the presence of branching and obtaining the DB in hydrophilic polymers such as PAA and PAPTAC is of great importance since it is rarely measured in the literature. Furthermore using CE-CC the distribution of branching can be identified in terms of the heterogeneity of branching in the homopolymer sample. The branching in the samples would further affect the properties of the materials made from the homopolymers and of the block copolymers produced from these homopolymers. With this information on the branching of the polymers a relationship between the synthesis of these polymers and their branching can be established as well as the link between their branching and their physical properties.

1.4 Composition of Block Copolymers

1.4.1 Effect of composition on copolymers properties

Copolymers require additional characterisation compared to homopolymers, since the ratio of the monomer units in each chain should also be measured. Similar to molar mass the composition in a block copolymer exists in a distribution of different compositions. Therefore an average composition of the sample or a distribution of the compositions can be obtained.
The composition of block copolymers has been shown to be the defining factor for the tensile strength of an industrial rubber [133]. In a biodegradable copolymer, as the fraction of one monomer unit increases so does the crystallinity of the material [134]. Properties of copolymers used for drug delivery are heavily influenced by their composition, with the rate of degradation of polylactone copolymers [135] and the gelation of PEO block copolymers [136] being affected by composition. Therefore ensuring the correct distribution of compositions is required to ensure all the copolymer chains present in the sample have the desired composition. By only following the monomer feed and average composition some chains may not be at the required level.

1.4.2 Methods for determining average composition

The composition of copolymers is normally assessed in the literature as an average value mainly due to a lack of alternatives. Spectroscopic techniques are normally used to determine the average composition. Infrared (IR) spectroscopy has been used in the literature to obtain the average composition of copolymers of acrylonitrile and acrylamide [137] as well as industrial polyolefins [138]. NMR spectroscopy is the most commonly used technique for the determination of composition, and has been applied to many copolymers [43,134,139]. Both techniques have also been compared in the literature and were found to be in agreement, except when a small ratio of monomer units is present [73]. However, these techniques are only appropriate to measure composition if there are differing signals from each monomer unit. It is also important to note that some industrial samples contain a mixture of copolymers and their homopolymers, in these situations spectroscopic techniques are unable to distinguish the signals of the homopolymers from those of the corresponding copolymer [73]. Mass Spectrometry (MS) also provides a means of determining the average composition since it can precisely determine the molar mass of individual chains and thus ratio of monomer units [140]. The average composition of poly(styrene-\(b\)-isoprene) was determined by MS and was found to be in agreement with NMR spectroscopy [141]. However assessing the composition of copolymers by MS is difficult since it requires specialised software and there is a potential bias in the
ionisation which can reduce the accuracy of the determined composition [142]. Therefore, in some cases a separation technique is preferred.

1.4.3 Methods for determining distributions of compositions

Obtaining the distribution of a copolymer’s compositions is preferred to an average value as it provides an indication of the composition of each individual chain in the sample. To obtain a distribution separation of the sample is required. No method which separates solely by the composition of a polymer has been currently in the literature. The current methodology being developed in the literature involves a separation technique coupled to a spectroscopic detector. Separation techniques such as SEC [143], LC-CC [144-146], Temperature Rising Elution Fractionation (TREF) [143,147-149], Crystallisation Analysis Fractionation (CRYSTAF) [149,150], High Temperature Liquid Chromatography (HT LC) [147,151] and Thermal Field Flow Fractionation (ThFFF) [152,153] have all been used to determine the distribution of compositions of a copolymer. In almost all cases these techniques involved an IR or NMR spectrometer as a detector. Unlike an ultraviolet (UV) or Refractive Index (RI) detector which provides detection at each data point, current IR and NMR detectors give an average value of a finite group of data points [150], where the results are more analogous to analysing fractions with the detector. Furthermore the separation techniques used generally require a mixture of solvents and these solvents then appear in the spectroscopic detection, meaning solvent suppression is needed in most cases since the desired signals commonly overlap with that of the solvent. Overall the main criticism of these methods for determining the distribution of compositions is the immense cost, analysis time and being applicable to a small range of samples [147]. Due to these limitations very few research group have been able to use these techniques.

SEC and LC-CC separate copolymers according to their size and are applicable to a number of polymer samples. The spectroscopic detector can provide a qualitative
value for the composition at each point in the chromatogram but it cannot measure the amount of copolymer with that composition [147]. TREF, CRYSTAF and HT LC separate polymers based on their crystallinity which is related to their composition [149], however few polymers form a semi-crystalline material and so this is not applicable to many copolymers. ThFFF can be applied to a number of copolymers however it separates according to both molar mass and composition [154], furthermore significant resolution is only achieved with large molar mass samples [153], but at the cost of sensitivity. Using ThFFF an absolute determination of a distribution of compositions was achieved using a UV and RI detector; although it was effective in providing a distribution, its accuracy was limited when compared to ThFFF with NMR detection [152]. In addition it suffers from incomplete separation by composition. Gradient SEC has also been used to separate copolymers by composition; this technique relies on similar principles as LC-CC and so it takes a great deal of time to obtain the correct solvent conditions [155]. LAC is also capable of separating copolymers by composition however this method’s separation is also influenced by molar mass [70,156]. This problem of techniques separating by both composition and molar mass is present in all of the techniques mentioned except CE-CC.

To overcome this limitation two Dimensional (2D) separations have been employed to separate by composition in one dimension and molar mass in another. This has occurred with the coupling of LAC with SEC [55,70,157] or Mass Spectrometry (MS) [158], LC-CC with SEC [159,160] and reverse phase-High Performance Liquid Chromatography (HPLC) with normal phase-HPLC [161,162]. Such techniques are very useful in providing a complete picture of the molar masses and compositions present in a copolymer sample. However, these techniques are very tedious for an individual sample, they have relatively high costs and they require two instrumental setups. Furthermore to obtain the composition at each point in the chromatogram or contour plot of an unknown sample by gradient SEC, LAC or 2D separations standards of well-known composition are required to provide a calibration curve; unfortunately such standards are very rare and seldom available for new polymers such as DHBCs. As discussed in the previous paragraph the need for standards can be overcome with a
spectroscopic detector. 2D LC-CC and SEC separations coupled to an NMR spectrometer have been reported but such an instrumental setup has incredibly high costs and is not readily available [163]. In summary a number of methods have been developed in the literature for the determination of distributions of compositions; however, they are unlikely to be applicable to DHBCs and in most cases such techniques can only be applied to specific copolymers. Therefore identifying a cost effective technique which is applicable to a large number of copolymers and provides a clear separation by composition is still desired.

1.4.4 Separation by composition of block copolymers using capillary electrophoresis

CE-CC is another separation method which separates copolymers according to their composition. For a block copolymer consisting of one charged block and one neutral block the number of effective monomer units can be related to the electrophoretic mobility in the following equation [164]:

\[ \mu = \mu_0 \frac{n_c}{n_c + \alpha n_u} \]  

(5)

where \( \mu \) is the electrophoretic mobility of the block copolymer, \( \mu_0 \) is the constant electrophoretic mobility of the charged homopolymer, \( n_c \) is the number of charged monomer units in the copolymer, \( n_u \) is the number of uncharged monomer units in the copolymer and \( \alpha \) is a rescaling factor which depends on the chemical nature of the monomer units in the block copolymer.

When a polyelectrolyte is in critical conditions, the electrophoretic mobility is constant (\( \mu_0 \)). Equation (5) represents the change in the charge to friction ratio caused by the addition of an uncharged block to the charged homopolymer [165]. Since the number of charged monomer units (\( n_c \)) is the only component contributing to the effective charge of the copolymer it totals the charge in the charge to friction ratio [164]. The frictions involved in CE-CC are the hydrodynamic friction which is caused by each
block and the electrostatic friction mainly caused by the charged block [166]. Therefore the electrophoretic mobility of a block copolymer is related to the ratio of the number of charged to uncharged monomer units, which is in essence the composition.

As the number of uncharged monomer units \((n_u)\) increases there is greater friction applied to the block copolymer. The rescaling factor \((\alpha)\) is an important part of the equation because it relates to the effective friction that the uncharged block adds to the chain. The friction is related to the size of the monomer units and the flexibility of polymer [167]. The more flexible the uncharged block is in the buffer solution the more friction it would add to the block copolymer as a whole [168]. Therefore the rescaling factor changes with the chemical nature of the charged and uncharged monomer units, but also with the buffer used, the ionic strength and the temperature [165]. The \(\alpha\) can be determined by rearranging Equation (5) to Equation (6) so that a plot of \(\mu_0/\mu - 1\) vs \(n_u/n_c\) yields \(\alpha\) as the slope.

\[
\frac{\mu_0}{\mu} - 1 = \alpha \frac{n_u}{n_c}
\]  

(6)

In free solution CE the composition of a polyelectrolyte is usually discussed in terms of its chemical charge density. The chemical charge density \((\xi)\) is defined as the number of charged monomer units divided by the total number of monomer units in the polymer chain, as shown in Equation (7) [169]:

\[
\xi = \frac{n_c}{n_c + n_u}
\]  

(7)

the distribution of the chemical charge densities of a block copolymer is therefore the distribution of compositions of this copolymer. Previous studies have shown that free solution CE can be used to determine the average chemical charge density of synthetic statistical and block copolymers by the use of calibration curves [169,170]. However using Equation (5) a distribution can also be determined. Equation (7) can be rearranged to produce Equation (8). Equation (8) can then by combined with Equation (6) to produce Equation (9), demonstrating that the electrophoretic mobility \((\mu)\) which is the x axis of the electropherogram can be converted to chemical charge density \((\xi)\) thus providing the distribution of compositions of the block copolymer.
Using a series of charged-uncharged block copolymer samples a rescaling factor \( \alpha \) could be determined. From the rescaling factor the distribution of chemical charge densities will be determined. This would then demonstrate the potential of CE-CC to be the first method to provide a distribution of compositions where all the compositions and the fraction of each composition can be identified, as opposed to the composition at different points in the chromatogram. Furthermore, the cost of this method would be a fraction of the cost of those currently in the literature and require significantly less running time.

1.5 Aims of Study

This work addresses the characterisation of block copolymers and of the initial parent homopolymers, in particular the characterisation of some ‘smart’ DHBCs which are generally incompatible with other characterisation techniques. This includes P(AA-\( b \)-AM), P(AA-\( b \)-EO), P(APTAC-\( b \)-NIPAM) and P(APTAC-\( b \)-AA) (Figure 4). The main problem at hand is the characterisation of cationic DHBCs, particularly P(APTAC-\( b \)-NIPAM), which are extremely difficult to be characterised by most techniques. Therefore this study focuses on the development of new characterisation methods for these DHBCs. Without the development of new characterisation methods for these cationic DHBCs their many potential applications would not be possible.

The first step in assessing the chemical structure of a block copolymer is the characterisation of the initial parent homopolymers, namely PAA and PAPTAC in this work. Of particular importance to this work was the molar mass distribution, the degree of branching (\( DB \)) and the heterogeneity of branching. These components of the chemical structure were analysed using SEC, NMR spectroscopy and CE-CC which all
provided different information about the chemical structure. With the combination of these techniques a near complete chemical structure of the homopolymers was determined.

The next stage involved the characterisation of the block copolymers. Firstly identifying the formation of a block copolymer was crucial. With this came an assessment of the purity of the block copolymers which is generally overlooked in the literature. When synthesising a block copolymer it is important to identify and quantify any parent homopolymers in the sample. Traditional characterisation techniques such as SEC are unlikely to identify these parent homopolymers. Therefore in this work a comparison between SEC and CE-CC for their ability to assess the purity of block copolymers was undertaken. A model block copolymer, P(AA-b-AM), was used for the comparison and to develop the CE-CC method, since the conditions for both techniques were known. The CE-CC method was then applied to challenging P(APTAC-b-NIPAM) block copolymers which are extremely difficult to characterise by SEC.

Finally this project aimed at developing the means of determining the distribution of compositions of DHBCs. This was undertaken using CE-CC which separates copolymers according to their composition without any influence of molar mass. Furthermore, using the theory developed for the separation of DNA by CE an absolute means of determining the distribution of compositions was examined.

From this work new characterisation methods for DHBCs were developed. A more complete characterisation of these block copolymers is presented and information regarding the purity, branching and composition was obtained which is rarely determined in the literature. Finally the cationic DHBC P(APTAC-b-NIPAM) was characterised which had not been achieved in the literature previously.
CHAPTER 2. MATERIALS AND METHODS

2.1 Polymer Samples

All polymer samples, shown in Table 1, were synthesised and purified by the PhD candidate Emmanuelle Read from the Paul Sabatier University in Toulouse, France. All samples were synthesised using the MADIX polymerisation technique with the \( O \)-ethyl-S-(1-methoxycarbonyl)ethylthiocarbonate MADIX agent called Rhodixan A1 (Figure 17).

\[
\text{Figure 17. Chemical structure of the MADIX agent } O\text{-ethyl-S-(1-methoxycarbonyl)ethylthiocarbonate (Rhodixan A1).}
\]

2.1.1 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 stabilised with 100 ppm MEHQ, p-methoxyphenol) was obtained from SNF. \( O \)-ethyl-S-(1-methoxycarbonyl)ethylthiocarbonate 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 deionised water were used as solvent for polymer syntheses. Diethyl ether was used to purify the diblock copolymers.
### 2.1.2 Synthetic procedure and polymer samples

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Polymer</th>
<th>Theoretical molar mass (g mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA2k</td>
<td>PAA</td>
<td>2 000</td>
</tr>
<tr>
<td>PAA2kPAM10k</td>
<td>P(AA-♭-AM)</td>
<td>2 000-♭-10 000</td>
</tr>
<tr>
<td>PAA10k</td>
<td>PAA</td>
<td>10 000</td>
</tr>
<tr>
<td>PAA10kPAM10k</td>
<td>P(AA-♭-AM)</td>
<td>10 000-♭-10 000</td>
</tr>
<tr>
<td>PEO2kPAA10k</td>
<td>P(EO-♭-AA)</td>
<td>10 000-♭-2 000</td>
</tr>
<tr>
<td>PAA50k</td>
<td>PAA</td>
<td>50 000</td>
</tr>
<tr>
<td>PAPTAC5k</td>
<td>PAPTAC</td>
<td>5 000</td>
</tr>
<tr>
<td>PNIPAM5k</td>
<td>PNIPAM</td>
<td>5 000</td>
</tr>
<tr>
<td>PAPTAC5kPNIPAM5k</td>
<td>P(APTAC-♭-NIPAM)</td>
<td>5 000-♭-5 000</td>
</tr>
<tr>
<td>PAPTAC2k</td>
<td>PAPTAC</td>
<td>2 000</td>
</tr>
<tr>
<td>PAPTAC2kPNIPAM8k</td>
<td>P(APTAC-♭-NIPAM)</td>
<td>2 000-♭-8 000</td>
</tr>
<tr>
<td>PAPTAC2kPNIPAM3k</td>
<td>P(APTAC-♭-NIPAM)</td>
<td>2 000-♭-3 000</td>
</tr>
<tr>
<td>PAPTAC1k</td>
<td>PAPTAC</td>
<td>1 000</td>
</tr>
<tr>
<td>PAPTAC1kPNIPAM9k</td>
<td>P(APTAC-♭-NIPAM)</td>
<td>1 000-♭-9 000</td>
</tr>
<tr>
<td>PAPTAC1kPNIPAM3k</td>
<td>P(APTAC-♭-NIPAM)</td>
<td>1 000-♭-3 000</td>
</tr>
<tr>
<td>PAPTAC3k</td>
<td>PAPTAC</td>
<td>3 000</td>
</tr>
<tr>
<td>PAPTAC3kPNIPAM3k</td>
<td>P(APTAC-♭-NIPAM)</td>
<td>3 000-♭-3 000</td>
</tr>
<tr>
<td>PAPTAC6k</td>
<td>PAPTAC</td>
<td>6 000</td>
</tr>
<tr>
<td>PAPTAC6kPNIPAM3k</td>
<td>P(APTAC-♭-NIPAM)</td>
<td>6 000-♭-3 000</td>
</tr>
<tr>
<td>PAPTAC7.5k</td>
<td>PAPTAC</td>
<td>7 500</td>
</tr>
<tr>
<td>PAPTAC4k</td>
<td>PAPTAC</td>
<td>4 000</td>
</tr>
<tr>
<td>PAPTAC4kPAA1k</td>
<td>P(APTAC-♭-AA)</td>
<td>4 000-♭-1 000</td>
</tr>
<tr>
<td>PAPTAC4kPAA2k</td>
<td>P(APTAC-♭-AA)</td>
<td>4 000-♭-2 000</td>
</tr>
</tbody>
</table>
PAA homopolymers were synthesised as in [12]. PNIPAM homopolymer was synthesised as in [28].

The general synthesis of the P(AA-b-AM) block copolymers is as follows, with PAA2kPAM10k as an example: 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.

The general synthesis of the P(EO-b-AA) block copolymers is as follows, with PEO2kPAA10k as an example. In a Schlenk flask, a solution of AA (1.99 g, 27 mmol), PEO macroMADIX agent (0.42 g, 0.21 mmol) and ACVA (1.26 × 10^{-2} g, 4.49 × 10^{-2} mmol) and 2.57 g of deionised water was degassed by bubbling argon for 30 min before heating at 60 °C for more than 4 h. Conversion was monitored by ^1H NMR of aliquots until the completion (>99 %) of the reaction. Finally the aqueous solution of P(EO-b-AA) was freeze dried to give a light yellow powder.

PAPTAC homopolymers and P(APTAC-b-NIPAM) block copolymers were typically synthesised as follows, with PAPTAC7.5kPNIPAM12.5k as an example: 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 deionised 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 °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 deionised 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.

**P(APTAC-b-AA) block copolymers were typically synthesised as follows:** in a Schlenk flask, a solution of AA, PAPTAC solution, as mentioned above, ACVA and deionised water was degassed by bubbling argon for 30 min before heating at 60 °C for more than 4 h. The aqueous solution was freeze dried to produce a white powder.

### 2.2 Nuclear Magnetic Resonance (NMR) spectroscopy

All NMR characterisation of polymers was conducted by the Master candidate.

Deuterated 1,4-dioxane (99 %D) was supplied by Cambridge Isotope Laboratories, Inc. Tetramethylsilane (TMS, 99 %) was purchased from Sigma Aldrich.

PAA samples were dissolved in 1,4-dioxane-\(d_8\) to achieve the highest concentration, which was 200 g L\(^{-1}\) for PAA2k and 100 g L\(^{-1}\) for PAA10k.

All NMR spectra were recorded using a Bruker DRX300 spectrometer (BrukerBiospin Ltd, Sydney) equipped with a 5 mm dual \(^1\)H/\(^{13}\)C probe, at Larmor frequencies of 300.13 MHz for \(^1\)H and 75 MHz for \(^{13}\)C at room temperature (~25°C). Conventional one dimensional (1D) \(^1\)H NMR spectra were recorded with a 10 000 Hz spectral width, a 30° flip angle. Conventional 1D \(^{13}\)C NMR spectra were recorded with a 20 000 Hz spectral width, inverse-gated decoupling, a 90° flip angle. All \(^{13}\)C DEPT-135 spectra were recorded with a 20 000 Hz spectral width, a 90° \(^{13}\)C flip angle and a 135° \(^1\)H flip angle. \(T_1\) values for \(^1\)H and \(^{13}\)C nuclei were overestimated using the inversion recovery pulse sequence (Figure 16), which involved a 180° pulse followed by a variable delay (\(\tau\)) and then a 90° pulse. The number of scans recorded and repetition
delay between scans for each experiment are shown in Table 2. $^1$H and $^{13}$C chemical shift scales were calibrated to the dioxane signal at 3.53 ppm and 66.48 ppm respectively. The dioxane signal was calibrated from tetramethylsilane (TMS) dissolved in dioxane, with the TMS signal set to 0 ppm. All experimental data was acquired using Bruker Topspin 1.3 and processed with Bruker Topspin 3.0 software, it was then plotted in Origin Pro 8.5. The SNR was determined using the Bruker command ‘sino real’ in Topspin 1.3 and 3.0, where the noise was measured between 12 and 20 ppm for $^1$H NMR spectra and between 133 and 168 ppm for $^{13}$C NMR spectra.

$^1$H-$^1$H COrelation SpectroscopY (COSY) spectra were acquired using the Bruker ‘cosyqf’ pulse sequence. The spectral width was 3 000 Hz in both dimensions. For PAA2k 2 048 increments were recorded in the direct dimension and 256 increments were used in the indirect dimension. Parameters were the same for PAA10k except that 128 increments were used in the indirect dimension. The $^1$H-$^1$H COSY spectra were plotted with a 4 096 × 512 increment matrix. $^1$H-$^{13}$C Heteronuclear Multiple-Quantum Correlation spectroscopy (HMQC) spectra were acquired using the Bruker ‘hmqcgpqf’ pulse sequence. For PAA2k the indirect dimension ($^1$H) had a spectral width of 3 000 Hz with 2 048 increments recorded, while the direct dimension ($^{13}$C) had a spectral width of 15 100 Hz with 256 increments used. The indirect dimension ($^1$H) is the same for PAA10k but the direct dimension ($^{13}$C) had a spectral width of 13 600 Hz with 128 increments recorded. The $^1$H-$^{13}$C HMQC spectra were plotted with a 1 024 × 1 024 increment matrix.
Table 2. Summary of the number of scans recorded, repetition delay between scans and the instrument time for NMR spectroscopy experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sample</th>
<th>Number of scans recorded</th>
<th>Repetition delay between scans (s)</th>
<th>Instrument time#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D $^1$H inversion recovery</td>
<td>PAA2k</td>
<td>n.r.^</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td></td>
<td>PAA10k</td>
<td>8</td>
<td>25</td>
<td>5 min</td>
</tr>
<tr>
<td>1D $^1$H</td>
<td>PAA2k</td>
<td>128</td>
<td>25</td>
<td>1 h</td>
</tr>
<tr>
<td></td>
<td>PAA10k</td>
<td>128</td>
<td>25</td>
<td>1 h</td>
</tr>
<tr>
<td>2D $^1$H-$^1$H COSY</td>
<td>PAA2k</td>
<td>128</td>
<td>3</td>
<td>1 d 3 h</td>
</tr>
<tr>
<td></td>
<td>PAA10k</td>
<td>64</td>
<td>1</td>
<td>2 h</td>
</tr>
<tr>
<td>1D $^{13}$C inversion recovery</td>
<td>PAA2k</td>
<td>24 394</td>
<td>6</td>
<td>1 d 23 h</td>
</tr>
<tr>
<td></td>
<td>PAA10k</td>
<td>62 808</td>
<td>6</td>
<td>5 d</td>
</tr>
<tr>
<td>1D $^{13}$C</td>
<td>PAA2k</td>
<td>81 920</td>
<td>6</td>
<td>5 d 18 h</td>
</tr>
<tr>
<td></td>
<td>PAA10k</td>
<td>81 920</td>
<td>6</td>
<td>5 d 18 h</td>
</tr>
<tr>
<td>1D $^{13}$C DEPT-135</td>
<td>PAA2k</td>
<td>81 920</td>
<td>6</td>
<td>5 d 18 h</td>
</tr>
<tr>
<td></td>
<td>PAA10k</td>
<td>81 920</td>
<td>6</td>
<td>5 d 18 h</td>
</tr>
<tr>
<td>2D $^1$H-$^{13}$C HMQC</td>
<td>PAA2k</td>
<td>164</td>
<td>3</td>
<td>17 h</td>
</tr>
<tr>
<td></td>
<td>PAA10k</td>
<td>900</td>
<td>3</td>
<td>3 d 23 h</td>
</tr>
</tbody>
</table>

^ n.r. stands for not recorded. * time is given to the nearest hour when instrument time was greater than 50 min, with d representing days, h hours and min minutes.
2.3 Capillary Electrophoresis (CE)

All CE characterisation was conducted by the Master candidate.

2.3.1 Materials

Water of MilliQ quality (Millipore, Bedford, MA, USA) was used for CE-CC. Standard fused-silica capillaries (50 µm i.d., 360 µm o.d.) were obtained from Polymicro (Phoenix, AZ, USA). 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 %) was purchased from BDH AnalR, Merck Pty Limited. Phosphoric acid (≥99.0 %) was purchased from Fusions (Homebush, Australia). Hexaamminecobalt(III) chloride (≥99.5 %), absolute ethanol, dimethyl sulfoxide (DMSO, >99 %), were supplied by Sigma Chemical company.

2.3.2 Free Solution Capillary Electrophoresis procedure

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. All electropherograms shown are at 200 nm. 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 where performed at 30 kV and 25 ºC unless specified. The injection concentration of each sample is 5 g L⁻¹ unless specified. Data was acquired using Chemstation A.10.01 and plotted, integrated, the absorbance was corrected and migration time was converted to electrophoretic mobility.
using OriginPro 8.5. The peak areas were corrected for time in the resonance window by dividing them by the migration time at the relevant apex.

2.3.2.1 PAA based polymer 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 400 µL sample to mark the electro-osmotic flow (EOF). 110 mM sodium borate buffer (NB110, pH 9.2) was prepared as stated in reference [100]. 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) or a standard fused-silica capillary with a total length 60.1 cm (effective length 51.6 cm) unless stated otherwise. The capillaries were 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 separations involved a 2 min flush with 1 M NaOH followed by a 5 min flush with NB110. After the last separation, 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.

2.3.2.2 PAPTAC based polymer samples

PAPTAC-based samples were dissolved at 5 g L\(^{-1}\) in 1 mM \([\text{Co(NH}_3)_6]\text{Cl}_3\) aqueous solution unless stated otherwise. 10 mM Na\(_2\)PO\(_4\) (PB10) was prepared by diluting 1 M H\(_3\)PO\(_4\) to 0.5 M; the resulting solution was titrated to pH 2 with 1 M NaOH, and then diluted to 100 mM and referred to as PB100. The PB100 solution was then diluted to 10 mM to produce PB10. Preconditioning involved a 20 min flush with 10 mM H\(_3\)PO\(_4\) 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 separation, the capillary was flushed for 20 min with 10 mM H\(_3\)PO\(_4\), for 5 min with ethanol, for 20 min with water and for 10 min with air.
When a FC coated capillary was used such as for the P(APTAC-\(b\)-AA) samples the capillary was pre-treated by flushing for 2 min with 0.1 M NaOH, 15 min with water and 10 min with PB100. The preconditioning involved a 2 min flush with water followed by 5 min flush with PB100. After the last injection, the capillary was flushed 2 min with water, 2 min with 0.1 M NaOH, 20 min with water and 10 min with air.

2.3.3 Pressure mobilisation and pressure assisted CE-CC

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

2.3.4 Determining electrophoretic mobility

The CE instrument records the time it takes the analytes to migrate to the detection window, indicated by the detector, thus giving a plot as a function of migration time. The migration time of the analyte varies with the capillary length and the applied voltage as well as the strength of the electro-osmotic flow (EOF). The EOF is influenced by the pH, viscosity and concentration of the background electrolyte (BGE) and the temperature. Therefore slight variations from measurement to measurement in capillary temperature, BGE concentration, pH and salt concentration can result in low repeatability of migration time. To provide a means of accounting for these variations and a means of universal comparison between capillary lengths and voltages the migration time \( t_m \) was converted to electrophoretic mobility \( \mu \) using Equation (10):

\[
\mu = \frac{l_d l_t}{V} \left( \frac{1}{t_m} - \frac{1}{t_{EOF}} \right)
\]
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, $t_{\text{EOF}}$ is the migration time of the (EOF) marker [171]. By correcting for changes in the EOF slight variations were accounted for.

In the case of the cationic polymers which were separated at pH 2, with a neutral coated capillary there is no measurable EOF within 60 min so the $t_{\text{EOF}}$ term is negligible in the determination of electrophoretic mobility. The internal standard hexaaminocobalt(III) chloride was used to correct for injection to injection variations to give an apparent mobility. Therefore Equation (11) was used to calculate the $\mu$ of the cationic polymers:

$$
\mu = \frac{l_d l_t}{V} \left( \frac{1}{t_{\text{m}}} \cdot \frac{t_{\text{ref}}}{t_{\text{is}}} \right)
$$

where $t_{\text{ref}}$ is the average migration time of the peak maximum of the internal standard and $t_{\text{is}}$ 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 taken as equal to 1. $t_{\text{ref}}$ was $0.771 \pm 0.009$ min (RSD 1.19 %, n=30) for CE-CC and $0.673 \pm 0.007$ min (RSD 1.07 %, n=7) for pressure assisted CE-CC.

### 2.4 Size Exclusion Chromatography (SEC)

SEC experiments were performed by PhD candidate Emmanuelle Read from the Paul Sabatier University in Toulouse, France and by PhD candidates Alison Maniego and Joel Thevarajah from the University of Western Sydney (UWS) in Parramatta, Australia. These results are referred to as Toulouse and UWS experiments respectively. PAA based samples were analysed in Toulouse and UWS while PAPTAC based samples were only analysed in Toulouse.
2.4.1 Materials

Water of MilliQ quality (Millipore, Bedford, MA, USA) was used all SECs. Sodium azide (99 %) was purchased from BDH AnalR, Merck Pty Limited. 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$_2$O) were supplied by Sigma Chemical company. Pullulan standards were purchased from PSS (Mainz, Germany).

2.4.2 SEC of PAA based samples

2.4.2.1 Toulouse SEC conditions

SEC analysis was conducted on a system equipped with an on-line degasser, a Waters chromatography (Milford, MA) model 1515 isocratic pump, an autosampler (Waters 717), a refractometer (RI-101, Shodex) thermostated at 25°C, a UV absorption detector (Prostar, Varian), refractive index detector (RI-101, Shodex) and a MALLS (Dawn Heleos, 18 angles, Wyatt Technology). The samples were eluted through a three-column set (8mm*300mm, 6µm particle size, 2* SB 806 M HQ columns and one SB 802.5 HQ protected by a guard column ShodexOHPak SB806-M) with an aqueous eluent containing 0.1 M NaNO$_3$ and 100 ppm NaN$_3$ at a flow rate of 1 mL·min$^{-1}$. All samples were filtered through 0.45 µm membrane filter before injection. Injection concentrations of the samples are shown in Table 3.
Table 3. Injection concentrations of polymer samples analysed by different SEC systems.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Injection concentration (g L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toulouse</td>
</tr>
<tr>
<td>PAA2k</td>
<td>~5</td>
</tr>
<tr>
<td>PAA10k</td>
<td>~5</td>
</tr>
<tr>
<td>PAA50k</td>
<td>~5</td>
</tr>
<tr>
<td>PAA2kPAM10k</td>
<td>~5</td>
</tr>
<tr>
<td>PAA10kPAM10k</td>
<td>~5</td>
</tr>
<tr>
<td>PAA2k, PAA2kPAM10k Mixture*</td>
<td>~2.5</td>
</tr>
<tr>
<td>PAA10k, PAA10kPAM10k Mixture*</td>
<td>~2.5</td>
</tr>
<tr>
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* injection concentration of each individual polymer sample is given. ^ A dash indicates the sample was not analysed in that system.

### 2.4.2.2 UWS SEC conditions

SEC of 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 Å, particle size of 5 µm and one 30 Å, particle size of 5 µm) from Polymer Standards Service (PSS, Mainz) with an aqueous eluent containing 0.1 mol L$^{-1}$ Na$_2$HPO$_4$ and 200 ppm NaN$_3$ at 50 °C and 1 mL·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 a 0.45 µm PES or PVDF membrane filter before injection.
The system was calibrated using 10 pullulan standards ranging from 342 to 708 000 g·mol⁻¹ (molar mass at the peak) with dispersity inferior to 1.27. The obtained calibration curve was fitted with a 4th order polynomial: \[ \log M = 142.9 - 20.76x + 1.184x^2 - 0.0301x^3 + 0.0002824x^4 \] \((R^2=0.9994)\). Injection concentrations of the samples are shown in Table 3.

### 2.4.3 SEC of PAPTAC based samples

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 \(\text{NH}_4\text{NO}_3\) solution of water/acetonitrile (80/20, w/w) containing 100 ppm of PDADMAC (flowrate 1 mL·min⁻¹). All samples were filtered through a 0.45 µm filter before injection. The data were recorded using Astra and plotted using OriginPro 8.5. Injection concentrations of the samples are shown in Table 3.
CHAPTER 3. CHARACTERISATION OF HOMOPOLYMERS

The aim of the work in this chapter was to obtain a comprehensive characterisation of the homopolymers used to produce the block copolymers of interest; these are the PAA and the cationic PAPTAC homopolymers. The degree of branching and the number-average molar mass were determined using NMR spectroscopy. The heterogeneity of branching and the molar mass were assessed by CE-CC. The molar mass determined by SEC was compared to that obtained NMR spectroscopy.

3.1 Structure Identification by Solution-State NMR Spectroscopy

3.1.1 One dimensional $^1$H NMR spectroscopy

During a polymerisation a number of different reactions can take place which may result in degradation of the end group, different end groups, chemical branches, etc. NMR spectroscopy can assist in identifying if these reactions have taken place. Routine NMR analysis is commonly used to monitor the conversion of the monomer units and estimate the number-average molar mass [172]. However, assigning all signals in an NMR spectrum is not common with routine conditions and polymers, especially since the broad nature of the peaks often prevents the observation of splitting patterns. Furthermore signals of expected equal intensity, such as those corresponding to end groups, may not be equal due to degradation and side reactions. Previous studies have used two dimensional (2D) NMR spectroscopy including $^1$H-$^1$H CORrelation SpectroscopY (COSY) and $^1$H-$^{13}$C Heteronuclear Multiple-Quantum Correlation spectroscopy (HMQC) to identify the degradation of polymer end groups [173]. Hence an accurate assessment of the polymer samples can be made using a comprehensive NMR analysis.
Performing a thorough NMR analysis including recording one-dimensional $^1$H, conventional one-dimensional $^{13}$C, Distortionless Enhancement by Polarisation Transfer (DEPT), $^1$H-$^1$H COSY and $^1$H-$^{13}$C HMQC spectra requires long instrument time so only PAA2k and PAA10k were analysed. The expected structure of PAA is shown in Figure 18a. The structure of the branches is shown in Figure 18b where the end of the branch shown as groups 17 to 19 would also produce signals which would also appear for ‘dead’ chains which have a hydrogen end group. Tables 4 and 5 show the identified signals from the $^1$H and $^{13}$C NMR spectra respectively.

![Figure 18](image_url)

**Figure 18.** a) Structure of PAA synthesised with MADIX, b) short chain branch in PAA chemical structure where R represents the continuation of the polymer chain. The short chain branch can range in length from one monomer unit to the length of a small oligomer chain. Numbers are the labels used for NMR signal assignment.

From the $^1$H NMR spectra of PAA2k and PAA10k the backbone signals are clearly present between 1.40 and 2.86 ppm (Figure 19 and 20). The hydroxyl signal is also present as a very broad signal between 5.5 and 10.0 ppm. Overlapping with the hydroxyl signal are multiple sharp signals between 5.5 and 6.5 ppm which correspond to the vinylic protons from the residual monomers (Figures 19a and 20a).
Identifying particular end groups in the $^1$H NMR spectra was difficult due to the broadness of the signals, meaning that splitting patterns were rarely present. This was not due to imperfect experimental setup (in particular, sufficient shimming was shown by the splitting patterns observed for the small molecules of residual acrylic acid), but to the intrinsic slow molecular motions of large polymer molecules in solution. Since no NMR spectrum of PAA synthesised with this particular MADIX agent was previously published the signals were assigned through comparison with literature values for similar PAA samples, NMR spectra of the MADIX agent provided by the Paul Sabatier University (Figure A-1 in appendix) and theoretical estimates of chemical shifts with ChemDraw Ultra 7.0 (Tables 4 and 5). Due to different solvents being used in the literature to obtain NMR spectra there was some variation between the observed chemical shifts and that reported in the literature. Some end group signals could be identified such as S4 (S represents an NMR signal and 4 is the assigned group in the molecule shown in Figure 18) as it exhibited a triplet splitting pattern and was at 1.35 ppm which was in agreement with the chemical shifts in the literature (Figure 19b and 20b, Table 4). However this signal is clearly overlapping with the backbone signals to account for this a resolved end group signal is needed to subtract its integral from the integral backbone region in order to use the backbone region in calculations such as $M_n$ determination. The majority of the other signals could not be accurately assigned due to similar chemical shifts between signals. To further assign some of the end groups signals a $^1$H-$^1$H COSY spectrum was obtained.
Figure 19. $^1$H NMR spectrum of PAA2k in dioxane-$d_8$: a) full spectrum with insert showing the vinylic signals from the residual monomers, b) region of the spectrum containing most signals of interest.
Figure 20. $^1$H NMR spectrum of PAA10k in dioxane-$d_8$: a) full spectrum with insert showing the vinylic signals from the residual monomers, b) region of the spectrum containing most signals of interest.
Table 4. Chemical shifts (in ppm) of $^1$H NMR signals identified for PAA2k and PAA10k in dioxane-$d_8$; comparison to reported literature values (with solvent indicated in brackets) and theoretical estimates with ChemDraw Ultra 7.0.

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*GN stands for group number; the group labels are shown in Figure 18. EST stands for estimates with ChemDraw Ultra 7.0. OL indicates that the signal is OverLapping with other signals in the spectrum and so no chemical shift could be accurately selected. Dashes (-) indicate that no chemical shift value for that signal was reported in that reference or that no signal would be present in that the ^1^H spectrum.
3.1.2 Two dimensional $^1$H-$^1$H COSY

From the COSY spectra some end groups signals could be assigned through to their correlation to other signals. In the COSY spectra a signal is produced with chemical shifts corresponding to the 1D NMR spectra which constitute each dimension. The signals with the corresponding chemical shifts in the 1D NMR spectra are thus shown to correlate to each other. S4 showed coupling to the signal at 4.60 ppm indicating that it is the CH$_2$ in the end group labelled as S5 (Figures 21 and 22). Furthermore the signal at 3.58 ppm showed no correlation to any other signals confirming that it is the methoxy group in the end group labelled as S16. A small quartet signal was observed around 3.6 ppm (Figure 19b) in PAA2k. These chemical shifts are very similar to those of ethanol in solvents similar to dioxane-$d_8$ [175], indicating a minute amount of residual ethanol is present in PAA2k sample while none was detected in PAA10k. The COSY results were also able to identify how many end group signals were overlapping with the backbone region. S14 (1.08 ppm) was assigned to the methyl group adjacent to the methyne carbon in the end group by comparison of the chemical shift to literature values. S14 exhibited correlation to the backbone region indicating that the methyne signal, S13, is overlapping with the backbone region (Figures 21 and 22). Although the COSY provided useful information a number of signals were still left unassigned, in order to link the information gathered from the $^1$H and $^{13}$C NMR spectra a $^1$H-$^{13}$C HMQC spectrum was recorded to complete the signal assignment, this is discussed in section 3.1.4.
Figure 21. $^1$H-$^1$H COSY spectrum of PAA2k in dioxane-$d_8$. Black lines indicate couplings. 1D spectra are shown as projections.

Figure 22. $^1$H-$^1$H COSY spectrum of PAA10k in dioxane-$d_8$. Black lines indicate coupling. 1D spectra are shown as projections.
3.1.3 One dimensional $^{13}$C NMR spectroscopy

The quantitative $^{13}$C NMR spectra and the DEPT-135 spectra of PAA2k and PAA10k are shown in Figures 23 and 24. The signals in Figure 23 and 24 were initially assigned in a similar manner to the $^1$H NMR spectra, by comparison with published chemical shift values and estimates from ChemDraw Ultra 7.0, with additional confirmation from DEPT-135 spectra (Table 5). In a DEPT-135 spectrum the signals from a quaternary carbon are absent, methyl and methyne groups exhibit positive signals and methylene groups exhibit negative signals. The backbone signals where clearly present between 33.8 and 46.3 ppm (Figure 23a and 24a). The carbonyl of the carboxylic acid group was also present between 174.0 and 180.0 ppm. The end group signals have significantly lower SNR than on the $^1$H NMR spectra but can still be identified. The xanthate carbon (S6) in the end group was easily assigned due to its distinctive chemical shift at 212.0 ppm (Figure 23a and 24c). The ester carbon (S15) in the end group was also identified since ester carbonyl tend to be more upfield than those in carboxylic acids, furthermore the signal at 171.7 ppm is shown to decrease in relative intensity between PAA2k and PAA10k indicating that it is part of the end groups (Figures 23a and 24c), since the higher molar mass PAA10k has less end groups per monomer unit. In Figure 23b a positive signal is present at 37.9 ppm amongst the backbone signals in the DEPT-135 spectrum. Based on the theoretically estimated chemical shifts in Table 5, this positive signal is the methyne carbon in the end group assigned to S13. As mentioned in section 3.1.2 ethanol was detected in PAA2k, a corresponding signal was observed at 57.8 ppm in the $^{13}$C NMR spectrum of PAA2k although the lower sensitivity of $^{13}$C NMR spectroscopy makes it difficult to identify the corresponding methyl signal amongst the end group signals. Furthermore the accompanying methyl signal could not be identified as it is likely overlapping with other signals.
Figure 23. $^{13}$C NMR spectra of PAA2k in dioxane-$d_8$: a) full quantitative $^{13}$C NMR spectrum with carbonyl signals shown in insert, b) 0 to 80 ppm region of DEPT-135 spectrum with insert showing the region of the branching signal, and c) 0 to 80 ppm region of quantitative $^{13}$C NMR spectrum with insert showing the region of the branching signal.
Figure 24. $^{13}$C NMR spectra of PAA10k in dioxane-$d_8$: a) full quantitative $^{13}$C NMR spectrum, b) carbonyl region of DEPT-135 spectrum, c) carbonyl region of quantitative $^{13}$C NMR spectrum, d) 0 to 80 ppm region of DEPT-135 spectrum, and e) 0 to 80 ppm region of quantitative $^{13}$C NMR spectrum.
Table 5. Chemical shifts (in ppm) of $^{13}$C NMR signals identified for PAA2k and PAA10k; comparison to reported literature values and theoretical estimates determined by ChemDraw Ultra 7.0.

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<th>Nuclei</th>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>171.6</td>
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<tr>
<td>14</td>
<td>CH$_3$-O</td>
<td>51.7</td>
<td>51.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>47.9</td>
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<tr>
<td>15</td>
<td>CH$_2$-CH$_2$</td>
<td>OL</td>
<td>OL</td>
<td>-</td>
<td>27.5-30.0</td>
<td>28.0</td>
<td>33.4</td>
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<td>24.6</td>
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<tr>
<td>16</td>
<td>CH$_2$-CH$_2$</td>
<td>OL</td>
<td>OL</td>
<td>-</td>
<td>30.5-33.0</td>
<td>37.5</td>
<td>31.5</td>
<td>-</td>
<td>33.0</td>
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<tr>
<td>GN</td>
<td>Group Label</td>
<td>Chemical Shifts</td>
<td>Integration</td>
<td>Dipole Coupling</td>
<td>1H NMR</td>
<td>13C NMR</td>
<td>Remarks</td>
<td></td>
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<tr>
<td>19</td>
<td>CH₂-COOH</td>
<td>OL</td>
<td>OL</td>
<td>-</td>
<td>-</td>
<td>181.3</td>
<td>175.0</td>
<td>OL</td>
<td></td>
<td></td>
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<tr>
<td>20</td>
<td>CH-CH₂-Cq</td>
<td>OL</td>
<td>OL</td>
<td>48.5-50.0</td>
<td>47.4</td>
<td>-</td>
<td>-</td>
<td>OL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>CH-CH₂-Cq</td>
<td>OL</td>
<td>OL</td>
<td>38.0-39.0</td>
<td>36.4</td>
<td>-</td>
<td>-</td>
<td>OL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>CH-CH₂-Cq</td>
<td>47.8-49.1</td>
<td>48.0-49.1</td>
<td>41.8-43.4</td>
<td>39.3</td>
<td>-</td>
<td>-</td>
<td>OL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*GN stands for group number; the group labels are shown in Figure 1. EST is estimate. OL indicates that the signal is OverLapping with other signals in the spectrum and so no chemical could be accurately selected. Dashes (-) indicate that no chemical shift value for that signal was reported in that reference or that no signal would be present in that the $^{13}$C NMR spectrum.
A relatively broad signal observed around 48 ppm is consistent with a quaternary carbon at a branching point. This signal was confirmed to be from a quaternary carbon in both samples with a DEPT-135 spectrum (Figure 23b and 24d). The DEPT-135 spectra were obtained with the same number of scans recorded as the $^{13}$C NMR spectra to ensure the disappearance of a signal was not due to insufficient sensitivity. Since no signal at 48 ppm was observed in the DEPT-135 spectra there was clearly branching present in both samples (Figure 22c and 23e).

Assigning each signal proved difficult since some signals did not clearly correspond to any chemical shifts in the literature. Furthermore, a signal is present in the DEPT-135 spectrum with the same chemical shift as the carbonyl signal of the carboxylic acid in PAA (S3), although carbonyl signal should not be present in the DEPT-135. To assign this peak and that of other signals a $^1$H-$^{13}$C HMQC was recorded for each sample to link the $^1$H NMR with the $^{13}$C NMR spectra.

3.1.4 Two dimensional $^1$H-$^{13}$C HMQC

In $^1$H-$^{13}$C HMQC spectra a signal is produced with chemical shifts corresponding to 1D $^1$H and $^{13}$C NMR spectra. The signals with the corresponding chemical shifts in the 1D spectra are thus shown to correlate to each other thus showing the hydrogen and carbon coupling in a sample. By knowing the correlation of the carbon and hydrogen nuclei, information gathered in one spectrum can be used to identify signals in another. For example the detection of S5 and S16 in the $^1$H NMR spectrum was discussed in 3.1.2. From the $^1$H-$^{13}$C HMQC spectrum the S5 signal at 4.60 ppm in the $^1$H NMR spectrum showed correlate to the signal at 71.3 ppm in the $^{13}$C NMR spectrum thus this $^{13}$C NMR signal can be assigned to S5 (Figures 25 and 26). The S16 signal at 3.58 ppm in the $^1$H NMR spectrum was shown to correlate to the signal at 51.7 ppm in the $^{13}$C NMR spectrum allowing this signal to be assigned to S16.

The signals between 50 and 51 ppm in the $^{13}$C NMR spectra are shown to correlate to the $^1$H NMR signal at 4.31 ppm (Figures 25 and 26). In turn this $^1$H NMR
signal in the COSY spectrum is shown to couple to the peak at 2.12 ppm in the $^1$H NMR spectrum (Figures 19b and 20b). The HMQC spectrum then shows that this signal correlates to the signals around 33 ppm in the $^{13}$C NMR spectrum, which are shown to be methylene carbons in the DEPT-135 spectrum. No further coupling of these signals could be clearly observed. Based on this information and on the comparison to literature values, this signal likely refers to the monomer units adjacent to the xanthate group. Further supporting this is that the signal at 2.12 ppm in the $^1$H NMR spectrum of PAA10k has a lower relative intensity than in that of PAA2k, which is expected since signals related to the end group would have a lower relative intensity in PAA10k and thus these signals were assigned S7 and S8 as shown in Tables 4 and 5.

In the HMQC spectrum a small cross peak was observed from the carbonyl region in the $^{13}$C NMR spectrum and in the backbone region in the $^1$H NMR spectrum. Carbon nuclei in a $\pi$ system can exhibit long range coupling to hydrogen on adjacent nuclei [176], so the signal in the DEPT-135 spectrum at 178 ppm may be caused by coupling to the methyne in the backbone. This weak coupling would not normally be observed expect for the large number of scans recorded for the DEPT-135 spectra. Although a pulse sequence was used to remove coupling of the carbon and hydrogen nuclei there may still be some weak, long range coupling present.

Although the majority of the signals in the spectra were identified some cannot be clearly assigned. These signals may relate to contamination or degradation in the PAA samples. Most of these signals are present with a very small relative intensity and thus may not have a significant effect on the properties of the sample. The signal at 60 ppm in the $^{13}$C NMR spectra is shown to be from a methylene group in the DEPT-135 spectra but cannot be clearly assigned. A methylene group is present in chain ends that consist of only a hydrogen (S18, Figure 18). However this chemical shift is expected at 33 ppm (Table 5), which does not correspond to the observed 60 ppm. Therefore the signals representing the ‘dead’ chains are likely overlapping with the backbone signals. Potential residual compounds from the synthesis also do not contain groups with a $^{13}$C NMR signal at 60 ppm. To assign the remaining unknown signals further
experimentation including other 2D techniques would be required. However these spectra would require long measuring times and the benefit from assigning these signals is unlikely to outweigh the time and effort required. With all end group signals and the branching signal clearly identified the $M_n$ and $DB$ can be determined. In addition the signal assignment confirms that the expected chemical structure of the PAA polymer occurred.

Figure 25. $^1$H-$^{13}$C HMQC spectrum of PAA2k in dioxane-$d_8$. Numbers represent the group assigned to cross peaks of interest (see Figure 18). 1D spectra are shown as projections.
Figure 26. $^1$H-$^{13}$C HMQC spectrum of PAA10k in dioxane-$d_8$. Numbers represent the group assigned to cross peaks of interest (see Figure 18). 1D spectra are shown as projections.

3.2 Molar Mass of Homopolymers

3.2.1 Determining conditions to perform quantitative NMR spectroscopy

To determine a quantity from an NMR spectrum quantitative conditions must be established. Although the branching has been identified and estimated in PAA there has been no clear determination of the degree of branching with quantitative conditions. This is usually due to the long time required to ensure quantitative conditions and then performing the experiment with such conditions. It is important to note that obtaining a quantitative spectrum is possible for any nucleus. To ensure a spectrum is quantitative, the relaxation time between radiofrequency pulses must be sufficient for all the nuclei of interest to return to their equilibrium state. If this time is not given, the corresponding
signal exhibits a smaller intensity, leading to distorted relative intensities. In the case of $^{13}$C NMR spectroscopy to prevent distortions due to the Nuclear Overhauser Effect (NOE) the inverse-gated decoupling sequence was used [121]. Otherwise carbon nuclei attached to more adjacent hydrogens would have a greater intensity then those with less adjacent hydrogens.

When a radiofrequency pulse is applied there is a net change in the magnetisation. Over time the magnetisation returns to equilibrium. The time constant involved in the process of returning to equilibrium is referred to as the longitudinal relaxation time $T_1$. The time required to allow the majority (99.33 %) of the nuclei to return to their equilibrium state is five times $T_1$ [120]. $T_1$ can be determined using the inversion recovery pulse sequence (Figure 16). The pulse sequence involves an initial 180º pulse which causes a change in the net magnetisation. A variable evolution time is then given ($\tau$). This is followed by a 90º pulse. If the time $\tau$ is sufficient to allow the magnetisation to reach equilibrium, a positive signal of maximal intensity is detected, while if there is not, the signals would be of lower intensity, ranging from fully negative to positive. The relationship between the signal intensity ($\sigma_t$) and the time ($\tau$) is related to the $T_1$ as shown in Equation (12) [121]:

$$\sigma_t = \sigma_0(1 - 2e^{-\tau/T_1})$$   \hspace{1cm} (12)

where $\sigma_0$ is the intensity when the magnetisation has reached complete equilibrium. Applying Equation (10) to determine $T_1$ accurately requires running several spectra, usually as a two-dimensional experiment. To record quantitative spectra where the relaxation time is five times greater than $T_1$ a simpler approach can be taken.

When $\tau$ is set to produce a signal with no intensity, thus $\sigma_t= 0$ the following equation can be determined from Equation (12):

$$T_1 = \frac{\tau}{\ln 2}$$   \hspace{1cm} (13)

If $\tau$ is such that it produces a positive signal then inputting that $\tau$ value will produce an overestimate of $T_1$. If the relaxation time is set to five times this value, it is surely sufficient as the $T_1$ value is overestimated.
The described test for $T_1$ was applied to $^1$H NMR of PAA10k and it was assumed to be sufficient for PAA2k. It was found that 25 s between the radiofrequency pulses was sufficient to achieve a quantitative $^1$H NMR spectrum of PAA for all signals (Figure A-2). With quantitative conditions established accurate values can be calculated from the spectrum. For example after subtraction of the hydroxyl signal it was found that less than 0.5% of the monomers were not converting into the polymer chains.

Determining whether a proton signal is positive or negative takes a relatively short amount of time, while it takes significantly longer for a carbon signal due to its lower receptivity. The $T_1$ for both PAA2k and PAA10k was estimated by testing a $\tau$ of 0.832 s to achieve a quantitative $^{13}$C NMR spectrum of PAA. Using Equation (13) the estimation of $T_1$ is 1.2 s. From the estimated $T_1$ quantitative conditions are established with 6 s between the scans. This is shown in Figure 27 where all signals related to the polymer sample, including the branching signals around 48 ppm, are positive while the solvent peak is negative. Some of the end group signals between 180 and 220 ppm are difficult to identify for PAA10k (Figure 27b). Using the inversion recovery pulse sequence, a positive signal means an overestimation of $T_1$ while no signal means an exact value of $T_1$ when calculated with Equation (13). Although the SNR is not high enough to detect a positive peak it is enough to estimate $T_1$, especially considering 62808 scans (obtained over 5 days) were used.
Figure 27. Estimation of $T_1$ using the one-dimensional inversion recovery pulse sequence (black) and original $^{13}$C NMR spectra (blue, also presented in Figure 23a and 24a) for a) PAA2k (24 394 scans, 2 days), and b) PAA10k (62 808 scans, 5 days). Inserts in figures show the quaternary carbon signal from the branching point.
3.2.2 Number average molar mass of PAA by NMR spectroscopy

3.2.2.1 Developing the NMR method

NMR spectroscopy has commonly been used in the literature to determine the number average molar mass ($M_n$). $M_n$ is defined as the total weight ($w$) of all polymer chains divided by the total number of polymer chains ($N_m$) present and is calculated using Equations (14 and 15):

$$M_n = \frac{w}{N_m} \quad (14)$$

$$M_n = \frac{\sum_i N_i M_i}{N_m} \quad (15)$$

where $M_i$ is the molar mass of a polymer chain of length $i$ and $N_i$ is the number of polymer chains of length $i$. Therefore the NMR signals representing the monomer units can be used to represent $w$ while the signals corresponding to the ends of the polymer chains can represent $N_m$. From this Equation (16 and 17) can be used to determine the $M_n$ of the polymers samples:

$$M_n = \left[ \frac{I(CH_{backbone} + CH_2_{backbone} + xCH_{y,end\,group}) - xl(CH_{y,end\,group})}{z I(CH_{y,end\,group})} \right]_{M_m} M_m \quad (16)$$

$$M_n = \left[ \frac{I(C = O_{carbonyl} + xC_{end\,group}) - xl(C_{end\,group})}{I(C_{end\,group})} \right]_{M_m} M_m \quad (17)$$

where $I$ is the integral of the signal, $M_m$ is the molar mass of the monomers unit, $x$ is the number of overlapping signals and $y$ relates to whether the signal is a methyl, methylene, etc. and $z$ is the number of relevant nuclei in the monomer unit backbone (2 for $^{13}$C NMR and 3 for $^1$H NMR). Equation (16) was used for both $^{13}$C and $^1$H NMR spectra while Equation (17) only applies to the $^{13}$C NMR spectra. When integrating the backbone and carbonyl region of the spectrum a number ($x$) of end group signals are also included, denoted by the end group term in the first integral of Equations (16 and
17). Since the area of the end group signals overlapping with the backbone and carbonyl region should have the same area as those not overlapping. Therefore different end group signals can be used to subtract the area of any overlapping end group signals, this is denoted in Equations (16 and 17) by the second integral in the numerator. To obtain accurate values from an NMR spectrum, quantitative conditions must be established for all signals of interest, shown in section 3.2.1.

It is often not acknowledged that in a polymer sample there is a distribution of end groups. Although one signal may correspond to the majority of chains, it is unlikely that one end group signal corresponds to all the chains in sample. Termination and non-reversible transfer reactions, potential autoinitiation and degradation of the end groups are possible causes of a distribution of end groups in the sample. Furthermore it is unlikely that all backbone signals exist in the same chemical shift region as those signals corresponding to monomer units closer to the end group may have significantly different chemical shift. Therefore the $M_n$ values given by NMR spectroscopy are assuming that all backbone signals are in that region and that all polymer chains have the same end group. These assumptions may explain some of the variations in the $M_n$ values reported in Table 6.

Comparing quantifications by $^{13}$C and $^1$H NMR spectroscopy is not common in the literature since establishing quantitative conditions for $^{13}$C NMR spectroscopy is time consuming. Furthermore it is often not possible to obtain sufficient resolution for some signals in $^1$H NMR spectroscopy and in the case of $^{13}$C NMR spectroscopy a high enough SNR may not be possible [177]. The accuracy of the $M_n$ determined by $^1$H NMR may thus be more limited than with $^{13}$C NMR, while the precision of the $M_n$ by $^{13}$C NMR is lower than with $^1$H NMR spectroscopy. However, in this work the $^{13}$C NMR spectroscopy conditions allow for an accurate comparison. Typically $^{13}$C NMR spectroscopy leads to resolved signals since it has a larger spectral width than $^1$H NMR spectroscopy [177]. However due to the limited receptivity of $^{13}$C NMR spectroscopy its sensitivity is far lower than that of $^1$H NMR spectroscopy. Hence if the measuring time
is available (2-5 days) to obtain sufficient SNR in $^{13}$C NMR spectroscopy it is more accurate than $^1$H NMR spectroscopy for determining $M_n$.

### 3.2.2.2 Determining $M_n$ values for PAA

**Table 6.** $M_n$ values (in g mol$^{-1}$) determined for PAA2k and PAA10k by $^1$H and $^{13}$C NMR spectroscopy using different end group signals.

<table>
<thead>
<tr>
<th>Sample</th>
<th>End group signal</th>
<th>$^1$H</th>
<th>$^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Backbone, Equation (16)</td>
<td>RSD (%) *</td>
</tr>
<tr>
<td>PAA2k</td>
<td>S14 CH$_3$-CH</td>
<td>1 750</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>S5 CH$_2$-O</td>
<td>1 970</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>S4 CH$_3$-CH$_2$</td>
<td>2 480</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>S6 C=S</td>
<td>ND $^\wedge$</td>
<td>ND</td>
</tr>
<tr>
<td>PAA10k</td>
<td>S14 CH$_3$-CH</td>
<td>5 210</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>S5 CH$_2$-O</td>
<td>11 800</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>S4 CH$_3$-CH$_2$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>S6 C=S</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^\wedge$ ND stands for not determined, which was the case was if there was no corresponding hydrogen signal, the end group signal was overlapping or the RSD on the signal was over 20 %. * RSD values determined from the SNR of the end group signal and calculated using Equation (4). The RSD calculated assumes that all error comes from the SNR although errors from inaccuracies in phasing and integration error may cause an RSD of around 1 %.
\(^{13}\)C NMR measurements were performed with sufficient number of scans (80 000) for PAA2k to obtain a strong SNR with the RSD of \(M_n\) values being between 0.7 and 4.3 \%. In the case of PAA10k the higher molar mass resulted in lower sensitivity of the end groups with their RSD between 10 and 40 \% in the \(^{13}\)C NMR spectrum, thus less signals could be used to determine the \(M_n\). For both samples more signals were available in the \(^{13}\)C NMR spectra than in the \(^1\)H NMR spectra to determine the \(M_n\) because of signals overlapping. Due to the RSD values in the \(^{13}\)C NMR spectra only end group signals with an RSD lower than 20 \% were used and values were only presented to 2 significant figures (Table 6).

There are slight differences between the \(M_n\) values determined using the backbone and carbonyl signals from the \(^{13}\)C NMR spectra. There was also a 10-20 \% difference between the \(M_n\) values from the \(^{13}\)C and \(^1\)H NMR spectra. There was one outlier for PAA2k when the signal S4 was used because of the significant variation between the \(M_n\) from the \(^{13}\)C and \(^1\)H NMR spectra. PAA10k also had an outlier, which S14 was used for the end group signal in the \(^1\)H NMR spectra. This is likely due to the signal overlapping slightly with other signals, although it was not expected to affect the result so strongly. For signals S4 and S14, since their corresponding \(^{13}\)C NMR signals gave \(M_n\) values in agreement with those determined from other end group signals, the outlying values are more likely due to the NMR spectra and not because there is a distribution of end groups in the sample. There is also a 10-20 \% difference between the \(M_n\) values determined from different end group signals, which is likely caused by the distribution of end groups in the sample. Thus from NMR spectroscopy the \(M_n\) of PAA2k is between 1 750 and 2 300 g mol\(^{-1}\) and that of PAA10k is between 8 900 and 11 800 g mol\(^{-1}\). The results show some variation between \(M_n\) values obtained using the different signals and nuclei.

Although a number of signals may reach this criteria there is still variation between the \(M_n\) values in Table 6. The signal which provides a value which is closest to the true molar mass would come from the signal least susceptible to degradation. The signals S13 and S14 which correspond to the methyl and methyne are attached to the
aliphatic backbone by carbon-carbon bonds. The groups connected by carbon-carbon bonds are less likely to be removed from the polymer chains due to the strength of the carbon-carbon bonds. The methyne signal is overlapping with the backbone signals while the methyl signal has sufficient sensitivity and resolution in most cases. Therefore the most accurate $M_n$ of PAA2k is $1\,800 \pm 75\, \text{g mol}^{-1}$, which is in agreement with the equivalent signal in the $^1\text{H}$ NMR spectrum and the error is based on the SNR of the end group signal in the $^{13}\text{C}$ NMR spectrum since it has an larger RSD than the $^1\text{H}$ NMR spectrum, thus the maximum error is shown. For PAA10k the most accurate $M_n$ is $10\,000 \pm 1\,600\, \text{g mol}^{-1}$, although the value is not in agreement with the equivalent signal in the $^1\text{H}$ NMR spectra due to insufficient resolution in the $^1\text{H}$ NMR spectra. These values are similar to the theoretical $M_n$ values of 2000 and 10000 g mol$^{-1}$, with the variation from the theoretical $M_n$ being likely due to the distribution of end groups in the sample.

3.2.3 Assessment of molar mass by free solution CE

3.2.3.1 Molar mass of PAA homopolymers

The trend between the electrophoretic mobility and the number of monomer units common to all polyelectrolytes is shown in Figure 13. Free solution CE separates oligomers by their molar mass [85], with separation also occurring by the end group and the tacticity of the oligomers [178], enabling the characterisation of oligomers in a polymer sample.

The molar mass of the oligomer peaks and the molar mass at which critical conditions are reached can be estimated by comparing the electrophoretic mobility of the PAA samples to the electrophoretic mobility of oligoAA standards. The sample known as AA4 which has a chain length of 4 monomer units at the maximum of the distribution determined by Electrospray Ionisation MS (ESI-MS) has been previously characterised by CE-MS [171,178]. As a result the molar mass of each peak in the oligoAA standard is known and by comparing the electrophoretic mobility of the peaks of the sample and of the standard, peaks in the sample their molar mass can be
estimated. The electrophoretic mobilities reported in the previous publications cannot be compared directly to those of PAA samples in this work because different BGEs were used, therefore the AA4 standard was injected using the same conditions as the PAA samples in this work (Figure 28). However the electrophoretic mobility of the oligomers in the PAA samples would differ slightly to those in the AA4 sample because of different end groups: the AA4 sample has an n-butyl trithiocarbonate and a hydrogen end groups while the PAA samples in this work have bulkier end groups (Figure 17). The larger end groups would provide additional hydrodynamic friction and result in a lower electrophoretic mobility [100].

From the electropherogram of AA4 it seems that the electrophoretic mobility for PAA in a NB110 BGE only varies slightly after 5 monomer units since no other peaks can be clearly identified (Figure 28). PAA2k has a broader peak than PAA10k and PAA50k because of a tail towards the higher electrophoretic mobilities (Figure 29). The tail is likely due chains having a slightly lower number of monomer units than what is needed to reach critical conditions. As shown in Figure 13 the electrophoretic mobility decreases slightly as the polyelectrolyte chain approaches critical conditions. The tail towards the higher electrophoretic mobility indicates that a significant number of chains are at a length which is near the required length for critical conditions. Therefore based on the theoretical molar mass of PAA2k the critical conditions are likely reached around 20 monomer units. This is significantly less than what has been observed for DNA and poly(styrene sulfonate) for which the electrophoretic mobility became similar after 18 and 10 monomer units respectively and the critical conditions reached at 155 and 100 monomer units respectively where the electrophoretic mobility varies by less than 0.1 % due to molar mass [94,179]. Bulkier end groups may cause the critical conditions to occur with a few more monomer units. This is due to it adding to the hydrodynamic friction and the critical conditions is reached when the hydrodynamic friction is negligible [97]. Consequently the PAA samples in this project with a MADIX end group may require a few more monomer units in the polymer chain to reach critical conditions.
Figure 28. Separation of AA oligomers by free solution CE. AAx indicates the number of AA units and R indicates the presence of RAFT agent end group. Separation was carried out in NB110 with an extended light path capillary with a total length of 60.6 cm (effective length 52.1 cm). Sample was dissolved in water to 10 g L\(^{-1}\). Sample synthesis is described in reference [180]. Peak identification was by comparing to references [171,178].

CE-CC provides baseline oligomeric separation of the monomer units between 1 and 3 monomer units long. Repeatable peaks at 2.30, 3.10 and 3.45 \(\times 10^{-8}\) m\(^2\) V\(^{-1}\) s\(^{-1}\) were produced in the samples (Figure 29). As mentioned above the bulkier end groups would likely produce a slightly lower electrophoretic mobility than the peak produced in the oligoAA standard. As a result the repeatable peaks are likely corresponding to chains of 1, 2, 3 monomer units long respectively. All three samples contained a peak for chains that are 3 monomer units long. Only PAA2k contains a detectable amount of chains with 1 monomer unit, while PAA10k only contains a detectable amount of chains with 2 monomer units. Between 3.3 and 3.6 \(\times 10^{-8}\) m\(^2\) V\(^{-1}\) s\(^{-1}\) the noise appears to increase.
However this is likely signals from different stereoisomers since the oligomers can be separated by their tacticity as well [178].

![Graph](image)

**Figure 29.** PAA electropherograms from CE-CC. Dash lines indicate repeat injection. PAA2k and PAA10k were separated in an extended light path capillary with a total length of 60.6 cm (effective length 52.1 cm) while PAA50k was separated in a standard fused silica capillary with a total length of 59.9 cm (effective length 51.4 cm). Injection concentration of each sample was 5 g L\(^{-1}\). Insert shows the lower electrophoretic mobility peaks which correspond to oligomers in the sample.

The PAA samples peaks present between 2.4 and \(3.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}\) correspond to oligomers in the sample which corresponds to chains with 1 to 3 monomer units (molar mass 72-216 g mol\(^{-1}\)). Such peaks could easily be misinterpreted as a large amount of noise however some peaks are repeatable between injections and between samples (Figure 29). PAA2k has some chains in the critical conditions since some of the peak has the same electrophoretic mobility as that of the other PAA samples. It is therefore likely that a majority of chains have more than 20 monomer units (1 440 g mol\(^{-1}\)), with a significant number of chains between 4 and 20 monomer units (molar mass 288-1440 g.)
The amount of oligomer peaks corresponding to chains with 3 or less monomer units takes up 8 % (w/w) of PAA2k (Table 7). This molar mass is consistent with the $M_n$ of 1 800 g mol$^{-1}$ determined by NMR spectroscopy. PAA10k and PAA50k contain only 2 and 1 % (w/w) of chains with 3 or less monomer units, respectively (Table 7). However, in the case of PAA50k an extended light path capillary, which has better sensitivity, was not used making it difficult to detect the minute amount of oligomers which would likely be present. The narrow main peaks for PAA10k and PAA50k indicate that critical conditions are reached and that the majority of the samples consist of polymer chains with more than 20 monomer units. For PAA10k this is in agreement with a $M_n$ of 10 000 g mol$^{-1}$ determined by NMR spectroscopy.

**Table 7.** Weight fraction of oligomers in PAA and PAPTAC homopolymer samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fraction of oligomers % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA2k</td>
<td>8</td>
</tr>
<tr>
<td>PAA10k</td>
<td>2</td>
</tr>
<tr>
<td>PAA50k</td>
<td>1</td>
</tr>
<tr>
<td>PAPTAC1k</td>
<td>13</td>
</tr>
<tr>
<td>PAPTAC2k</td>
<td>13</td>
</tr>
<tr>
<td>PAPTAC5k</td>
<td>1</td>
</tr>
</tbody>
</table>

As the theoretical molar mass increases the fraction of oligomers decreases (Table 7). This indicates the presence of these oligomers is likely not due to early termination reactions and thus formation of dead chains as this would result in the same fraction of oligomers in each sample. Furthermore dead oligomer chains were detected in the oligoAA standard, which should have a similar electrophoretic mobility to dead oligomers in the PAA sample since they have similar end groups. No repeatable peaks were identified in the PAA samples which correspond to the dead oligomer chains in the oligoAA standard. The oligomers identified would correspond to the tail of the molar mass distribution which is decreasing as the theoretical molar of the samples increases.
3.2.3.2 Molar mass of PAPTAC homopolymers

Unlike the PAA samples PAPTAC homopolymers adsorb onto the capillary surface under standard conditions. Previous projects have shown that even at pH 2 PAPTAC provides almost no signal as the majority of the polymer chains adsorbs onto the capillary surface. It was then showed that a fused silica capillary with a PEO or fluorocarbon (FC) coating prevented a significant amount of adsorption. A more thorough assessment of the adsorption of PAPTAC homopolymers and block copolymers is given section 4.2.2.2.

Obtaining accurate assessments of the molar mass of cationic polymers is difficult due to their adsorption as stated in section 1.2.2.1. However, minimal adsorption is present using CE-CC when using a ‘WAX’ PEO coated capillary. Oligomers were detected in the PAPTAC homopolymers, between 0.15 and 0.40 × 10^{-8} m² V^{-1} s^{-1} (Figure 30). PAPTAC1k and PAPTAC2k were found to contain a similar fraction of oligomers, which constituted a significant fraction of the polymer (Table 7). Only a small peak a 0.36 × 10^{-8} m² V^{-1} s^{-1} was detected in PAPTAC5k indicating a small fraction of oligomers in the sample. Although the weight fraction of the oligomers is higher in PAPTAC samples than in PAA samples, the PAPTAC samples have a lower targeted chain length. PAPTAC1k and PAPTAC2k theoretically have 5 and 10 monomer units respectively. If PAPTAC followed a similar trend as PAA it would be expected that PAPTAC1k would produce an electropherogram similar to the oligoAA standard AA4. However majority of the chains in PAPTAC1k have a similar electrophoretic mobility (Figure 30). This may indicate that the electrophoretic mobility is less influenced by the molar mass of PAPTAC than that of poly(styrene sulfonate), DNA and PAA [85,94,178] or that the majority of the sample is made of polymer chains of the theoretical molar mass or higher. To confirm this CE-CC with an ElectroSpray Ionisation Mass Spectrometry (ESI MS) detector would be required. However, it appears that the PAPTAC samples have a majority of their polymer chains with more than a few monomer units.
3.2.4 Molar mass of PAA from SEC and comparison with NMR

3.2.4.1 Theory of SEC

SEC is one of the most common means of determining the molar mass of polymers [52]. The main advantage of SEC is its ability to provide molar mass distributions and thus the dispersity ($D$) of a polymer, although there are several cases where SEC can only provide estimates for molar mass [61]. Despite the limited accuracy of SEC the molar mass of polymers is rarely confirmed with other methods in the literature.
When a molar mass distribution is determined, not only the \( M_n \) can be determined but also the weight average molar mass (\( M_w \)) and the Dispersity (\( D \)). The \( M_w \) is defined by Equation (18 and 19):

\[
M_w = \frac{\sum_i w_i M_i}{\sum_i w_i}
\]  

\[
M_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}
\]  

where \( w_i \) is the total weight of the polymer chains of length \( i \). \( D \) is a statistical figure used to describe the heterogeneity of the chain lengths in a polymer sample and is calculated using Equation (20). Dispersity was commonly referred to as the Polydispersity Index (PDI) or polymolecularity index until the International Union of Pure and Applied Chemistry (IUPAC) recommendation in 2010 [181].

\[
D = \frac{M_w}{M_n}
\]  

The most common means of determining molar mass in SEC is by conventional calibration. This involves the use of a calibration curve relating the elution volume of standards to their molar mass. The major problem with this method is that SEC separates by hydrodynamic volume (\( V_h \)), which is proportional to the product of a molecule’s molar mass (\( M \)) and intrinsic viscosity ([\( \eta \)]), shown in Equation (21):

\[
V_h \equiv M[\eta]
\]  

resulting in a calibration curve which provides a relationship between the \( V_h \) of the standard and its elution volume. The \( V_h \) of the standards are then related to the \( V_h \) of the sample as shown in Equation (22):

\[ M_{standard}[\eta]_{standard} = M_{sample}[\eta]_{sample} \]  

only when the [\( \eta \)] of the standard is the same as the [\( \eta \)] of the sample is the actual molar mass of the sample determined by conventional calibration. This situation only occurs when the standards used have the same chemical nature and structure as the polymer being analysed. Unfortunately only a few polymers standards can be produced with well-defined molar masses. To overcome the problems of conventional calibration,
universal calibration can be used. Universal calibration involves the use of a viscometer to determine the \([\eta]\) of both the standards and the sample so that the molar mass of the sample can be determined [53]. Alternatively absolute determination of molar mass (without the use of a calibration curve) is possible in SEC. A light scattering detector determines the molar mass as stated in the Rayleigh equation, given as Equation (23)

\[
\frac{K c}{R_0} = \frac{1}{M_w P(\theta)} + 2A2c
\]  

(23)

where \(K\) is a constant, \(c\) is the concentration determined by a concentration dependent detector, \(R_0\) is related to the excess of light scattered, \(P(\theta)\) is extrapolated to 1 when using Multi Angle Laser Light Scattering (MALLS) or assumed to be 1 with Low Angle Laser Light Scattering (LALLS), and \(A\) is a constant which is only significant at high concentrations. It is important to note that with a light scattering detector the \(M_w\) is determined at each point of the chromatogram. Thus the molar mass can be determined by different means in SEC to validate the accuracy of the values.

3.2.4.2 PAA homopolymers

SEC traces were obtained from Paul Sabatier University (Toulouse) using MALLS and the conditions described in section 2.4.2.1 for PAA and section 2.4.3 for PAPTAC samples. However, it should be noted that the SEC separations from Paul Sabatier were conducted on uncalibrated columns so there is a possibility that there is incomplete separation by hydrodynamic volume. In addition SEC data and values obtained by PhD candidates Alison Maniego and Joel Thevarajah at the University of Western Sydney (UWS) using universal calibration and the conditions described in section 2.4.2.2 for PAA are also reported (Table 8).
Table 8. Molar mass averages and dispersity of PAA samples determined by NMR spectroscopy and SEC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Technique</th>
<th>$M_n$ (g mol$^{-1}$)</th>
<th>$M_w$ (g mol$^{-1}$)</th>
<th>$D$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NMR</td>
<td>MALLS*</td>
<td>UC#</td>
<td>MALLS*</td>
</tr>
<tr>
<td>PAA2k</td>
<td>1 800</td>
<td>12 400</td>
<td>2 200</td>
<td>15 700</td>
</tr>
<tr>
<td>PAA10k</td>
<td>10 000</td>
<td>20 300</td>
<td>21 000</td>
<td>22 300</td>
</tr>
<tr>
<td>PAA50k</td>
<td>ND^</td>
<td>81 800</td>
<td>108 000</td>
<td>92 400</td>
</tr>
</tbody>
</table>

^ ND stands for not determined. * results from Toulouse as described in section 2.4.2.1. # results from UWS as described section 2.4.2.2, values determined by universal calibration.

There is considerable variability between the molar mass values determined by different methods for the PAA samples (Table 8). The molar mass of PAA2k determined by universal calibration and NMR are in agreement, however, the values obtained with the SEC system using MALLS detection are several times higher. SEC of ‘smart’ polymers such as PAA suffers from aggregation and light scattering is often sensitive to large aggregate particles causing an overestimation in molar mass. However, the same result did not occur for the other PAA samples and since larger molar masses tend to aggregate more this is unlikely to be the cause of the molar mass discrepancy. Light scattering is not sensitive to small compounds such as oligomers. As determined by CE-CC PAA2k contains a significant amount of oligomers while PAA10k and PAA50k do not, as a result the molar mass may be overestimated due to the lack of detection of oligomers. To correct for this the concentration would have to be carefully adjusted to provide sufficient sensitivity for the oligomers [52].

For PAA10k the molar masses determined by both SEC systems are in agreement but are twice higher than that determined by NMR. If the molar mass determined by NMR was larger than that by SEC it could be attributed to the branching of PAA causing incomplete separation by molar mass in SEC causing a local dispersity above unity and hence an underestimation in the molar mass determined [122]. When there is incomplete separation the dispersity is greater than 1 at a given point in a chromatogram, the dispersity at each point is thus a local dispersity. However, the
values from SEC and NMR may not be significantly different taking into account the precision of both values. The precision of the $M_n$ determined by NMR is $\pm 1600$ g mol$^{-1}$. The error on the $M_n$ of polystyrene in THF by SEC was found by IUPAC to be 16\% [182] and in another case more than one order of magnitude higher at 800\% [61]. The error on the SEC of PAA is likely much higher than that of polystyrene so there would be a minimum error of $\pm 3300$ g mol$^{-1}$ on the $M_n$ from SEC. Therefore the molar mass values may be within experimental error of each other. For PAA50k both the molar mass values and the $D$ are significantly different between the SEC systems and no comparison with NMR was conducted. Excluding the MALLS results only the PAA2k molar mass is found to be near the theoretical molar mass. To summarise, there is a significant variation between the molar masses determined by different methods and no clear trend is present to identify if one particular method for determining molar mass is more accurate. In order, to properly assess the molar mass a more comprehensive study is required which was not the goal of this study as other components of the chemical structure are equally as important as the molar mass. Due to this the SEC chromatograms of PAA are presented to show a more qualitative information (Figure 31).

Large discrepancies between the molar mass of polyacrylates determined by SEC and NMR have been previously shown in the literature [183]. Those differences were attributed to the difficulty of determining the molar mass of PAA by SEC. The SEC of PAA has been found to have a large variability. IUPAC found the inter-laboratory reproducibly of the molar mass of PAA determined by SEC to be 2 000\% [61]. The low reproducibility was attributed to a non-standardised SEC procedure for PAA [184]. Recently IUPAC has presented recommended SEC conditions for PAA [185] which were used in this work at UWS. These conditions were found to minimise aggregation and ion exclusion making the determined molar masses more reproducible. However, even with these recommended conditions the complexity of PAA due to its branching makes determining its molar mass by SEC not a simple task [185] and large variations in molar mass are common, especially for PAA. To clarify the most accurate
molar masses of the PAA samples additional experiments are required to ensure reproducibility of the results.

**Figure 31.** SEC chromatograms of PAA samples. Refractive index detector traces shown. Injections performed by PhD candidates Alison Maniego and Joel Thevarajah at UWS. Injection concentrations given in Table 3.

From the SEC chromatograms there is a shift towards the lower elution volumes for the higher theoretical molar mass samples indicating that larger molar masses were likely produced (Figure 31). In the case of PAA2k there appears to be some peak tailing toward the higher elution volumes which may indicate that oligomers are present. However, the oligomers may co-elute with the small molecules creating the negative peak at 32 mL, so they cannot be clearly identified. Furthermore the same tailing in PAA10k and PAA50k is difficult to observe due to the setting of the baseline. Therefore the detection of oligomers is more easily performed by CE-CC as shown in Figure 29.
3.2.4.3 PAPTAC homopolymers

Obtaining useful SEC data of cationic polymers is difficult. Often SEC analysis of PAPTAC homopolymers resulted in no recovery (no polymer elutes out of the column) [62]. However, the group from the Paul Sabatier University (Toulouse) were able to achieve separations of PAPTAC homopolymers using the conditions developed for PDADMAC homopolymers [63]. The SEC data obtained was then compared with the results obtained by CE-CC. The SEC 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 distribution of the PAPTAC homopolymer. Unfortunately the molar mass cannot be estimated with sufficient accuracy because universal calibration and light scattering detector require the change in refractive index with the change in concentration (d\(\eta\)/dc). The d\(\eta\)/dc relates to the [\(\eta\)] in Equation (22) and K in Equation (23) and for these specific conditions cannot be determined easily. Furthermore conventional calibration cannot be conducted easily since obtaining well defined standards which are soluble in the mobile phase and have a similar chemistry to PAPTAC is not possible.

The SEC chromatograms of PAPTAC show a shift in elution time towards the lower elution time as the theoretical molar mass of the samples increases (Figure 32). The shift indicates that the samples are increasing in molar mass following the trend of the theoretical molar mass. These shifts are seen with both the refractive index (RI) detector and the light scattering (LS) detector, although differences in the peak shapes are observed making it difficult to compare the width of the peaks. In the light scattering detector another peak forms between 12 and 14 min which are not observed with the RI detector (Figure 32). This peak represents some aggregation in the sample: the LS detector is sensitive to aggregation, while the RI is not [186]. The aggregation would mean that not the entire polymer sample is present in the main chromatogram peak so it only represents the majority of the sample.
Figure 32. SEC chromatograms of PAPTAC samples: a) refractive index detector traces, and b) the light scattering detector traces. Injection concentrations given in Table 3. The intensities are adjusted to give a peak maximum of 1.

CE-CC of the PAPTAC1k and PAPTAC2k showed the presence of a significant fraction of oligomers while the higher molar mass sample PAPTAC5k showed a very little fraction of oligomers. The SEC chromatograms of the higher molar mass sample PAPTAC6k shows a shift towards the lower elution volume with minute tailing towards the higher elution volumes. In the SEC chromatograms of PAPTAC1k there appears to be a small peak at 20 min, although it appears much smaller with the LS detector, which likely represents the smallest oligomer in the sample, which is in agreement with CE-CC. PAPTAC2k does not show this oligomer peak as clearly. Since CE-CC found
similar amounts of oligomers in PAPTAC1k and PAPTAC2k, however this was not observed in the SEC chromatograms. The discrepancy between the SEC chromatograms is likely due to poor SEC separation of oligomers resulting in only the smallest oligomers being selectively detected [171]. Therefore the SEC is not well suited to resolve and identify oligomers especially for the low fractions determined by CE-CC.

3.3 Branching in PAA Homopolymers

Side reactions during polymerisation result in the formation of mid-chain radicals (Section 1.3.1). The mid-chain radical can then further react with monomers creating a branch in the polymer chain. Short chain branches are usually produced by intramolecular transfer to polymer (Figure 14a) [187]. Long chain branches are generally produced by intermolecular chain transfer to polymer (Figure 14b) [188]. Short chain branches tend to affect the polymer's crystallinity while long chain branches influence its viscosity [109]. Therefore finding synthetic conditions which favour the desired branching conditions is highly valuable.

3.3.1 Average degree of branching

The $DB$ is determined by $^{13}$C NMR spectroscopy and calculated using Equation (3). To use this equation a signal representing the branched units and a signal representing all monomer units is required. In the case of PAA the branched units can be represented by the area of the quaternary carbon peak at 48 ppm. All monomer units can be represented by either the backbone carbon signals or by the carbonyl signal from the carboxylic acid on each monomer unit. In all cases except using the backbone of PAA2k there is overlap of some of the end group signals with that of the backbone and carbonyl signals. To correct for this the area of one of the fully resolved end group signals (methyl group at 13.7 ppm) was subtracted the required number of times to negate the overlap. Thus the two Equations (24 and 25) were used to determine the $DB$ in PAA. The values for the $DB$ are presented in Table 9.
The DB was the same for each polymer when using the backbone signals and the carbonyl signals within the experimental error achieved. It has been previously shown that when there is low error in the DB significantly different values for DB can be determined when using different signals such as the backbone or functional group [189-191]. Very low DB values of less than 1 % were measured for PAA2k and PAA10k (Table 9). With such a low DB, the RSD is high even though 80 000 scans were recorded. However, with the large number of scans it can be said within experimental error that there is branching in both samples. This is especially important for PAA10k since branching would not be detected in polymer samples with such a small DB using typical conditions due to insufficient measuring time resulting in insufficient sensitivity. The DB is not significantly different between the samples which only differ in molar mass. This is in agreement with reports that no correlation was found through simulations between the DB and the molar mass of a related polymer, poly(n-butyl acrylate) [107,192].

### Table 9. DB of PAA homopolymers determined by $^{13}$C NMR.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$DB$ (%)</th>
<th>$DB$ (%)</th>
<th>RSD (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Determined by Equation (24)</td>
<td>Determined by Equation (25)</td>
<td></td>
</tr>
<tr>
<td>PAA2k</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>30</td>
</tr>
<tr>
<td>PAA10k</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>63</td>
</tr>
</tbody>
</table>

*denoted using Equation (4).
As mentioned in section 1.3.2 the DB has been estimated for PAA in the literature using possibly quantitative conditions. PAA synthesised by RAFT and NMP were reported to have a DB of 2 % [119] and 4-6 % [115] respectively. These values are in agreement with the published data obtained at the UWS, which found that the DB for PAA synthesised by RAFT (the oligoAA standards reported in section 3.2.3.1) and NMP (samples used in reference [100]) was around 1 % and 4 % respectively. However there is likely a large error in the branching measurement for PAA synthesised by RAFT in the literature [119]. To estimate the error of this measurement the SNR of the branching signal used was determined from the published spectrum (Figure 33). The SNR was calculated by the signal (distance between the blue lines on Figure 33) divided by the noise of the spectrum which was defined as the distance between the red lines on Figure 33 divided by 2.5 which yields a similar result as the calculation applied when using the ‘sino real’ command in the Bruker Topspin software [106]. The SNR was calculated to be 8.68 for the signal which used to determine the DB. The SNR ratio assumes that noise measured from the section of the spectrum which was published is an accurate representation of the noise in the entire spectrum. Using Equation (4) the RSD of this signal was found to be 15 %, thus the DB from reference [119] is (1.9 ± 0.3) %. The PAA in this work were synthesised by MADIX at 60 °C in a mixture of ethanol and water, as stated in section 2.1.2. Compared to MADIX, there is a clear increase in the DB when PAA is synthesised by NMP, there is also a significant increase when synthesised by RAFT.
Figure 33. $^{13}$C NMR spectrum of PAA synthesised by RAFT. Figure adapted from reference [119]. * indicates the signal used to represent branched units. Distance between red lines represents the noise while distance between the blue lines represents the signal used to determine the SNR.

It has been noted in the literature that poly($n$-butyl acrylate)s synthesised by RDRP have significantly less branching than when made by conventional radical polymerisation [116], however, the reason as to why this occurs is still not completely understood [107,192]. Furthermore, no significant difference between the three RDRP techniques has been reported in the literature. Therefore these $DB$ values may assist in determining a link between the synthesis and $DB$. Since there is no perfect agreement between the MADIX and RAFT polymerised samples the difference in branching may be due to conditions other than the mechanism of polymerisation since RAFT and MADIX have the same mechanism. The oligoAA samples were synthesised by RAFT in dioxane at 60 °C [180] while the other RAFT sample with a $DB$ reported in the literature was synthesised in water and ethanol at 80 °C [119]. To synthesise PAA by NMP dioxane and temperatures around 120 °C are required which are substantially different to those used in MADIX. It has been reported that higher polymerisation temperatures produces a $DB$ which increases from 2.3 % when polymerised at 100 °C to 4.7 % when polymerised at 140 °C for poly($n$-butyl acrylate) synthesised by bulk radical polymerisation [108]. The $DB$ in PAA seems to be linked to the polymerisation temperature since PAA synthesised by NMP has the most branching and highest
temperatures while MADIX has the lowest polymerisation temperature and branching. Although the solvent used in the polymerisation may influence the *DB* as well. To limit the frequency of branching in PAA produced by RDRP, MADIX is recommended since it allows for the synthesis with the lowest polymerisation temperature and a water and ethanol mixture as the solvent.

3.3.2 Heterogeneity of branching in PAA homopolymers

When polyelectrolytes have a sufficient chain length their electrophoretic mobility in CE is independent of molar mass (Figure 13). For poly(styrene sulfonate) in 20 mM borate buffer the electrophoretic mobility of PSS is slightly lower than $5.4 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ for polymer chains with 10 monomer units, less than $4.4 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ for chains with 100 monomer units and more than $4.2 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ for chains with 1000 monomer units (Figure 1 of [94]). The change is thus less than 17% decrease in electrophoretic mobility between 10 and 100 monomer units while over 100 monomer units there was less than 5% decrease and so critical conditions were reached [94,96]. PAA also follows a similar trend as discussed in section 3.2.3.1, thus the samples PAA10k and PAA50k which theoretically have 139 and 694 monomer units respectively should mainly be separated by their branching architectures and end group as observed for some PAAs obtained by NMP [100]. PAA2k may contain some chains which are not in the critical conditions thus the peak width may still be influenced by molar mass and not only the branching and end groups. For PAA in NB110, which are the conditions used in this work, there is a 2.2% decrease in the electrophoretic mobility for a 3 arm star polymer compared to that of a linear PAA architecture with both samples having a molar mass well above critical conditions [100]. Demonstrating that the selectively of CE-CC to separate different branching structures is minimal. The electrophoretic mobility was shown to decrease further for a hyperbranched PAA [100]. Therefore CE-CC is capable of separating by branching structures such as number,
position and length of branches and the width of the homopolymer peak should represent the heterogeneity of the branching.

The PAA50k and PAA10k have no significant difference in electrophoretic mobility with the difference determined to be 0.79 % and the RSD of the electrophoretic mobility taking into account electrodispersion (discussed in section 5.3.1) being 1.47 % (n=16) and 1.04 % (n=11) respectively. The peak shape and width of the peaks are also very similar (Figure 34). Therefore each polymer has a very similar branching distribution. There is no significant difference between the electrophoretic mobility of the PAA10k and PAA50k samples and that of a linear PAA (Figure 34). Furthermore branching has been shown to decrease the electrophoretic mobility indicating that the PAA samples are the same as the linear PAA. Since branching was detected by NMR spectroscopy the branching present in the samples are most likely short chain branches which should have minimal effect on the electrophoretic mobility. If long chain branches were present then significant differences in electrophoretic mobility would likely be present as observed between linear and 3 arm star architectures [100].

The triangular asymmetrical peak shape observed for PAA is typical of a peak obtained for small molecules in CE due to electrodispersion. In CE-CC since there is no influence of molar mass, polyelectrolytes with little distribution in end group or branching should exhibit the same peak shape as a small molecule which corresponds to a single molecular species. This and the similar peak widths also indicate a similar heterogeneity of branching between the samples and overall the branching is fairly homogenous in the PAA samples. Such a result is to be expected since a very low DB was quantified for two of the PAA samples by NMR spectroscopy. However, the PAA samples synthesised by NMP mentioned in the section 3.3.1 which have a large DB exhibit broad Gaussian like peaks as shown in reference [100]. In addition branching in these samples is compared to branching in other PAA samples synthesised by NMP and ATRP in a publication in preparation. This demonstrates that CE-CC is capable of assessing the heterogeneity of branching in polyelectrolytes.
Figure 34. Electropherogram of PAA in CE-CC showing the heterogeneity of branching. PAA50k (red) and PAA10k (green) were separated in a fused silica capillary with a total length of 60.1 cm (effective length 51.6 cm). CE-CC trace of linear PAA (dotted blue line) and error from reference [100]. Error of PAA50k and PAA10k is from the RSD of PAA50k which is shown in section 4.2.1. Injection concentration of each sample was 5 g L⁻¹.

To quantitatively compare heterogeneity of branching a similar approach to what is used to describe the broadness of a molar mass distribution can be used. The $D$ is used to compare the heterogeneity of molar mass between samples. In a similar way the dispersity of the electrophoretic mobilities ($DEM$) of the polymer can be used to describe how broad the peak is. $D$ can be expressed as follows by substituting Equations (14 and 18) into Equation (20):

$$D = \frac{\sum_i w_i M_i}{\sum_i w_i} \frac{\sum_i w_i}{N_m}$$

(26)
\[ D = \frac{N_m \sum_i w_i M_i}{(\sum_i w_i)^2} \]  

(27)

where \( N_m \) can be expressed as:

\[ N_m = \sum_i w_i M_i^{-1} \]  

(28)

Therefore \( D \) can be written as:

\[ D = \frac{\sum_i w_i M_i^{-1} \sum_i w_i M_i}{(\sum_i w_i)^2} \]  

(29)

In a molar mass distribution the molar mass \((M)\) is the abscissa value in the distribution and weight \((w)\) is the ordinate value. The distribution obtained by CE-CC has the electrophoretic mobility \((\mu)\) as the abscissa value and \((w)\) as the ordinate (after the UV signal has been corrected as described in section 5.2.2). Each distribution has statistically significant points referred to as moments determined from the abscissa and ordinate values. These moments are then used to calculate the averages from the distribution [193,194]. The moments in a distribution of electrophoretic mobilities are shown in section 7. Therefore \( DEM \) can be calculated in the same manner as \( D \) by substituting \( M \) with \( \mu \) as that is the corresponding abscissa variable, which corresponds to moment -1 multiplied by moment 1 divided by moment 0 squared (moments shown in Tables A-3 and A-4). As shown in Equation (29) for chains of electrophoretic mobility \( z \):

\[ DEM = \frac{\sum z w_z \mu_z^{-1} \sum z w_z \mu_z}{(\sum z w_z)^2} \]  

(30)

In contrast to an electropherogram with the UV signal as a function of migration time, where the abscissa values have an equal distance apart as the detector records at regular intervals, the UV signal as a function of electrophoretic mobility does not have electrophoretic mobility values an equal distance apart. To take into account the difference in the electrophoretic mobility interval each point can be multiplied by the change in electrophoretic mobility \((d\mu)\). \( d\mu \) is equal to the subsequent electrophoretic mobility value minus the current value. Therefore \( DEM \) is calculated as shown in Equation (31).
\[ DEM = \frac{\sum w_z \mu_z^{-1} (\mu_{z+1} - \mu_z) \sum w_z \mu_z (\mu_{z+1} - \mu_z)}{[\sum w_z (\mu_{z+1} - \mu_z)]^2} \] (31)

Since the peaks are narrow the DEM values are small and required to more significant figures than D. The DEM of PAA10k and PAA50k were 1.000049 and 1.000051 respectively. The samples have the same heterogeneity of branching and the molar mass of PAA does not affect the heterogeneity of branching, PAA50k likely has a similar DB as PAA10k and PAA2k since it exhibits a similar DEM as PAA10k. Therefore in terms of branching architecture a PAA synthesised by MADIX has very similar chains which are likely linear or contain a short chain branch.

The approach used to determine the heterogeneity of branching of the PAA samples could not be applied to the PAPTAC because the peak width may still be influenced by their molar mass as they have not reached critical conditions. Assuming PAPTAC follows a similar trend as PAA, critical conditions would be reached at around 20 monomer units which would be a molar mass of 3 600 g mol\(^{-1}\). If the PAPTAC followed a similar trend as poly(styrene sulfonate) the critical conditions would be reached at 100 monomer units which equates to PAPTAC with a molar mass of 18 000 g mol\(^{-1}\). Therefore it is unlikely that majority of the PAPTAC chains in the samples in this work will be in the critical conditions. Furthermore the number of monomer units in these samples is small, in the case of PAPTAC1k less than 10, so they could only exhibit short chain branches, if any branches were to occur. These short chain branches may not provide a significant change in electrophoretic mobility to assess the heterogeneity of branching.

### 3.4 Chapter Conclusion

The PAA samples were characterised in terms of their molar mass and branching. The molar mass determined by \(^{13}\)C and \(^1\)H NMR spectroscopy were compared and \(^{13}\)C NMR spectroscopy was found to be more accurate than \(^1\)H NMR spectroscopy when enough scans are performed to achieve a sufficient SNR. It was
found that accurate determination of molar mass is difficult especially when comparing NMR and SEC. Further work would be required to assess the reproducibly of the molar masses. Moreover, using free solution CE oligomers could easily be identified and their relative amount quantified in the PAA samples. The DB was found to be lower than 1% for PAA2k and PAA10k which is lower than values measured for PAAs synthesised by other RDRP. The heterogeneity of branching was then assessed by CE-CC which showed the branching to be very homogenous.

Similar work was also carried out on challenging cationic homopolymers, PAPTAC. The molar mass was qualitatively assessed by SEC and CE-CC and oligomers were detected by both techniques but CE-CC provided superior resolution. In summation the two types of homopolymers were well characterised and this will improve the understanding of the block copolymers formed from these homopolymers.
CHAPTER 4. PURITY OF BLOCK COPOLYMERS

The purity of block copolymers is rarely assessed, even though it impacts the properties of the block copolymers and the way they are characterised as discussed in section 1.2. There is currently no established method for determining the purity of block copolymers and majority of the proposed methods in the literature have only been applied to hydrophobic block copolymers. In this chapter a method for assessing the purity of DHBCs in terms of their homopolymer content in corresponding block copolymers is demonstrated and analytically validated, which is an innovation in terms of quantification of homopolymers.

The work in this chapter was a part of the following invited publication, in the Journal of Chromatography A:


4.1 Nomenclature used to Describe Homopolymers in Block Copolymer Samples

There is not only no established determination method, there is also no established nomenclature for the homopolymers found in block copolymers. This results in a number of different terms can be used, one example is ‘parent homopolymer’ which refers to the homopolymer that constitutes a block in the block copolymer, and so a diblock copolymer has two ‘parent homopolymers’ [79]. In terms of the synthesis the
goal is often to produce a pure block copolymer sample so terms such as ‘undesired’ and ‘unwanted’ homopolymers are used to describe these as impurities [150]. However, the presence of these homopolymers can be beneficial or detrimental to the properties of the block copolymer sample. Therefore the term ‘unintended homopolymers’ can also be used to describe the presence of these homopolymers since they were not intended in the synthesis but may not negatively influence the properties. This term does not reflect an error in the synthesis but rather an inevitable formation of the homopolymers.

In the case of the initial homopolymer being present in the block copolymer sample the terms ‘unreacted’, ‘remaining’ and ‘residual’ homopolymer are often used as these homopolymer chains were not converted to a block copolymer [45,91,92]. However, such terms can only be used to describe the initial homopolymer since any homopolymer corresponding to the second block is a by-product of the reaction and thus these terms are not grammatically suited to refer to both of the parent homopolymers. In summary a number of terms can be used to describe homopolymers in a block copolymer sample, in this work the term ‘parent homopolymers’ is used to describe them from a synthetic perspective while ‘unintended homopolymers’ is used to describe them from an applications perspective and ‘residual homopolymer’ is used when measuring the initial homopolymer.


4.2 Comparison of SEC and CE-CC to Identify Block Copolymer Formation and Unintended Homopolymers

4.2.1 Separating PAA homopolymers from block copolymers.

4.2.1.1 P(AA-b-AM) block copolymers

Block copolymers are generally characterised by SEC, including whether a block copolymer has formed and whether there is any homopolymer present [23,195,196]. Nevertheless, fully resolved SEC chromatograms of homopolymers and copolymers are scarce in the literature [197]. SEC was compared to CE-CC for separating homopolymers from block copolymers. The separation of a PAA homopolymer from a P(AA-b-AM) block copolymer is achieved by CE-CC with complete resolution, using standard conditions (fused silica capillary and borate buffer [100]) in under 14 min for both PAA10k and PAA2k with their corresponding block copolymer (Figures 35c,d and 36c,d). Migration times are repeatable, with RSDs at the peak maximums being all below 3.5 % (Table 10). The electrophoretic mobilities are even more repeatable with RSDs below 2 % for all samples. The electrophoretic mobility is expected to be more repeatable since it is corrected for measurement to measurement variations of the electro-osmotic flow (influenced in turn by the BGE concentration, the viscosity of the BGE, the zeta potential of the capillary surface as stated in section 2.3.4) through the use of the EOF marker.
Table 10. RSD values obtained for PAA samples using CE-CC. The same conditions were used except PAA2k and PAA10k were separated in an extended light path capillary with a total length of 60.6 cm (effective length 52.1 cm) while PAA50k was separated with a standard fused silica capillary with a total length of 59.9 cm (effective length 51.4 cm).

<table>
<thead>
<tr>
<th>Sample</th>
<th>RSD of migration time (%)</th>
<th>Number of repeats</th>
<th>RSD of electrophoretic mobility (%)</th>
<th>Number of repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA2k</td>
<td>2.75</td>
<td>8</td>
<td>1.53</td>
<td>8</td>
</tr>
<tr>
<td>PAA10k</td>
<td>2.97</td>
<td>8</td>
<td>0.50</td>
<td>8</td>
</tr>
<tr>
<td>PAA50k</td>
<td>3.29</td>
<td>8</td>
<td>1.23</td>
<td>10</td>
</tr>
</tbody>
</table>

When the same samples were analysed by SEC, no baseline separation of the homopolymer from the block copolymer was observed (Figures 35a,b and 36a,b). Only a 0.6 and 3.8 % shift in elution time (calculated from the average at the peak maximum of the repeat injections), shown in Table 11, was detected for the block copolymer compared to the homopolymer in SEC for PAA10kPAM10k and PAA2kPAM10k respectively using established conditions for SEC of PAA and PAM [198-200] (Figures 35b and 36b, and Table 11). The variation between duplicate injections was below 1 % for all samples which is below the expected repeatability of SEC [182]. Therefore the small shift in elution time is not due to poor repeatability but to a small change in hydrodynamic volume. Using other SEC conditions [12,25] which have been used for PAA and P(AA-b-AM) a lower shift was observed (Figures 35a and 36a). However the column used in Toulouse had not been calibrated with standards so the lower separation may be due to ageing columns. In contrast Figures 35d and 36d show a strong shift in electrophoretic mobility of the block copolymer compared to the PAA homopolymer by CE-CC.
Table 11. Shift in elution time of the block copolymer relative to homopolymer sample. SEC injections performed with different conditions from groups at Paul Sabatier University (Toulouse) and University of Western Sydney (UWS). Elution times taken at the peak maximum of the sample peak.

| Sample               | Elution time (min) | Shift in elution time of block copolymer (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toulouse</td>
<td>UWS</td>
</tr>
<tr>
<td>PAA2k</td>
<td>23.21</td>
<td>27.98±0.07</td>
</tr>
<tr>
<td>PAA2kPAM10k</td>
<td>22.40</td>
<td>26.92±0.01</td>
</tr>
<tr>
<td>PAA10k</td>
<td>21.91</td>
<td>25.83±0.05</td>
</tr>
<tr>
<td>PAA10kPAM10k</td>
<td>21.84</td>
<td>25.67±0.02</td>
</tr>
</tbody>
</table>

* Shift calculated from the average at the peak maximum of the repeat injections, using the refractive index detector traces. ~ Toulouse results do not include a repeat injection. * error determined by the mean deviation as opposed to the standard deviation since only two injections was performed.

The complete separation of PAA homopolymer from P(AA-b-AM) block copolymer by CE-CC was confirmed by spiking the block copolymer sample with its corresponding homopolymer (Figures 35c,d and 36c,d). Spiked block copolymers samples were also analysed by SEC (Figures 35a and 36a). In the case of the mixture of PAA10k and PAA10kPAM10k the difference between the individual homopolymer and block copolymer samples was lower than 0.2 % and so no significant difference between the mixture and the individual samples was observed. Since there was a greater shift in elution time of PAA2kPAM10k from PAA2k is was suspected that there may be the appearance of a shoulder representing the homopolymer in the block copolymer signal. However what was observed in Figure 35a was that the mixture produced a broader peak than that of the block copolymer with no distinctive shoulder or tail. Furthermore a shift in elution time from the pure PAA2k sample was observed for the mixture towards the lower elution volumes. In this situation if homopolymer was present in the block copolymer sample it could easily be interpreted as the formation of a block copolymer with no homopolymer present, in addition to an inaccurate molar mass determined.
Figure 35. Separation of PAA10k homopolymer (blue) from PAA10kPAM10k block copolymer (red) using a) Toulouse SEC conditions, b) UWS SEC conditions, c) CE-CC shown as a function of migration time and d) CE-CC shown as a function of electrophoretic mobility. For a) the best separation from three columns is shown as a refractive index trace. A mixture of homopolymer and block copolymer (black) was injected at 5 g L$^{-1}$ and 2.5 L$^{-1}$ for each individual polymer for CE-CC and SEC respectively. Dashed lines show repeat injections.
Figure 36. Separation of PAA2k homopolymer (blue) from PAA2kPAM10k block copolymer (red) using a) Toulouse SEC conditions, b) UWS SEC conditions, c) CE-CC shown as a function of migration time and d) CE-CC shown as a function of electrophoretic mobility. For a) the best separation from three columns is shown as a refractive index trace. A mixture of homopolymer and block copolymer (black) was injected at 5 g L$^{-1}$ and 2.5 g L$^{-1}$ for each individual polymer for CE-CC and SEC respectively. Dashed lines show repeat injections.

In CE-CC a narrow PAA peak at a higher electrophoretic mobility and a broad P(AA-$b$-AM) at a lower electrophoretic mobility were resolved. The narrow peak of PAA confirms that it is under critical conditions. 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 [164]. In a RDRP, 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 different relative lengths of each block in the sample.

**4.2.2.2 P(EO-\textit{b}-AA) block copolymers**

The separation of PAA homopolymers from PAA block copolymers in CE-CC was tested with P(EO-\textit{b}-AA) in the same conditions (Figure 37). In the PEO2kPAA10k sample a narrow peak was observed with an electrophoretic mobility within experimental error to that of PAA10k, indicating a significant fraction of PAA homopolymer in the sample. A broader peak is observed with a lower electrophoretic mobility than PAA10k, indicating that a block copolymer was produced in the sample. Unlike the P(AA-\textit{b}-AM) samples shown above this P(EO-\textit{b}-AA) block copolymer has a neutral block with significantly lower targeted molar mass. Therefore a small difference in electrophoretic mobility between the PAA and the P(EO-\textit{b}-AA) block copolymer was expected. This would explain why baseline separation was not as easily obtained between PAA and P(EO-\textit{b}-AA). It should also be noted that the PAA block is the secondary block in the P(EO-\textit{b}-AA) sample unlike the P(AA-\textit{b}-AM) samples. This demonstrates CE-CC's ability to separate anionic block copolymers with different ratios of charged to neutral blocks from homopolymers. When the neutral block is significantly shorter than the charged block, a longer capillary should be used to obtain baseline resolution of the homopolymer and block copolymer.
Figure 37. Electropherograms of PAA10k (blue) and PEO2kPAA10k (red). Dashed lines show repeat injections. Separations were performed with a standard fused silica capillary with a total length of 60.1 cm (effective length 51.6 cm). The injection concentration of each sample was 5 g L\(^{-1}\).

4.2.2.3 Discussion of CE-CC separation

The success of a block copolymer synthesis is often probed through a shift in SEC elution time towards the higher molar masses [195,196,201-203]. 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 [17,56]. In the case of the symmetric block copolymer PAA10kPAM10k, SEC analysis failed to indicate that a block copolymer had been formed. Even if an additional method such as diffusion NMR (also named DOSY) indicated the presence of block copolymer [25], SEC does not allow the detection or quantification of any homopolymer contaminant (Figures 35a,b and 36a,b). That means 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 (Figure 35c and 36c) and in
electrophoretic mobility (Figure 35d and 36d) is observed, providing evidence that a block copolymer was produced and whether any homopolymers are present. 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 [87] and does not require sample filtration [96,101]. As a result CE-CC can be a faster and cheaper method than SEC for qualitatively identifying the formation of a block copolymer as soon as one of the blocks can be charged.

4.2.2 Separating PAPTAC homopolymers and block copolymers.

4.2.2.1 SEC of PAPTAC homopolymers and block copolymers.

SEC and CE-CC separations are now compared for more challenging cationic DHBCs. Using the conditions developed for PDADMAC, the SEC of PAPTAC was able to produce repeatable separations (Figure 32) [63]. This procedure involves the addition of PDADMAC to the mobile phase, creating a positively charged coating on the stationary phase. 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 (Figure 38). The identical elution profiles of the homopolymer and block copolymer samples could have meant that no block copolymer was formed; however, the decrease in peak area more likely indicates 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. No definite conclusion can be drawn from SEC, even using optimised conditions, about the formation of the block copolymers and the
molecular weight distribution of this copolymer. Therefore CE-CC was used to investigate these block copolymer samples.

Figure 38. SEC chromatograms of PAPTAC homopolymers (blue) and P(APTAC-b-NIPAM) block copolymers (red): a) PAPTAC1k and PAPTAC1kPNIPAM3k, b) PAPTAC2k and PAPTAC2kPNIPAM3k, c) PAPTAC3k and PAPTAC3kPNIPAM3k, d) PAPTAC6k and PAPTAC6kPNIPAM3k. Solid and dashed lines represent signals obtained with refractive index and light scattering detectors respectively. Black dash lines mark the peak maximums in the refractive index traces of PAPTAC homopolymers. Injection concentrations given in Table 3.
4.2.2.2 Preventing adsorption of PAPTAC homopolymers and block copolymers

Cationic polymers have a tendency to adsorb onto surfaces with a negative zeta potential as discussed in section 1.2.2.1. A neutral coating such as fluorocarbon (FC) or poly(ethylene oxide) – PEO - on the capillary surface was used to prevent this adsorption of the PAPTAC homopolymers. This is shown by the well defined peaks in Figure 39. A previous UWS undergraduate student Maryanne Selim had shown that electropherograms of P(APTAC-b-NIPAM) were not repeatable when using a FC coating likely due the adsorption attributed to hydrophobic interactions between the PNIPAM block and the hydrophobic (fluorinated) coating. In this work a PEO coated capillary led to good sensitivity and repeatability. The RSD of the electrophoretic mobility of the homopolymer at the peak maximum was 0.6 % (n=7) with a SNR of >2800 when 5 g L\(^{-1}\) was injected for PAPTAC2k and 2.4 % (n=3) for PAPTAC5kPNIPAM5k. Thus similar RSD results to the PAA homopolymers shown in Table 10 were achieved for the PAPTAC homopolymers. However adsorption of the block copolymer still appeared to be occurring and led to slightly less repeatable migrations in the presence of block copolymer.

Figure 39. Electropherograms of a) PAPTAC7.5k using a PEO coated capillary with PB10 as the BGE and b) PAPTAC4k using a FC coated capillary with PB100 as the BGE. Injection concentrations were 4 g L\(^{-1}\) for PAPTAC7.5k and 5 g L\(^{-1}\) for PAPTAC4k. Repeat separations are shown as dotted lines.
Pressure mobilisation was then used to identify if the analyte interacted with the capillary surface. Pressure mobilisation involves pushing the analyte through the capillary with pressure in the absence of an electric field, so no separation is expected. If no interaction with the capillary surface occurs then a Gaussian peak (or superimposed Gaussian peaks) is (are) observed with the cause of peak broadening being the diffusion of the analyte [204]. Some tailing is observed at high migration time: a small fraction of PAPTAC is interacting with the capillary surface (Figure 38). 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 (Figure 40). The block copolymer P(APTAC-b-NIPAM) showed permanent adsorption with the peak tailing giving a consistent elevated baseline (Figure 40).

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 before each injection. This prevented the adsorption from impacting subsequent experiments.
Figure 40. Pressure mobilisation of PAPTAC5k (black solid line), PNIPAM5k (red dashed line) and PAPTAC5kPNIPAM5k (blue dashed-dotted line) through a PEO coated capillary with a total length of 74.8 cm (effective length 66.3 cm). Injection concentration of each polymer was 5 g L$^{-1}$ in PB10.

4.2.2.3 Separation of PAPTAC homopolymers from PAPTAC based block copolymers by CE-CC and pressure assisted CE-CC

The separation of PAPTAC homopolymer from P(APTAC-$b$-NIPAM) block copolymer by CE-CC is shown in Figure 41. Although P(APTAC-$b$-NIPAM) is adsorbing, as clearly shown by the elevated baseline in Figure 41, a prominent PAPTAC signal was noticeable in all samples. Therefore CE-CC was able to provide some separation of PAPTAC homopolymers from P(APTAC-$b$-NIPAM) block copolymers. 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 (Figure 41).

![CE-CC electropherograms of PAPTAC1k homopolymer (red) and PAPTAC1kPNIPAM9k block copolymer (black). The PAPTAC homopolymer and the internal standard (labeled as PH and IS respectively) have mobilities between $4 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$, and between $6 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$, respectively. PAPTAC oligomer peaks (labeled as PO) occur around $2 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ as shown in the insert. The injection concentrations were 0.625 g L$^{-1}$ for PAPTAC1k and 5 g L$^{-1}$ for PAPTAC1kPNIPAM9k.](image)

**Figure 41.** CE-CC electropherograms of PAPTAC1k homopolymer (red) and PAPTAC1kPNIPAM9k block copolymer (black). The PAPTAC homopolymer and the internal standard (labeled as PH and IS respectively) have mobilities between $4 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$, and between $6 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$, respectively. PAPTAC oligomer peaks (labeled as PO) occur around $2 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ as shown in the insert. The injection concentrations were 0.625 g L$^{-1}$ for PAPTAC1k and 5 g L$^{-1}$ for PAPTAC1kPNIPAM9k.

A similar procedure was followed for P(APTAC-$b$-AA) block copolymers using a FC coated capillary in PB10 so the PAA block is uncharged. FC coated capillaries were used instead of PEO coated capillaries since PAA in its protonated form was shown to adsorb onto PEO coated capillaries in a previous UWS undergraduate student's project (Neil Sharma) with PAA. The block copolymers showed some adsorption due to the elevated baseline although a broad peak is observed (Figure 42). There is a significant shift to lower electrophoretic mobilities from that of the homopolymer indicating that a block copolymer was produced. There is some overlap of the electrophoretic mobility of the block copolymer and that of the corresponding homopolymer indicating that some residual PAPTAC homopolymer may be present.
There is no prominent PAPTAC peak as was observed with P(APTAC-\textit{b}-NIPAM) which may be caused by a smaller amount or residual homopolymer. By adjusting the maximum of the PAPTAC4k peak to the height of the overlay at that electrophoretic mobility, the residual homopolymer content was estimated to be between 10 and 15 \%. PAPTAC4kPAA2k has a greater shift towards the lower electrophoretic mobilities than PAPTAC4kPAA1k indicating that the PAA block is indeed larger in PAPTAC4kPAA2k than PAPTAC4kPAA1k. Therefore CE-CC is capable of analysing different cationic block copolymers.

**Figure 42.** CE-CC electropherograms of PAPTAC4k homopolymer (red), PAPTAC4kPAA1k (green) and PAPTAC4kPAA2k (purple) block copolymers. Dashed lines show repeat separations. Separations occurred in a FC coated capillary with a total length of 66.7 cm (effective length 58.2 cm) with PB100 as the BGE. The injection concentrations were 5 g L\(^{-1}\) in water, for each sample.

To further combat the adsorption of the cationic block copolymers, pressure assisted CE-CC was used on the P(APTAC-\textit{b}-NIPAM) samples. This technique uses the same conditions as regular CE-CC 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-CC has previously been used to aid in the separation of polyelectrolytes [205]. Pressure assisted CE-CC helps migrate the adsorbed block copolymer along the capillary enabling its detection. Furthermore, the neutral PNIPAM homopolymer is not detected by CE-CC: with a PEO coating at pH 2 there is no measurable EOF, meaning the detection of neutral species such as DMSO or PNIPAM would take too long, with no peak observed within 60 min. With the aid of pressure the presence of neutral species in the sample can be detected within a reasonable migration time (below 10 min).

The pressure assisted CE-CC of a mixture of PAPTAC and PNIPAM homopolymers produced well separated and relatively narrow peaks (Figure 43). The pressure assisted CE-CC of the P(APTAC-b-NIPAM) samples detected some species migrating at the same speed as the PAPTAC homopolymer, further 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 (Figure 43). The block copolymer chains with the highest NIPAM fraction likely migrate slower than the neutral species due to the adsorption of the block copolymer on the capillary surface. 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.

In a single pressure assisted CE-CC run, both parent homopolymers and the block copolymer can be detected in P(APTAC-b-NIPAM). The detection of both homopolymers in a block copolymer sample has already been shown for block copolymers produced by anionic polymerisation [79] and RAFT/MADIX [45]. In the case of RAFT/MADIX block copolymers this is likely due to the need of an external source of radicals for initiating the polymerisation of the second monomer unit. Therefore, the formation of a secondary homopolymer would be less likely with living anionic polymerisation or NMP which do not require an external initiator for the second
The presence of one or two parent homopolymers in the sample would influence the properties of the final product [46,50,92].

Figure 43. Pressure assisted CE-CC of a mixture of PAPTAC5k and PNIPAM5k homopolymers (red) and a) PAPTAC2kPNIPAM8k or b) PAPTAC5kPNIPAM5k block copolymers (black). Dashed line is a repeat electropherogram. Injection concentration of each individual polymer was 5 g L\(^{-1}\).

4.3 Quantification of PAA and PAPTAC Homopolymers by CE-CC

4.3.1 Analytical assessment of CE-CC to quantify homopolymers

4.3.1.1 Establishing a homopolymer calibration curves

Since the homopolymers could be separated from their corresponding block copolymers the amount the homopolymer can be quantified in the block copolymer sample. Assessing how accurately a separation method can be used (with appropriate
detection) to quantify a polymer is rarely demonstrated in the literature. Free solution CE was shown to be a reliable method to determine the cationic PDADMAC homopolymer within PAA-PDADMAC statistical copolymers [91]. In this work, the ability of CE-CC to quantify PAA homopolymers in P(AA-b-AM) block copolymers was analytically validated and then the quantification method was extended to cationic block copolymers.

To accurately quantify the amount of the PAA homopolymer in this work, a calibration curve between the homopolymer peak area and the homopolymer concentration was established (Table 12). Since oligomers were shown to make up a significant fraction of the weight of some of the homopolymer samples (Table 7), the peak area was excluding the oligomers so only the polymer like chains were measured. The concentration \( c \) used in the calibration is shown in Equation (32), where \( c_{\text{inj}} \) is the injection concentration:

\[
c = c_{\text{inj}} \frac{\text{polymer peak area}}{\text{polymer peak area} + \text{oligomer peak area}}
\]

The alternative means of quantifying the homopolymer would be to compare the area of homopolymer signal to that of the block copolymer signal. This is less accurate due to the different absorptivities of the different monomer units, although this approach is commonly used in the literature [78,80,92,196]. Furthermore demonstrating the linearity of an analytical technique on a polymer has rarely been reported in the literature [73,206-208].

The UV detection in CE-CC showed sufficient linearity with all samples having a regression coefficient \( R^2 \) greater than 0.979 (Table 12, Figure A-3). The end group was shown to affect the calibration curve since the same homopolymers with different molar masses produced different calibration curves. Therefore the precursor homopolymers were used to quantify homopolymer in the corresponding copolymers to prevent bias from the end group.
Table 12. Linearity of homopolymer quantification, recovery and LOD of CE-CC for PAA and PAPTAC homopolymers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration range (g L(^{-1}))</th>
<th>Equation</th>
<th>(R^2)</th>
<th>Number of points</th>
<th>Recovery*</th>
<th>LOD(^#) (g L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA2k</td>
<td>2.50 – 20.0</td>
<td>(y = 0.431x - 0.402)</td>
<td>0.979</td>
<td>10</td>
<td>113</td>
<td>0.121</td>
</tr>
<tr>
<td>PAA10k</td>
<td>1.25 – 20.0</td>
<td>(y = 0.475x - 0.487)</td>
<td>0.984</td>
<td>12</td>
<td>95</td>
<td>0.095</td>
</tr>
<tr>
<td>PAPTAC2k</td>
<td>0.63 – 5.0</td>
<td>(y = 0.835x - 0.096)</td>
<td>0.998</td>
<td>10</td>
<td>ND(^\wedge)</td>
<td>0.005</td>
</tr>
<tr>
<td>PAPTAC5k</td>
<td>0.63 – 5.0</td>
<td>(y = 0.769x - 0.005)</td>
<td>0.999</td>
<td>10</td>
<td>ND</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Recovery was measured for the homopolymer when mixed with its corresponding block copolymer; when the homopolymer was already present in the block copolymer the recovery was measured through the change in homopolymer peak upon spiking with homopolymer. "The Limit Of Detection (LOD) was defined as the concentration at which the signal-noise ratio is equal to 3. ^ ND stands for 'not determined'.

CE-CC has the advantage of using a DAD unlike LC-CC and LC-LCD which usually require an Evaporative Light Scattering Detector (ELSD) due to the use of organic solvent (cutting UV at low wavelength) and solvent gradients. Although the linearity of the UV detector response for the PAA homopolymers is not as high as wanted, establishing linearity with an ELSD detector is at least equally as difficult and in some cases is no linear response between the detector signal and concentration of the polymer can be established [73,209], as the molar mass of the polymer also influences the ELSD response, thus the response of disperse polymers is not linear. The imperfect linearity of the DAD detector response for the PAA homopolymers is likely due to variations in injection volume between injections since the samples were injected hydrodynamically and the pressure may vary from injection to injection. At higher concentrations the PAA homopolymers also change the solution viscosity which can influence the injection volume. These variations can be corrected if needed by the use of an internal standard such as naphthalene sulfonic acid which has been used previously in alkaline BGE [210].
4.3.1.2 Assessment of CE-CC to quantify homopolymers

To account for differences between injections, such as those mentioned above, the internal standard, hexaaminecobalt(III) chloride, was used for the PAPTAC based polymers, which resulted in higher linearity of the calibration curve (R² ≥ 0.998) than for the PAA homopolymers (Table 12). The internal standard migrated before the PAPTAC homopolymer, had strong molar absorptivity and showed no sign of interaction with capillary coating or polymers as shown previously with chitosan [211]. The internal standard was injected at a concentration of 1 mM so that the peak area would not be influenced by low SNR.

The linearity of the calibration curve of PAPTAC samples was found to be greater than 0.99 with the use of the internal standard. Based on the strong linearity it seems that the adsorption of PAPTAC mentioned in section 4.2.2.2 has little impact on the precision of the quantification.

The recovery of LC based techniques is rarely tested but has been shown to be a problem for polymeric samples [80,150,209,212]. The recovery of CE-CC is quantitative in the case of PAA (Table 12). The recoveries of PAA2k and PAA10k were 113 % and 95 % are considered quantitative within experimental error. Greater than 100 % recovery is likely due to the error in the linearity of PAA and so the recovery value reflects the accuracy of the quantification.

The LOD was estimated by a plot of the concentration calculated using Equation (32) against the SNR of the polymer peak (Figure A-4). The LOD was then extrapolated from the curve as the concentration at which the SNR would equal 3. The LOD of the PAPTAC homopolymers was found to be significantly lower than that of the PAA homopolymers likely due to the higher UV absorption coefficient of the amide in the
APTAC unit compared to the carboxylate in the AA unit. Therefore minute amounts of PAPTAC homopolymer can be detected in block copolymer samples.

If a polymer was lacking a chromophore and thus could not be analysed by UV detection, indirect UV detection and conductivity detection would both be able to provide successful quantification as previously shown with PDADMAC with even lower LOD than the direct UV detection used in this work [91]. Therefore CE-CC is capable of quantifying a range of polyelectrolytes with equal or superior linearity to chromatography based techniques for polymers.

4.3.2 Quantification of homopolymers in block copolymer samples.

4.3.2.1 Experimental fraction of parent homopolymers

Even when trying to avoid the presence of homopolymers in a block copolymer sample, the formation of ‘dead chains’ inevitably results in residual homopolymer in the block copolymer sample [26,81]. Knowing the fractions of homopolymer and block copolymer in a sample can then provide useful information about the ‘dead’ chains. The fraction of homopolymer is presented in terms of the weight of the polymer chains and as the number of polymer chains as shown in Equations (33) and (34) respectively:

\[
\% \frac{w\text{H}}{w}\text{S} = \frac{w\text{H}}{w\text{S}} \times 100
\]

(33)

\[
\% \frac{mol\text{H}}{mol\text{S}} = \frac{w\text{H}/M\text{H}}{w\text{H}/M\text{H} + w\text{B}/M\text{B}} \times 100
\]

(34)

where \(w\text{H}\) is the weight of the homopolymer, \(w\text{B}\) is the weight of the block copolymer, \(w\text{S}\) is the weight of the sample such that \(w\text{H} + w\text{B} = w\text{S}\), \(M\text{H}\) and \(M\text{B}\) are the molar masses of the homopolymer and block copolymer respectively. In Equation (34) it is assumed that all homopolymer and block copolymer chains have their theoretical molar masses.
Using the calibration curves shown in Table 12, 2 % (w/w) of PAA homopolymer was found in PAA10kPAM10k while none was detected in PAA2kPAM10k (Table 13) therefore based on the LOD its concentration was less than 2.4 % (w/w). 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. Pressure-assisted CE-CC is not needed in this case (or in general for separation with strong EOF).

Table 13. Quantification of residual PAA or PAPTAC homopolymer in block copolymer samples for determination of sample purity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>( C_{H}^- ) (g L(^{-1}))</th>
<th>RSD (%)</th>
<th>Fraction of residual homopolymer</th>
<th>Estimated fraction of block copolymer#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 3 )</td>
<td></td>
<td>% (w/w)</td>
<td>% (mol/mol)*</td>
</tr>
<tr>
<td>PAA2kPAM10k</td>
<td>&lt;0.12</td>
<td>CD+</td>
<td>&lt;2.4</td>
<td>100</td>
</tr>
<tr>
<td>PAA10kPAM10k</td>
<td>0.11</td>
<td>ND^</td>
<td>2.14</td>
<td>98</td>
</tr>
<tr>
<td>PAPTAC5kPNIPAM5k</td>
<td>1.3</td>
<td>0.9</td>
<td>18.9</td>
<td>80</td>
</tr>
<tr>
<td>PAPTAC2kPNIPAM8k</td>
<td>0.1</td>
<td>4.0</td>
<td>1.8</td>
<td>96</td>
</tr>
</tbody>
</table>

\( C_{H}^- \) stands for concentration of homopolymer. * CD stands for ‘could not be determined’. ^ ND stands for ‘not determined’. # assuming no significant amount of PAM in P(AA-b-AM) block copolymers *calculated from the theoretical molar masses shown in Table 1.

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 13). A significant fraction of PAPTAC homopolymer was found in all P(APTAC-b-NIPAM) samples although the fraction was found to vary greatly between samples (Table 13). 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 can help to indicate the reactivity of the end group and whether the synthetic conditions used favour the formation of a block copolymer. The end group absorbs at 285 nm while the monomer units do not. 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 fraction of PNIPAM present in the P(APTAC-\textit{b}-NIPAM) samples was estimated by adjusting the maximum of the PNIPAM peak to the height of the shoulder observed in the block copolymer peak. It was then estimated that the P(APTAC-\textit{b}-NIPAM) samples likely contained between 1 and 3 % (w/w) of PNIPAM homopolymer (Figure 43). Therefore, the P(APTAC-\textit{b}-NIPAM) samples contain a majority of block copolymer but also significant fractions of both homopolymers. The fraction of block copolymer in the sample was then calculated by subtracting the fraction of parent homopolymers from the total sample with the remaining being assumed to be the block copolymer.

The PEO2kPAA10k sample was injected with a standard fused silica capillary and so the fraction of residual PAA could not be determined from the calibration curves shown in Table 12 since they were determined with an extended light path fused silica capillary. The amount of unintended PAA was estimated by fitting the pure PAA10k peak to the size of the PAA homopolymer peak observed in the electropherogram of PEO2kPAA10k (Figure 37). This creates a one point calibration to assess the purity of the PEO2kPAA10k sample. The fraction of PAA homopolymer was found to be around 14 % (w/w) which equates to around 1 % (mol/mol). It is important to note that PAA is the secondary block in this block copolymer. Therefore the formation of both homopolymers may be inevitable in RAFT/MADIX polymerisation. Only a small fraction of PAA chains were produced which suggests that the formation of the second parent homopolymer is minimal. A small but significant fraction of PAA homopolymer was detected which would influence the properties of the material.
4.3.2.2 Comparison with theoretical number of dead chains

Assuming ideal polymerisation kinetics, which is that no side reactions occur resulting in ‘dead’ chains or additional polymer chains, and that all the initiator and MADIX agent molecules reacted, the theoretical number of ‘dead’ PAA chains can be estimated. This is because ‘dead’ chains are produced in RAFT/MADIX polymerisation based on the ratio of initiator to MADIX agent, shown in Equation (35):

\[
De (\%) = \frac{I_0}{I_0 + A_0} \times 100
\]  

(35)

where \(De\) is the fraction of ‘dead’ chains, \(I_0\) is the amount of initiator (in mol) and \(A_0\) is the amount of RAFT/MADIX agent (in mol). Each polymer chain starts with either an initiator or the RAFT/MADIX agent and so the total theoretical number of chains can be determined. To maintain a chain’s ‘livingness’ it must also contain the RAFT/MADIX agent at the end of the chain.

Using Equation (35) the theoretical fraction of ‘dead’ chains was found to be 7.5 % (mol/mol) in the PAA samples (see section 7.5 for calculations). However as shown in Table 13 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 (initial homopolymer) was converted to a block copolymer. The discrepancy between the theoretical and measured fractions of ‘dead’ chains the may be due to the difference between values of the actual and theoretical molar masses (the non-ideal kinetics of polymerisation of acrylic acid [110] leading to branching [122] and other polymer chains) or to incomplete reaction of all the initiator (especially since no initiator moiety was detected with NMR, see section 3.1). In the case of PAA2kPAM10k, the theoretical maximal fraction of ‘dead chains’ would result in 1.4 % (w/w) or 0.07 g L\(^{-1}\) of PAA homopolymer, which is below the LOD at the injection concentration used.
The large fraction of PAPTAC homopolymer in PAPTAC5kPNIPAM5k cannot be solely attributed to the use of excess initiator because $De$ is 25% assuming complete decomposition of the initiator. However it may explain the residual homopolymer in PAPTAC2kPNIPAM8k. Since some PNIPAM is also detected it may be that the PAPTAC macroMADIX agent is not as reactive as required. Since the PAPTAC2kPNIPAM8k has a significantly lower fraction of residual PAPTAC than PAPTAC5kPNIPAM5k it is unlikely to be due to the xanthate group. However, the PAPTAC chain has to be a sufficient leaving group for it to disassociate from the xanthate allowing it to propagate with the NIPAM monomers to form another block [26,44]. Therefore the higher molar mass PAPTAC chain may be a less effective leaving group resulting in some of the macroMADIX agents not reacting over the course of the reaction time. However since the ‘livingness’ of the polymerisation cannot be measured it may be that the PAPTAC homopolymers have undergone a number of side reactions that have resulted in ‘dead’ chains and so the PNIPAM propagating chains were unable react with a significant number of PAPTAC chains. Further experimental work to assess the livingness if the PAPTAC homopolymers is required to identify which step in the synthesis of the block copolymer is causing the large amount of residual homopolymer. CE-CC gives the first direct evidence of the formation of a block copolymer.

4.4 Assessing the Purification of Block Copolymers

In response to the large fraction of residual PAPTAC in the P(APTAC-$b$-NIPAM) samples the group from Paul Sabatier University performed dialysis on the samples. Since the block copolymers are expected to have a larger molar mass than the homopolymers it would be assumed that with a sufficient molar mass cut off of the dialysis membrane the homopolymers could be removed from the block copolymer sample. CE-CC can then be used to assess the effectiveness of the dialysis of the samples.

The fraction of PAPTAC homopolymer in the P(APTAC-$b$-NIPAM) samples before and after dialysis is shown in Table 14. After dialysis it was found that the
fraction of PAPTAC in the block copolymer samples may be reduced by a small amount. However, there was still a significant fraction of PAPTAC homopolymer present in the block copolymers. As mentioned in section 4.2.2.3 PAPTAC oligomers were also detected in the original block copolymer. After dialysis oligomers were no longer detected indicating that the dialysis is removing the PAPTAC oligomers from the block copolymer but leaving most of the residual PAPTAC homopolymer in the sample (Figure 44). Therefore it would seem that the PAPTAC homopolymer is too high in molar mass for the dialysis membrane used, however the small oligomers were removed.

Table 14. Fraction of PAPTAC homopolymer and P(APTAC-b-NIPAM) in the block copolymer samples before and after dialysis. Error determined from n=3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polymer component</th>
<th>Before dialysis</th>
<th>After dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% (w/w)</td>
<td>% (mol/mol)*</td>
</tr>
<tr>
<td>PAPTAC2KPNIPAM8K</td>
<td>PAPTAC</td>
<td>1.8 ± 0.1</td>
<td>8.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>P(APTAC-b-NIPAM)</td>
<td>96</td>
<td>89</td>
</tr>
<tr>
<td>PAPTAC5KPNIPAM5K</td>
<td>PAPTAC</td>
<td>18.9 ± 0.2</td>
<td>31.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>P(APTAC-b-NIPAM)</td>
<td>80</td>
<td>67</td>
</tr>
</tbody>
</table>

*calculated from the theoretical molar masses shown in Table 1.
Figure 44. Electropherograms obtained by CE-CC of PAPTAC1k homopolymer (red) and of PAPTAC1kPNIPAM9k block polymers before (black) and after dialysis (blue). The PAPTAC homopolymer and the internal standard (labeled as PH and IS respectively) have mobilities between 4 and 5 × 10⁻⁸ m² V⁻¹ s⁻¹, and between 6 and 9 × 10⁻⁸ m² V⁻¹ s⁻¹, respectively. PAPTAC oligomer peaks (labeled as PO) occur around 2 × 10⁻⁸ m² V⁻¹ s⁻¹ as shown in the insert. Injection concentration of the internal standard was 1 mM, while PAPTAC1kPNIPAM9k samples were injected at 5 g L⁻¹ and PAPTAC1k was injected at 0.63 g L⁻¹.

Pressure assisted CE-CC was performed on the block copolymer samples after dialysis to assess its impact on the PNIPAM content of P(APTAC-b-NIPAM). The dialysis may have lowered the fraction of PNIPAM since the shoulder in the electropherograms is no longer as prominent (Figure 45). Identifying the effect of dialysis on the block copolymer chains is difficult since the adsorption is difficult to reproduce, this is especially prevalent in the electropherogram of PAPTAC5kPNIPAM5k (Figure 45b). However by determining the fraction of block copolymer in the sample through subtraction of the fraction of each homopolymer it appears that the fraction of block copolymer may have slightly increased, indicating that the block copolymer was not removed during the dialysis (Table 14).
Figure 45. Electropherograms obtained by pressure assisted CE-CC of a mixture of PAPTAC5k and PNIPAM5k homopolymers (red) as well as of block copolymers a) PAPTAC2kPNIPAM8k and b) PAPTAC5kPNIPAM5k before (black) and after dialysis (blue). Dashed lines indicate repeat separations. Injection concentration of each individual polymer was 5 g L$^{-1}$.

It appears that the dialysis was largely unsuccessful in removing the residual PAPTAC homopolymer. In order to remove the residual PAPTAC a larger molar mass cut off dialysis membrane is required, although this may cause loss of some of the block copolymer chains as well. Purification of DHBCs is difficult since identifying a solvent to selectively dissolve the homopolymers and not the block copolymer would be difficult. Although CE-CC can separate PAPTAC homopolymer from the P(APTAC-b-NIPAM) block copolymer, the amount injected in the capillary is in nL quantities thus CE-CC is not well suited for purification. Ion-exchange columns may be able to provide purification although the adsorption would be difficult to overcome. Therefore there are little alternatives to dialysis in trying to purify the block copolymer. To minimise the amount of homopolymer in the block copolymer sample it may be more efficient to find the synthetic conditions to produce this sample directly instead of trying to subsequently purify the sample.
4.5 Chapter Conclusion

Determining the purity of block copolymers is important for a complete characterisation 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 reinitiation of a homopolymer can be assessed by CE-CC, which is not possible with SEC and NMR. In the case of a P(AA-b-AM) and P(AA-b-EO) block copolymers with varying ratios of charged and uncharged block lengths, CE-CC was shown to easily identify the formation of a block copolymer and measure the fraction of parent PAA homopolymer. In contrast, SEC was unable to identify the presence of any homopolymers in the block copolymer and for PAA10kPAM10k it was even 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 there is no stationary phase. This makes CE-CC an excellent method to characterise highly adsorbing polymers such as P(APTAC-b-NIPAM) and other cationic DHBCs, especially since a range of different coatings can be used to prevent interaction with the capillary walls. UV detection in CE-CC was found to have linearity and recovery equal to 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 fractions 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 in the future. Furthermore, the effectiveness of purification techniques such as dialysis was assessed by CE-CC. With CE-CC kinetic and ‘livingness’ studies of the polymerisation are possible since the quantity of both the block copolymer and the homopolymers can be determined.
CHAPTER 5. HETEROGENITY AND DISTRIBUTION OF COMPOSITIONS

As stated in section 1.1.3 the chemical structures of polymers exist as distributions. The width of a composition distribution is often assumed to be narrow for a copolymer; however they can differ greatly between samples [156]. This chapter aimed at developing a relatively easy and inexpensive means of determining the distribution of compositions or at least their heterogeneity of DHBCs. This method was developed using CE-CC which does not require the use of standards or spectroscopic detectors which have been previously used in the literature with LC based methods to determine the distribution of compositions of hydrophobic block copolymers.

5.1 Electropherograms of Block Copolymers

In CE-CC the electropherogram is the detector response as a function of migration time. In this work a DAD detector was used for UV detection. Block copolymers are separated according to the ratio of the block lengths as shown in Equation (5) and thus separated according to their composition. Therefore CE-CC provides a means to assess how broad the distribution of compositions is within a block copolymer sample. The simplest way to assess the composition is to compare the UV signal as a function of migration time between block copolymer samples.

From the electropherograms of PAA2kPAM10k and PAA10kPAM10k it is observed that each block copolymer has a distinct range of migration times with the peaks occurring between 3 and 6 min, and between 3 and 9 min, respectively (Figure 46). Since PAA10kPAM10k has a larger range of migration times compared to PAA2kPAM10k it seems that it has a wider range of compositions. Since some migration times of PAA10kPAM10k are higher than that of PAA2kPAM10k.
PAA10kPAM10k may contain a higher fraction of PAA in some chains than PAA2kPAM10k.

**Figure 46.** Electropherograms of PAA2kPAM10k (red) and PAA10kPAM10k (blue) with the UV absorbance as a function of migration time. The EOF marker migrates at 3 min and a PAA homopolymer peak is present in PAA10kPAM10k between 9 and 10 min. Injection concentration was 5 g L\(^{-1}\) for each sample. PAA2kPAM10k was separated with an extended light path capillary with a total length of 60.6 cm (effective length 52.1 cm) while PAA10kPAM10k was separated with a standard fused silica capillary with a total length of 59.9 cm (effective length 51.4 cm).

In should be noted that comparing the width of distributions is biased using migration time since species that migrate at different velocities (giving them different migration times) will spend different amounts of time in the detection window and absorb more or less light due to this [213]. To account for the differences in analyte velocities the detector signal can be divided by the migration time, as shown in Equation (36). Therefore the distribution of compositions in PAA10kPAM10k may not be significantly broader than PAA2kPAM10k as it appears (Figure 46). Furthermore the migration times can only be compared between separations carried out with the same capillary length and voltage. Using electrophoretic mobility instead of migration time allows for better comparison between systems and corrects for the bias caused by longer
residence time in the detection window. Furthermore as described in section 2.3.4 and shown in Table 10 the electrophoretic mobility is more repeatable than migration time. The electrophoretic mobility can then be converted to chemical charge density which is the composition using Equation (9). Through composition distributions samples can be compared regardless of separation conditions. Furthermore, not only the width of the composition distribution can be identified but also the actual compositions present in the sample. Therefore using the electrophoretic mobility to assess composition is more precise than migration time and the composition distribution is more accurate than both migration time and electrophoretic mobility. Converting from migration time to chemical charge density is preferred to correctly assess the distribution of compositions in a block copolymer sample.

5.2 Theory of Obtaining a Distribution of Compositions and its Dispersity

5.2.1 Effect of changing variables

Obtaining a distribution of compositions requires relating one variable (migration time) to the composition. This can be done through a calibration curve or in the case of CE-CC a direct mathematical relationship, as shown in Equations (5 and 9). In order to calculate composition a variable is converted. Obtaining distributions requires a separation technique and most separation techniques record the detector response $R(t)$ as a function of time ($t$). When converting the time to another variable ($y$), the detector response must also undergo a correction. When the relationship between $t$ and $y$ is not linear, the shapes of the original electropherogram and of the converted distribution differ. This is because $R(t)$ is present as a function of $t$ and not of $y$. Not applying the correction can then result in an inaccurate assessment of the resulting distribution [214].
5.2.2 Calculating electrophoretic mobility distributions

The most common electropherograms obtained in free solution CE are a UV absorption signal as a function of migration time. The latter can be converted to electrophoretic mobility as described in section 2.3.4. The relationship between migration time and electrophoretic mobility is hyperbolic and thus not linear. In order to plot the electrophoretic mobility distribution, the UV signal should be “corrected” otherwise the distribution would be distorted. The distortion is negligible with relatively narrow peaks produced by small molecules and polymers in critical conditions. When identifying peaks and obtaining peak areas of narrow peaks, the effects of the distortion can also be avoided by using migration time and the corrected peak area as described in section 2.3.4, thus the electrophoretic mobility is not needed. However, to characterise polymers using distributions, such as distributions of compositions and heterogeneity of branching the observed broad peaks would be influenced by the distortion resulting in a bias in the distribution. Therefore transforming the UV absorption signal when converting from a function of migration time to a function of electrophoretic mobility allows an accurate determination of the weight fraction.

The weight fraction can be calculated using a similar process to what is used in SEC to convert from a \( w(\log M) \) distribution to \( w(M) \) [193]. In SEC when converting the abscissa from elution volume to molar mass the detector response is converted by dividing it with the derivative of the calibration curve [194]. In CE-CC a mathematical relationship exists between migration time \( (t_m) \) an electrophoretic mobility \( (\mu) \) and between \( \mu \) and chemical charge density \( (\xi) \) and thus these equations are used instead of the calibration curve. When converting \( t_m \) into \( \mu \), to obtain \( w(\mu) \) from the initial UV signal \( (S_{UV}) \) as a function of migration time Equation (36) can be used which takes into account the different analyte velocities by dividing with \( t_m \).

\[
w(\mu) = \frac{S_{UV}}{(\mu \frac{d\mu}{dt_m}) t_m}
\]  

(36)
Given the relationship between $t_m$ and the electrophoretic mobility ($\mu$) in Equation (10) the derivative with respect to $t_m$ is shown in Equation (37), determination of the derivatives are shown in section 7.6:

$$\frac{d\mu}{dt_m} \propto \frac{1}{t_m^2}$$

Note that the absolute value of the derivative is taken. Furthermore a constant relating to the capillary length and applied voltage is also present in the derivative; however, this is only important for comparing the peak area between electropherograms obtained with different voltages or capillary lengths. They are not required for the determination of relative values such as averages or dispersity values from a distribution. By substituting Equation (37) into Equation (36) the following relationship is obtained:

$$w(\mu) \propto S_{UV} \times t_m$$

### 5.2.3 UV signal for block copolymer in CE-CC

For the block copolymers the UV absorption detector proportional to the number of monomer units, giving the weight fraction. This is the same for the SEC of polymers with UV or RI detection. However the weight fraction is the total mass of monomer units at a point in a distribution divided by the total mass of monomer units in the sample. For homopolymers the number of monomer units is directly proportional to the mass of monomer units since the monomer units all have the same molar mass. However for copolymers the molar mass is different for the different monomer units. Therefore UV and RI detection which are sensitive to the number of monomer units do not directly provide the weight fraction. The weight fraction presented for the block copolymers in this work is assuming the monomer units all have the same molar mass; it follows that the UV signal which is proportional to the number of monomer units in both blocks would be directly proportional to the total mass of the monomer units. In the case of the P(AA-b-AM) the assumption is valid since the monomer units have the same molar mass while for P(APTAC-b-NIPAM) there is a 37 % difference in the molar mass of the monomer units which may cause some error in the determined distribution.
At each point in the distribution of the block copolymers in CE-CC there are polymer chains of a specific composition where the polymer chains can have different molar masses. For branched polymers a similar situation occurs where at each point in the SEC distribution there are chains with the same hydrodynamic volume but different molar masses due to the branched structures [193]. Band broadening also occurs resulting in chains with different hydrodynamic volumes eluting at the same point [193]. With all separation method band broadening occurs, and in CE-CC it would cause some polymer chains of different compositions to overlap. Thus the $S_{UV}$ which is a function of migration time ($t_m$) can be expressed similarly to the RI detector signal of branched polymer in SEC [193], as shown in Equation (39):

$$S_{UV} = A \varepsilon \int_0^1 \int_0^\infty G(t_m, C)M N(M, C) dM dC$$

(39)

where $A$ is a calibration instrument constant, $\varepsilon$ is the molar absorptivity of the monomer units assuming all monomer units have the same molar absorptivity, $G(t_m, C)$ is a band broadening function and $N(M, C)$ is the number distribution of the polymer chains which have the composition $C$ and the molar mass $M$. The calibration constant is unique to each detector and can be determined experimentally; however, it is not required to compare points in a distribution since the factor is the same for each point. The composition is a fraction of one block in the copolymer and it has values only between 0 and 1, unlike molar mass which values have no upper limit. Band broadening in CE-CC should be minimal since in the absence of adsorption diffusion of the species would be the only cause, as described later in section 5.3.1, hence the $G(t_m, C)$ term would be close to a Dirac function.

The signal produced by the DAD detector is complex making the direct comparison of the distribution obtained by CE-CC to distributions obtained by other methods difficult, thus requiring more work outside the scope of this thesis.

5.2.4 Calculating the distribution of compositions
The weight distribution of compositions (weight fraction as a function of composition), \( w(\xi) \), can be obtained from \( w(\mu) \) by dividing it with the derivative of Equation (9) which is used to convert \( \mu \) to \( \xi \). Hence \( w(\xi) \) can be calculated from Equation (40):

\[
w(\xi) = \frac{w(\mu)}{\left(\frac{d\xi}{d\mu}\right)}
\]  

(40)

where the term \( d\xi/d\mu \) is expressed as follows (determination of derivative shown in section 7.6):

\[
\frac{d\xi}{d\mu} = \frac{\alpha\mu_0}{[\mu(\alpha - 1) + \mu_0]^2}
\]  

(41)

therefore by substituting Equation (41) into Equation (40) the following relationship is shown:

\[
w(\xi) = w(\mu) \frac{[\mu(\alpha - 1) + \mu_0]^2}{\alpha\mu_0}
\]  

(42)

although this conversion is not as simple as Equation (38) all the values would be known when attempting to obtain a distribution of compositions. Hence \( w(\xi) \) can be calculated for each composition distribution.

It is important to note that although \( w(\mu) \) and \( w(\xi) \) can be calculated their accuracy is dependent on the assumptions done above on detection itself. Some error in the distributions is due to a difference in molar absorptivity between the different monomer units. However, this error is removed if the block copolymer is measured at the isobestic point between the two blocks, which is the wavelength at which the absorption coefficients of both units are equal.
5.2.5 Average compositions and dispersity of a composition distribution

As mentioned in section 3.3.2 the broadness of a distribution is normally quantified by a statistical dispersity term. The composition distribution of a copolymer can thus be described in terms of the Dispersity of Composition (DC). In this work the DC is defined in a similar manner to D used for molar mass distributions. The ‘weight average composition’ (C_w) can be determined from a weight-composition distribution (which is the most common composition distribution) however the ‘number average composition’ (C_n) cannot be determined since the number of polymer chains corresponding to a particular composition is unknown from that distribution. It is theoretically possible to simultaneously determine the C_w and C_n, if the weight and number composition distributions are found with multiple detection which detects the monomer unit and the end group, assuming all polymer chains have the same end group. With a DAD detector it is possible to selectively detect the end group, for instance the MADIX agent used can be detected at 285 nm while the monomer units cannot. However the sensitivity is often insufficient to detect the end group making the determination of C_n to be not possible or inaccurate in most.

An infinite number of averages can be calculated from a distribution using the ratio of the moments in the distribution, moments are shown in section 7.7 [194]. M_n can be calculated by dividing moment -1 by moment 0 therefore an equivalent composition average can be determined by dividing moment -1 by moment 0, denoted by C_m, of the composition weight distribution (moments shown in Tables A-3–A-5). By analogy with D, DC can be calculated similar to DEM in section 3.3.2 as shown in Equation (43).

\[ DC = \frac{C_w}{C_m} \]  

with C_m, C_w and C_n defined by analogy with M_n and M_w in Equations (15, 28 and 18) as:

\[ C_m = \frac{\sum_k w_k}{\sum_k w_k c_k^{-1}} \]
\[ C_w = \frac{\sum_k w_k C_k}{\sum_k w_k} \quad (45) \]

\[ C_n = \frac{\sum_k N_k C_k}{\sum_k N_k} \quad (46) \]

where \( w_k \) and \( N_k \) are the total weight and number of the polymer chains respectively of number \( k \) and \( C_k \) is the particular composition of these polymer chains of number \( k \). \( C \) is the fraction of one type of monomer unit (relative to all monomer units in the chain), which can be expressed as a percentage or a unit fraction. Specifically in the case of charged-uncharged copolymers \( C \) can be expressed as the chemical charge density (\( \zeta \)), thus the terms \( C \) and \( \zeta \) can be used interchangeably.

### 5.3 Electrophoretic Mobility of Charged Homopolymers in the Critical Conditions

#### 5.3.1 How to determine the electrophoretic mobility

As mentioned in section 1.4.4 block copolymers are separated by CE-CC according to their composition, as shown in Equation (5). The first step in relating the electrophoretic mobility of a block copolymer to its composition using Equation (5) is to determine the electrophoretic mobility of the charged homopolymer in the critical conditions (\( \mu_0 \)). This electrophoretic mobility can directly be determined from the electropherogram of the charged homopolymer. However determining the true electrophoretic mobility of an analyte may be more complex than selecting the peak maximum corresponding to the homopolymer and applying Equation (10) to this migration time. This is due to the asymmetrical peak shapes commonly observed in free solution CE.

In the absence of Joule heating and adsorption of the analyte onto the capillary surface, the band broadening of a pure analyte is mainly caused by diffusion [83]. As the analyte migrates through the capillary diffusion occurs equally in each direction, which
produces Gaussian shaped peaks. In this situation the migration time at the maximum of the peak gives the best approximation of the electrophoretic mobility value of the analyte. However in free solution capillary electrophoresis the phenomenon of electrodispersion, also known as electromigration dispersion, results in the measurement of asymmetrical, triangular shaped peaks. Electrodispersion takes place when there is a difference between the electrophoretic mobilities of an analyte and of the background electrolyte (BGE). The difference results in a stable and unstable boundary between the analyte the BGE. The stable boundary is produced by the stacking of the ions while the unstable boundary does not have this stacking and thus the ions are more spread out as they migrate [215]. The concentration of the analyte also influences how far the ions are spread out at the unstable boundary [216]. Therefore the peak maximum can vary slightly with the concentration due to electrodispersion and thus no longer provides the best approximation of the electrophoretic mobility. When the analyte is ‘highly’ dilute electrodispersion does not take place since there is not enough analyte ions to create the boundary [215]. Consequently the electrophoretic mobility of analyte at the peak maximum is determined more accurately when in the absence of electrodispersion. To prevent electrodispersion the analyte needs to either have the same electrophoretic mobility as the BGE or to be highly dilute. Such criteria are rarely met since electrophoretic mobilities are rarely identical and reaching these ‘highly’ dilute conditions reduces sensitivity. However, with triangular peaks it is possible to determine the electrophoretic mobility of the analyte in the absence of electrodispersion [217]. Since only one side of the peak is influenced by the electrodispersion, the other side is only broadened by diffusion (Figure 47). The longer side of the peak has broadening due to both diffusion and electrodispersion. The distance \( \Delta \) changes with concentration while the distance \( d \) is equal on either side of the apex. As a result the peak which is only under the influence of diffusion and not electrodispersion would have an apex marked by the red line which would correspond to the true electrophoretic mobility (Figure 47).
Figure 47. Illustration of the average peak shape obtained in free solution capillary electrophoresis. The dashed line shows the peak broadening due to diffusion creating the distance $d$. The distance $\Delta$ is caused by electrodispersion. The red line indicates the true electrophoretic mobility of a pure analyte.

There is a 5.5 and 2.1 % difference in the electrophoretic mobility for PAA10k and PAA50k, respectively, when taken at the peak maximum and when correcting for electrodispersion (Table 15). There is a significant difference between the electrophoretic mobility at the peak maximum and after correcting electrodispersion for PAA10k but not for PAA50k. Although there is no significant difference in electrophoretic mobility between PAA10k and PAA50k, based on the averages obtained there is a 2.7 % difference at the peak maximums and 0.8 % after correcting for electrodispersion (Table 15). As discussed in section 3.3.2 the electrophoretic mobility for poly(styrene sulfonate) varied by less than 5 % due to the molar mass for polymer chains that were more than 100 monomer units long [94]. For PAA the variation is lower than for poly(styrene sulfonate). Furthermore the difference in electrophoretic mobility between taking it at the peak maximum or correcting for electrodispersion as shown in Figure 47 is significantly larger than the difference in electrophoretic mobility due to molar mass for PAA10k and PAA50k. Therefore correcting for electrodispersion should be undertaken when determining the electrophoretic mobility of a polyelectrolyte.
Table 15. Difference in electrophoretic mobility for PAA's of different molar masses when taken at the peak maximum and when correcting for electrodispersion.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Electrophoretic mobility at peak maximum $(\times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1})$</th>
<th>Electrophoretic mobility correcting for electrodispersion $(\times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1})$</th>
<th>Number of injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA10k</td>
<td>3.61 ± 0.07</td>
<td>3.82 ± 0.04</td>
<td>11</td>
</tr>
<tr>
<td>PAA50k</td>
<td>3.71 ± 0.05</td>
<td>3.79 ± 0.06</td>
<td>16</td>
</tr>
</tbody>
</table>

It is also important to note that the block copolymer sample is separated into many different species based on their composition. Each species would then be highly dilute and likely not influenced by electrodispersion, therefore each point in the block copolymer's distribution should only correspond to one composition. To prevent the impact of oligomers and peak tailing due to adsorption on the distances $d$, the tangent at half maximum was taken on each side of the triangular shaped peaks. Where the tangent crossed the baseline was taken as the start of distance $d$ (Figure 47).

5.3.2 Electrophoretic mobility of PAA and PAPTAC homopolymers in CE-CC

To ensure critical conditions were reached the electrophoretic mobility of PAA was determined from PAA10k and PAA50k. The largest molar mass PAPTAC sample used was PAPTAC5k which would still have some minor influence of molar mass on its electrophoretic mobility thus the precise electrophoretic mobility of PAPTAC in the critical conditions cannot be determined. In the case of the P(APTAC-$b$-NIPAM) block copolymers pressure assisted CE-CC is needed to improve the recovery. Considering the adsorption of the P(APTAC-$b$-NIPAM) block copolymer only relative comparisons between samples were possible thus the error from the electrophoretic mobility of PAPTAC should be negligible overall. The electrophoretic mobility of the PAPTAC samples cannot be determined since the EOF is too weak to be measured within 1 h. The
apparent mobility is thus measured (and its value is close to the electrophoretic mobility due to the weak EOF) as according to Equation (11). Since in Equation (6) the ratio in electrophoretic mobility is used it does not matter if electrophoretic or apparent mobility is used because the EOF is negligible in the cases where apparent electrophoretic mobility is used. Thus in the conditions used for PAPTAC based polymers the apparent mobility would not be significantly different from the electrophoretic mobility.

The electrophoretic mobility of PAA was highly repeatable with an RSD of 1.35\% (Table 16). This value includes injections at different concentrations, separations in different capillaries and on different days, showing the ruggedness of CE-CC. The apparent mobility of PAPTAC was less repeatable but still acceptable with an RSD of 3.66\% (Table 16). The lower repeatability obtained for PAPTAC may be due to the adsorption or to the lower number of injections.

**Table 16. Electrophoretic or apparent mobilities of charged homopolymers.**

<table>
<thead>
<tr>
<th>Homopolymer</th>
<th>Electrophoretic mobility value used for $\mu_0$ ($\times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$)</th>
<th>Number of injections</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA</td>
<td>3.80 ± 0.05</td>
<td>27</td>
<td>1.35</td>
</tr>
<tr>
<td>PAPTAC$^*$</td>
<td>5.7 ± 0.2</td>
<td>6</td>
<td>3.66</td>
</tr>
</tbody>
</table>

$^*$apparent electrophoretic mobility given relative to the internal standard in pressure assisted CE-CC.

**5.4 Rescaling Factor $\alpha$**

**5.4.1 Effect of the rescaling factor**

The electrophoretic mobility of a block copolymer is related to its composition as shown in Equation (5). Based on the electrophoretic mobility of the block copolymer relative to the electrophoretic mobility of the charged parent homopolymer the composition can be qualitatively assessed. Furthermore comparisons between block copolymers can be made as those with a larger charged block compared to the
uncharged block exhibit an electrophoretic mobility closer to that of the electrophoretic mobility of the charged homopolymer. As explained in section 1.4.4 this is due to the hydrodynamic friction which the uncharged block adds to the block copolymer chain. The way in which the uncharged block influences the hydrodynamic friction of the block copolymer chain varies between different combinations of blocks. Therefore to determine quantitative values of chemical charge density and hence composition from the electrophoretic mobility, the rescaling factor ($\alpha$) must be known.

Predicting $\alpha$ for a block copolymer can be difficult. To simulate the effect of $\alpha$ on the distribution of compositions different $\alpha$ values were used in Equation (9) to calculate the composition distribution of PAA2kPAM10k and PAA10kPAM10k (Figure 48). The arbitrary values of 1, 2 and 10 were used to observe the effect of doubling $\alpha$ and changing it by a factor of ten. With these values it can be estimated how accurately the $\alpha$ value is needed to produce the true composition distribution. If doubling the $\alpha$ resulted in an insignificant change in the distribution then knowing $\alpha$ within a factor of two would be sufficient to determine the composition distribution. $\alpha$ has previously been determined for DNA conjugated to PEO [164], an 18 amino acid protein [218] and a 30 amino acid protein [219] to be 0.138, 0.2 and 0.26 respectively. Therefore different block copolymers exhibit different $\alpha$ values which can differ by a factor of 2. Since $\alpha$ is related to the ratio of the Kuhn lengths of the charged and uncharged blocks these reported $\alpha$ values are similar as they all have DNA as the charged block. Furthermore DNA would have a noticeably different Kuhn lengths to the other blocks used, giving a value lower than 1. The blocks in the synthetic block copolymers in this work would have more similar Kuhn lengths due there relatively similar chemical structures. Thus an $\alpha$ closer to 1 is expected for these synthetic polymers, which is closer to the arbitrarily chosen $\alpha$ values. The different $\alpha$ values chosen caused strong shifts in the chemical charge density, as well as expansions and contractions in the distribution (Figure 48). A significant shift was observed when $\alpha$ was arbitrarily doubled, demonstrating that different $\alpha$ values can result in completely different chemical charge density distributions and thus composition distributions.
Figure 48. Effect of $\alpha$ value on the chemical charge density distribution of a) PAA10kPAM10k and b) PAA2kPAM10k: $\alpha$ values of 1 (green), 2 (black) and 10 (dark blue) were arbitrarily used. The vertical straight lines indicate the $C_w$ of the corresponding distribution.

To observe whether the distortion in the distribution caused by different $\alpha$ values is relative between samples the change in $DC$ between samples was compared (Table 17). It was found that when a different value of $\alpha$ is used the $DC$ changes. The relative change between samples is not equal so the $DC$ cannot be compared between samples unless an accurate value of $\alpha$ is known because the distortions in the distribution are not comparable for different samples.
Table 17. Effect of $\alpha$ on the $DC$ of a composition distribution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha$</th>
<th>$DC$</th>
<th>Change in $DC$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA2kPAM10k</td>
<td>1</td>
<td>1.121</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.067</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.008</td>
<td>10.1</td>
</tr>
<tr>
<td>PAA10kPAM10k</td>
<td>1</td>
<td>1.123</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.055</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.005</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*Change is calculated relative to the initial value when $\alpha$=1, with (initial-x)/initial × 100, where x is a subsequent value.

5.4.2 Determining the rescaling factor

The difficulty in using Equation (5) lies in the determination of $\alpha$ since the other variables must be known in order to calculate $\alpha$. The previous studies used to develop Equation (5) involved the use of monodisperse DNA or proteins, this coupled with good resolution enabled the determination of $\alpha$ [164]. Unfortunately with synthetic block copolymers no monodisperse (uniform) block is present as both blocks exhibit a distribution of molar masses. However, as mentioned in section 1.4.4 Equation (6) can be used to determine $\alpha$ by a plot of $\mu_0/\mu - 1$ vs $n_u/n_c$ yielding $\alpha$ as the slope. The determination of $\mu_0$ is as described in Table 16, section 5.3.2, while the other values are more difficult to determine since in each electropherogram a number of different $\mu$, $n_u$ and $n_c$ values are present.
5.4.2.1 Determination of $\alpha$ through weight average molar mass

In CE-CC with UV detection sensitive to the monomer units of each block (carboxylate or amide functional groups as chromophores), the signal intensity is a weight sensitive detection. If each composition corresponded to a particular molar mass then each electrophoretic mobility would correspond to a particular molar mass, and in turn each electropherogram would be similar to the molar mass weight distribution of the block copolymer. In a molar mass weight distribution the molar mass at the maximum usually corresponds to the $M_w$ of the polymer sample [220]. It should be noted that in most causes the chromatograms produced in SEC are the log weight molar mass distribution of the polymer and need to be converted to a weight distribution [193]. There is an individual electrophoretic mobility which can be related to specific values of $n_u$ and $n_c$. In the plot of $\mu_0/\mu - 1$ vs $n_u/n_c$ the value for $\mu$ can be taken as the electrophoretic mobility at the maximum of the peak of the block copolymer, while $n_u$ and $n_c$ can be determined from the $M_w$ of the block copolymer and initial homopolymer. Since SEC is conducted on most polymer samples it could be used to determine the $M_w$. Alternatively, $M_w$ could be determined by static light scattering [221,222] and recent developments in Taylor Dispersion Analysis may enable the determination of $M_w$ using the capillary electrophoresis equipment [204,205,223-225].

Using the above process to determine $\alpha$ means that the accuracy of $\alpha$ is dependent on the accuracy of $M_w$. Considering the reproducibility of the SEC of polystyrene in THF to determine $M_w$ was found to ± 9 % [182] there will always be some error on the $M_w$. Furthermore to obtain both $n_u$ and $n_c$ the $M_w$ of the initial homopolymer must be subtracted from the $M_w$ of the block copolymer. This assumes that the chains which correspond to the $M_w$ in the homopolymer are the same chains that are converted into the block copolymer chains corresponding to $M_w$ of the block copolymer. Overall these errors are likely to be in an acceptable range considering the assumptions made in SEC of copolymers. These assumptions include the composition
not influencing the hydrodynamic volume and the $dn/dc$ value which affects the separation and detection of the copolymers in SEC, respectively [56].

5.4.2.2 Concluding about the determination of $\alpha$

To determine the $\alpha$ of a block copolymer the $\mu_0$ of the charged homopolymer must be known. A reasonable number of block copolymer samples with different targeted compositions are needed to enable a meaningful linear fit of $\mu_0/\mu - 1$ vs $n_u/n_c$. Either the $M_w$s of the block copolymer and of the initial homopolymer must be known, or each monomer unit must be able to be discriminated by a quantitative spectroscopic technique to determine $n_u/n_c$. Finally the electropherograms of the block copolymer samples is required; these would then be used to obtain the distribution of compositions.

Unfortunately the number of block copolymer samples available in this project was not sufficient to accurately determine a value of $\alpha$. Furthermore in the case of the block copolymer P(AA-b-AM) determining the $M_w$ accurately is not easy as stated in section 3.2.4. Since Equation (6) requires the ratio of the numbers of uncharged to charged monomer units techniques which can determine the average composition such as NMR spectroscopy may provide a means of determining $\alpha$. However, distinguishing between the AA and AM monomer units using $^1$H and $^{13}$C NMR spectroscopy and a spectrometer with a field strength equal to or lower than a $^1$H Larmor frequency of 600 MHz is difficult [226-229]. Furthermore a composition value which relates to a particular point in the electropherogram is required. The $C_w$ appears to occur near the maximum of the electropherogram; however, techniques such as NMR and IR spectroscopy do not clearly provide the $C_w$. Furthermore due to the complexity of the detection as described in section 5.2.3 there is no clear link between the composition determined by NMR and any of the composition averages which can be determined from the composition distribution. Therefore additional samples and other experiments are required to obtain an $\alpha$ and in turn obtain the true distribution of compositions and $DC$.  

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Since $\alpha$ is unique to each block copolymer, for the composition distribution of each block copolymer to be determined the $\alpha$ must be determined. To determine $\alpha$ certain parameters must be achieved such as a well-defined $M_w$. This could be particularly challenging to obtain for a number of block copolymers and may limit its use in the development of new block copolymers. However once $\alpha$ has been determined for a block copolymer, this value can be used indefinitely for samples of that particular block copolymer. Therefore for industrial applications which involve producing multiple bulk quantities of a particular polymer the difficulties in determining $\alpha$ once would not likely be a concern. This occurs for a number of constants in polymer science such as Mark-Houwink-Sakurada parameters [230] and kinetic coefficients of polymerisation [106].

5.5 Distribution of Electrophoretic Mobilities of Charged-Uncharged Block Copolymers

5.5.1 Influence of chemical structure on electrophoretic mobility

Although the $\alpha$ of the block copolymers could not be determined to obtain the true distribution of compositions the distribution of electrophoretic mobilities can be used to assess the composition of the block copolymers. Since each electrophoretic mobility corresponds to a particular composition as stated by Equation (5), the broadness of the electrophoretic mobility distribution gives an indication of the $DC$ of the sample. In a similar way, the distribution of electrophoretic mobilities of a charged homopolymer provides information on the heterogeneity of branching structures in the polymer. The distribution of electrophoretic mobilities of a charged-uncharged block copolymer provides heterogeneity of composition. Consequently the Dispersity of Electrophoretic Mobility ($DEM$) used in section 3.3.2 to compare the heterogeneity of
branching between samples can also be used to compare the composition distributions in different samples.

It should be noted that branching does influence the electrophoretic mobility of the block copolymer in addition to composition. Therefore a similar situation as that in SEC is present, with branching affecting the separation. However, unlike SEC where branching has a large impact on the hydrodynamic volume [122], the difference in electrophoretic mobility of a hyperbranched and linear polymer is about 3 % [100]. In contrast larger differences in electrophoretic mobility are observed between a pure charged homopolymer and a block copolymer, thus the error due to incomplete separation of compositions due to branching is very minimal. Furthermore for the P(AA-\textit{b}-AM) samples in this work the \textit{DB} of the PAA was found to be very low as shown in section 3.3.1. The branching in the PAM block is also likely non-existent or very low since the only documented evidence of branching in PAM is in a publication before the time branching could be detected or quantified by NMR spectroscopy and which only found branching following polymerisations above 70 °C [112]. Therefore it would be unlikely that there would be any significant effect of branching on the electrophoretic mobility of the block copolymers.

5.5.2 Comparing electrophoretic mobility distributions

5.5.2.1 P(AA-\textit{b}-AM) block copolymers

Both PAA2kPAM10k and PAA10kPAM10k have broad peaks but they have distinct electrophoretic mobilities (Figure 49). PAA10kPAM10k has an electrophoretic mobility ranging from $0.5 \times 10^{-8}$ m$^2$ V$^-1$ s$^-1$ near the neutral species to $3.7 \times 10^{-8}$ m$^2$ V$^-1$ s$^-1$ near PAA indicating that most possible compositions are present in the sample. In contrast the electrophoretic mobilities in PAA2kPAM10k range from $0.3 \times 10^{-8}$ m$^2$ V$^-1$ s$^-1$ closer to the neutral species to only $3.0 \times 10^{-8}$ m$^2$ V$^-1$ s$^-1$. This range indicates that no chains are present with a composition which has a large fraction of PAA, which is agreement with the theoretical composition. Furthermore, there is a significant overlap
in the distributions showing that many compositions are present in both samples. Assuming the maximum of the distribution represents the $C_w$ of the block copolymer, the $C_w$ of PAA2kPAM10k is considerably lower than that of PAA10kPAM10k. Therefore the average composition is likely close to the theoretical compositions.

The shapes of the electropherograms (UV signal) and of the electrophoretic mobility distribution are similar for PAA2kPAM10k (Figure 49). However, the distribution of PAA10kPAM10k is slightly narrower than the electropherogram with the majority of the weight towards the higher electrophoretic mobilities, which indicates that most of the block copolymer chains have a higher fraction of PAA. From the electropherogram only it would be difficult to observe where the majority of the sample lies in the distribution. It was expected that PAA10kPAM10k would be more affected by the distortion than PAA2kPAM10k since it had the broader distribution.
Figure 49. a) Electropherograms with UV signal and b) mobility distributions $w(\mu)$ for PAA2kPAM10k (red) and PAA10kPAM10k (blue). PAA peak present in PAA10kPAM10k between 3.7 and 4.0 × 10^{-8} m^2 V^{-1} s^{-1}. Injection concentration was 5 g L^{-1} for each sample. PAA2kPAM10k was separated in an extended light path capillary with a total length of 60.6 cm (effective length 52.1 cm) while PAA10kPAM10k was separated in a standard fused silica capillary with a total length of 59.9 cm (effective length 51.4 cm).

Both samples have a sharp shoulder at 0.5 × 10^{-8} m^2 V^{-1} s^{-1} indicating that there is a concentration of copolymer chains with a small PAA block relative to the PAM block. This may be due to the kinetics of the copolymerisation where short homopolymer chains may initiate the second block before the longer chains resulting in block copolymer chains with the initial blocks smaller and the second block larger.
relative to the other block copolymer chains. These shoulders may also be signals from by-products in the synthesis such as degradation products. To isolate the cause of these shoulders further experiments are needed to find the electrophoretic mobility of possible impurities and find out if they correspond to the observed shoulder. The area of the shoulder is very small compared to the area of the rest of the peak, constituting 5% of it for PAA2kPAM10k and 1% of it for PAA10kPAM10k, thus they would have a minor influence on the composition and electrophoretic mobility distribution.

DEM and DC values (Tables 17 and 18) cover a more limited range than D values. This is because a molar mass can theoretically range from one to infinity allowing for broad distributions. In contrast the composition of a block copolymer can only range from zero (neutral homopolymer) to one (charged homopolymer); thus the electrophoretic mobility range of a copolymer is also limited to the range between zero and the mobility of the charged homopolymer. Therefore broader distributions can be observed for molar mass, thus DEM and DC values are typically smaller and the variation between samples lower than that seen for D values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DEM before signal correction</th>
<th>DEM after signal correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA2kPAM10k</td>
<td>1.123</td>
<td>1.121</td>
</tr>
<tr>
<td>PAA10kPAM10k</td>
<td>1.144</td>
<td>1.123</td>
</tr>
</tbody>
</table>

*Since the UV signal was not converted to $w(\mu)$ the dispersity of the electropherogram with the UV signal is calculated.

Converting the UV signal into the weight distribution results in a noticeable decrease in the dispersity values (Table 18). Additionally the distortion is not relative between samples so to accurately compare between samples the correction is required.

Very similar DEM values were observed for both P(AA-b-AM) samples indicating that the samples have the same heterogeneity of composition (Table 18). However, if the correction was not applied it would have appeared that PAA2kPAM10k
might have a slightly broader distribution of composition. It is important to note that if electrophoretic mobility and \( w(\mu) \) were not used and the UV signal as a function of the migration time was used (Figure 46), PAA10kPAM10k would appear to have a broader distribution, thus leading to an incorrect conclusion. In order to correctly compare the heterogeneity of composition between samples the electrophoretic mobility distribution needs to be used.

The distribution determined for PAA2kPAM10k is fairly symmetrical while for PAA10kPAM10k the distribution is more asymmetrical (Figure 49). The asymmetry illustrates that the majority chains have a larger fraction of PAA, but a small amount of the chains have a small fraction of PAA.

5.5.2.2 PAPTAC based block copolymers

Unlike the PAA based block copolymers the PAPTAC based block copolymers exhibit adsorption which would influence their observed electrophoretic mobility. However if it is assumed that the effect of adsorption on the electrophoretic mobility is the same between chains of different compositions the electrophoretic mobility can still be used to compare the composition between samples. This assumption appears fairly reliable since the peak maximums observed in CE-CC and pressure assisted CE-CC of the PAPTAC based block copolymers are fairly repeatable and not all the sample adsorbed as shown by pressure mobilisation (Figure 40) as discussed in section 4.2.2.2.

For the P(APTAC-\( b \)-AA) samples the peak was defined between 2 and \( 4 \times 10^{-8} \) m\(^2\) V\(^{-1}\) s\(^{-1}\) so that it does not include the elevated baseline caused by the adsorption (Figure 50), while the rest of the distribution is assumed not to be affected by the adsorption. It should be noted that an apparent mobility is determined for the cationic polymers as described in section 2.3.4 so the Dispersity of Apparent Mobility (\( DAM \)) is determined. Although with pressure assisted CE-CC the EOF can be detected allowing for the determination of the electrophoretic mobility, a clear peak representing the EOF in the block copolymer samples cannot be identified so the apparent mobility is still...
used. The $DAM$ was not significantly different between PAPTAC4kPAA1k and PAPTAC4kPAA2k (Table 19). Therefore the broadness of their composition distributions is very similar. Both samples have very low $DAM$ indicating a narrow distribution of compositions; however it is difficult to indicate the range of electrophoretic mobilities due to the adsorption. The majority of the peak has a similar electrophoretic mobility to PAPTAC homopolymer showing that the majority of the chains have a large fraction of PAPTAC.

**Figure 50.** Electropherograms of PAPTAC4kAA1k (green) and PAPTAC4kAA2k (purple) obtained by CE-CC. Dashed lines show repeat injections. Injection concentration of each individual polymer sample was 5 g L$^{-1}$. 

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Table 19. *DAM* value obtained for PAPTAC based block copolymers. P(APTAC-\textit{b}-AA) samples were separated by CE-CC between 2 and $4 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ while P(APTAC-\textit{b}-NIPAM) samples were separated by pressure assisted CE-CC between 1 and $5 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$. All values are determined with the true weight fraction obtained after correcting UV signal and are an average of repeat injections.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPTAC4kPAA1k</td>
<td>1.023</td>
</tr>
<tr>
<td>PAPTAC4kPAA2k</td>
<td>1.021</td>
</tr>
<tr>
<td>PAPTAC5kPNIPAM5k</td>
<td>1.270</td>
</tr>
<tr>
<td>PAPTAC2kPNIPAM8k</td>
<td>1.162</td>
</tr>
<tr>
<td>PAPTAC1kPNIPAM9k</td>
<td>1.133</td>
</tr>
</tbody>
</table>

The electropherograms of P(APTAC-\textit{b}-NIPAM) obtained by pressure assisted CE-CC with a corrected UV signal were used to determine their *DAM* values. The majority of the P(APTAC-\textit{b}-NIPAM) distributions have an electrophoretic mobility close to that of PNIPAM homopolymer, and although this is most likely due the adsorption there is a clear trend in the electrophoretic mobility at the peak maxima (Figure 51). The peak maxima decrease in electrophoretic mobility with PAPTAC2kPNIPAM8k, PAPTAC5kPNIPAM5k and PAPTAC1kPNIPAM9k in descending order. A lower electrophoretic mobility indicates a lower fraction of PAPTAC in the block copolymer chains. Therefore this order is not in agreement with the theoretical values. The lower electrophoretic mobility of PAPTAC5kPNIPAM5k compared to PAPTAC2kPNIPAM8k is likely because of the differences in residual PAPTAC. Since PAPTAC5kPNIPAM5k has significantly more residual PAPTAC than PAPTAC2kPNIPAM8k the PNIPAM blocks in PAPTAC5kPNIPAM5k would be longer than expected. Near complete conversion of the monomers occurs therefore the NIPAM monomers had to have formed a homopolymer or a block. Since little PNIPAM homopolymer was found it would appear that the monomers have formed larger blocks than intended resulting in a lower electrophoretic mobility for PAPTAC5kPNIPAM5k.
Figure 51. Electropherograms of PAPTAC1kPNIPAM9k (purple), PAPTAC2kPNIPAM8k (blue), PAPTAC5kPNIPAM5k (red) and a blend of PAPTAC5k and PNIPAM5k (black) obtained by pressure assisted CE-CC. Dashed lines show repeat injections. Injection concentration of each individual polymer sample was 5 g L\(^{-1}\) and 1 mM for hexaminecobalt(III) chloride. Hexaminecobalt(III) chloride migrates between 7 and 9 × 10\(^{-8}\) m\(^2\) V\(^{-1}\) s\(^{-1}\). PAPTAC homopolymer migrates between 5 and 6 × 10\(^{-8}\) m\(^2\) V\(^{-1}\) s\(^{-1}\) and the insert shows the PNIPAM peak and the majority of the block copolymer peaks.

As the fraction of the PAPTAC theoretical block length decreases in P(APTAC-\(b\)-NIPAM) block copolymers so does the DAM, indicating a narrowing of the composition distribution. Although the parent PNIPAM homopolymer is present in the distribution it only constitutes a small fraction of it and thus it should have a minor impact on the DAM values. Therefore the narrowing of the composition distribution would appear to be a real trend in the samples. This trend may suggest that when less monomers are used to produce the second block a broader distribution of compositions is formed. However there is no trend between the fraction of residual homopolymer and the DAM.
The $DAM$ of the P(APTAC-$b$-AA) samples is lower than that of the P(APTAC-$b$-NIPAM) samples. This indicates that the composition is more homogenous in the P(APTAC-$b$-AA) than the P(APTAC-$b$-NIPAM) samples. This may be due to the PAPTAC homopolymer chains reinitiating at different rates with the NIPAM monomers than with the AA monomers. Since more PAPTAC homopolymer was found in the P(APTAC-$b$-NIPAM) than P(APTAC-$b$-AA) samples (sections 4.2.2.3 and 4.3.2.1) the NIPAM monomers may be less reactive than the AA monomers resulting in the greater fractions of residual homopolymer and higher $DAM$.

5.6 Chapter Conclusion

The distribution of compositions of a block copolymer is rarely determined in the literature. Furthermore there was no reported means of assessing the distribution of compositions of DHBCs. CE-CC allows the examination of the composition of DHBCs including highly adsorbing cationic block copolymers. Obtaining the distribution of compositions of block copolymers requires taking into account a number of parameters. Although the distribution of compositions of a block copolymer was not determined in this work the theory behind obtaining it with CE-CC was further developed. The possible ways of determining the rescaling factor $\alpha$ were examined along with the effect of $\alpha$ value on the resulting composition distribution. From this work a means of comparing average compositions and the distribution of compositions was stated with the definition of the terms moment-average Composition ($C_m$), Weight-average Composition ($C_w$) and Dispersity of Composition ($DC$) which have not been previously described in the literature. Furthermore a method of correcting the distortion along the y axis of an electropherogram when converting its x axis from migration time to electrophoretic mobility is provided which has not been previously discussed in the literature. With this theory further studies will obtain the true distribution of compositions of a DHBC.

By comparing the distributions of electrophoretic mobilities the composition between block copolymer samples can be compared. Using the Dispersity of
Electrophoretic Mobilities (DEM) the broadness of the compositions can be compared between samples. Two P(AA-b-AM) block copolymers exhibited significantly different distributions and by comparing the DEM they were found to have distributions of compositions that were equally broad, while a visual comparison on their conventional electropherograms plotted as a function of migration time showed a much broader signal for PAA10kPAM10k. This shows the importance of using electrophoretic mobility and $w(\mu)$ to accurately compare the heterogeneity of composition between samples. Furthermore the same procedure was applied to adsorbing cationic DHBCs where the sample PAPTAC5kPNIPAM5k was found to have a different composition to what was targeted which is linked to the presence of the residual homopolymer in the sample.

In conclusion, CE-CC provides a means of identifying how controlled the composition is in a sample. In addition through the shift in electrophoretic mobility the composition can be qualitatively assessed in terms of which block has the predominant fraction.
CHAPTER 6. CONCLUSIONS

6.1 Conclusions and Implications

The aim of this work was to characterise a range ‘smart’ DHBCs including cationic block copolymers which are very challenging to be characterised by traditional means. NMR spectroscopy and SEC along with newly developed CE-CC methods were used to assess the molar mass, degree of branching (DB), heterogeneity of branching of the initial homopolymers, as well as the purity and composition of the corresponding DHBC samples. Furthermore the potential of CE-CC for the characterisation of homopolymers and block copolymers was demonstrated.

The initial PAA homopolymers were examined by a range of one-dimensional and two-dimensional NMR spectroscopy techniques for near complete signal assignment. Using quantitative NMR spectroscopy the DB was determined for the PAA samples with an RSD below 65 % after recording over 80 000 scans. The DB was found to be below 1 %, which was significantly lower than that of PAA samples synthesised by RAFT and NMP. Thus samples synthesised by MADIX polymerisation at room temperature, in water and ethanol exhibit a low DB. The heterogeneity of the branching was then assessed by CE-CC: the branching was very homogenous in the PAA samples. Measuring the DB of polymers is rare hence the knowledge of the kinetics of polymerisation does not allow yet for the prediction of DB for most polymers including PAA. Therefore obtaining DB of the PAA enables a better understanding of the kinetics. A comparison can be made between synthetic procedures to determine how to produce PAA with the desired DB, for example drug carriers may benefit from a highly branched structure thus NMP could be used while for water purification a low DB may be desired and so MADIX may provide the needed chemical structure. Furthermore it may be possible to elucidate how branching is influenced by different polymerisation mechanisms since NMP, ATRP and RAFT/MADIX have different mechanisms including different means of initiation.
The molar mass of PAPTAC and PAA homopolymers were examined by NMR spectroscopy, SEC and CE-CC. There was a significant variation in the $M_n$ values determined by NMR spectroscopy when different end group signals were used. However, using both $^{13}$C and $^1$H NMR spectroscopy the signal leading to the most accurate $M_n$ values was found to be the methyl group S14, shown in Figure 18, since it was bonded to the backbone through carbon-carbon bonds. The molar mass determined by NMR spectroscopy and SEC with MALLS detection and universal calibration were compared and found to vary significantly. Therefore the exact molar mass could not be accurately determined. Using CE-CC the fraction of short chain oligomers was estimated in the PAPTAC and PAA homopolymers to be at least 13 and 8 % (w/w) respectively.

After the characterisation of the initial homopolymers the composition and purity of the resulting block copolymers was assessed by CE-CC. To show the effectiveness of CE-CC to separate block copolymers from their parent homopolymers, mixtures of P(AA-b-AM) and PAA were separated by SEC and CE-CC. SEC was unable to separate the block copolymers from the homopolymers. In particular for the symmetric PAA10kPAM10k, SEC was not able to show the formation of a block copolymer. In contrast CE-CC provided baseline separation and clearly demonstrated the formation of a block copolymer in PAA10kPAM10k. Cationic block copolymers P(APTAC-b-NIPAM) did not elute out of SEC columns. CE-CC was able to separate the PAPTAC homopolymer from the block copolymer. Using pressure assisted CE-CC the PAPTAC homopolymer, P(APTAC-b-NIPAM) block copolymer are separated and the PNIPAM homopolymer is presented as a shoulder, thus showing the formation of a block copolymer which had not been previously shown in the literature.

Since CE-CC could separate block copolymers from their parent homopolymers the ability of CE-CC to quantify homopolymers was determined. CE-CC had linearity and recovery equal to or greater than that reported for LC based techniques. Furthermore a significant amount of PAPTAC homopolymer and a small amount of PNIPAM was
detected in all the P(APTAC-\textit{b}-NIPAM) block copolymers. Therefore CE-CC provides a cost-effective and efficient means of identifying the formation of block copolymer and quantifying residual homopolymers. Furthermore with a means of quantifying homopolymers in block copolymers a number of different aspects of a synthesis can be addressed. The ratio of polymers in polymer blends could be measured, the efficiency of the initiation of a RDRP transfer agent could be determined, the kinetics of the formation of a secondary block could be examined, the degradation of RDRP transfer agents could be measured and many other possible components of a synthesis could be examined using CE-CC. Furthermore the purity of a block copolymer could be related to its functional properties such as micelle formation and phase separation.

CE-CC is capable of providing information about the composition of copolymers. The theory of accurately obtaining the weight fraction for distribution of compositions of a block copolymer was developed as well as how to interpret the distribution in terms of composition averages and \textit{DC}. From this future studies can obtain the distribution of compositions of a block copolymer and how to interpret a distribution of compositions. Additionally the effect of converting the migration time to electrophoretic mobility on the detector signal in CE was shown which would benefit CE users determining distributions. Although the true composition distributions of the block copolymers were not determined, the heterogeneity of the compositions in the samples could be compared through the \textit{DEM}. It was observed that samples had significant differences in their \textit{DEM} indicating different distributions of compositions. With a means of comparing the composition of copolymers with CE-CC the effect of composition heterogeneity on a polymer’s properties can be examined. Furthermore the control of a copolymers synthesis in terms of their composition can be made.

In summation the branching of PAA was assessed by NMR spectroscopy and CE-CC. The purity and composition of DHBCs was assessed by CE-CC. In particular cationic DHBCs could be characterised by pressure assisted CE-CC while SEC was ineffective. Through this analysis near complete characterisation occurred which is rare in the literature. Thereby this characterisation provides a means to create a clear link
between the chemical structure and the materials properties established, thus allowing the block copolymers to be more easily designed to produce a material with the desired properties.

6.2 Future Directions

To provide the most accurate molar mass of the PAA samples as possible a thorough SEC analysis is required for example using triple detection [188] or 2D separation techniques such as SEC coupled to CE [230]. This will allow obtaining most reproducible molar mass values.

The molar mass of the PAPTAC samples could be assessed by CE-MS. The molar mass of each oligomer peak can be determined following the same procedure as that performed on AA oligomers [178]. This procedure requires the use of volatile buffers and so the separation must first be reproduced in a BGE such as formic acid.

The DB of PAA was determined in this work. However, branching may also be present in the other parent homopolymer, PAM, and it has not been accurately quantified in the literature. Therefore similar NMR spectroscopy studies should be undertaken on PAM, PNIPAM and PAPTAC to detect and possibly quantify any branching present.

P(AA-b-AM) block copolymers and PAA were separated and quantified. However the separation and quantification of PAM should also been undertaken, especially since both parent homopolymers have identified in many block copolymer materials [45,80,231]. This could also be performed on the P(EO-b-AA) block copolymer, firstly by improving the separation in PEO2kPAA10k with a longer capillary or different BGE. Subsequently indirect detection could then be used to measure any residual PEO.
The PAPTAC based block copolymers suffer from adsorption onto the capillary wall in CE-CC even with the coated capillaries used in this work. The adsorption may be prevented through the use of different coatings on the surface. Only commercial coated capillaries were used in this work while positively charged dynamic coatings through polyelectrolyte multilayers may prevent the adsorption of these cationic block copolymers [232]. Alternatively having ethanol introduced to the BGE may prevent adsorption or allow the migration of the adsorbed polymer past the detector, since ethanol flushes were able to remove any adsorbed polymers. In this way the solvent is used to remove adsorbed polymers in a similar way to what occurs in LAC [233].

Obtaining an accurate distribution of compositions by CE-CC would provide a means of characterising block copolymers which has not been previously explored in the literature. From this work the ground work was established however the rescaling factor $\alpha$ was not determined. Therefore future work should be conducted to calculate an $\alpha$ enabling the determination of a composition distribution. In order to determine $\alpha$ several block copolymers which can accurately be assessed by SEC or have each block easily distinguished by NMR spectroscopy is required. Taylor Dispersion Analysis (TDA) may also provide a means of determining $\alpha$ since it has been shown to provide information about the size of polymers [223]. In addition to accurately obtain the composition distribution the ordinate UV detection must be equal for each monomer unit, thus when the molar absorptivity is identical. If the isobestic point of each parent homopolymer should be determined so this wavelength can be used for the UV detection.

Further development of Equation (5) which relates the electrophoretic mobility of a block copolymer to its composition has occurred in the literature to improve its accuracy. That is because in Equation (5) it is assumed that each monomer unit contributes equally to the electrophoretic mobility of a block copolymer. The electrophoretic mobility is proportional to the charge to friction ratio however, it is more accurate to describe it as the effective charge to friction ratio [234]. This is because effective charge is related to the surface charge of the species. Therefore the monomer units on the outside of the polymer contribute more towards the effective charge as well
as the hydrodynamic interactions [235]. Hence the monomers units near the ends of the polymer contribute more to the overall electrophoretic mobility of the polymer because they are more likely to be positioned on the outside of the random coil [165]. Consequently the electrophoretic mobility is weighted based on the position of the monomer as shown [236]:

$$\mu = \frac{1}{N_t} \int_0^{n_c} \Psi\left(\frac{n}{N_t}\right) \mu(n) \, dn$$

(47)

where $N_t$ is the effective number of monomer units in the whole copolymer, $\Psi(n/N_t)$ is the weighing function and $n$ is the $n$th monomer in the chain, counting from the charged end of the copolymer. The weighing function only applies to the charged monomers because the uncharged monomers exhibit zero electrophoretic mobility in CE [236].

The $N_t$ is expressed as $n_c + a n_u$ to incorporate the number of charged monomers and the number of effective uncharged monomers units [237]. Therefore Equation (48) can be rearranged to provide the following [165]:

$$\mu = \mu_0 \frac{\int_0^{n_c} \Psi\left(\frac{n}{n_c + a n_u}\right) \, dn}{n_c + a n_u}$$

(48)

the weighing function has been shown to work effectively when related to a Gaussian distribution, as follows [236]:

$$\Psi\left(\frac{n}{N_t}\right) \approx -0.65 + 0.62 \left(\frac{n}{N_t}\right)^{-\frac{1}{4}} + 0.62 \left(1 - \frac{n}{N_t}\right)^{-\frac{1}{4}}$$

(49)

once a distribution of compositions is obtained using Equation (5) future work should be conducted to improve the accuracy of the distribution using Equation (48). From an accurate composition distribution the effect of different synthetic parameters on the heterogeneity of the composition can be studied. Furthermore materials dependent on their composition can be synthesised to their desired composition.

Lastly with a greater understanding of DHBCs chemical structure further studies can be undertaken to establish synthesis-structure and structure-property relationships.
From these relationships the applications of DHBCs can become a reality. For example studies can be conducted to assess the purity of DHBCs when different initiators are used. The complexation of DHBCs with negatively charged polyelectrolytes leads to applications in drug and gene delivery. Therefore studies can be conducted to compare the effect of the purity and composition of the DHBCs on the complexation. The pH responsive PAA and the thermoresponsive PNIPAM DHBCs can be assessed in terms of purity, branching and compositions, providing a clear assessment of how responsive these block copolymers are, allowing for the incorporation of these properties into different applications.
CHAPTER 7. APPENDICES

7.1 NMR Spectra of MADIX agent

The MADIX agent used to synthesis the polymers in this work was Rhodixan A1 shown in Figure 17. The $^1$H and $^{13}$C NMR spectra of Rhodixan A1 obtained from Paul Sabatier University are shown in Figure A-1. The chemical shifts are reported in Tables 4 and 5.

Figure A-1. NMR spectra of the MADIX agent Rhodixan A1. a) $^1$H NMR spectrum and b) $^{13}$C NMR spectrum.
7.2 Estimation of \( T_1 \) for \(^1\text{H} \) NMR of PAA

To obtain a quantitative \(^1\text{H} \) NMR spectrum of PAA the repetition delay is required to be at least five times \( T_1 \) for the signals of interest. Using the inversion recovery pulse sequence shown in Figure 16, different \( \tau \) values were trialled until a value produced a spectrum with all the signals positive (Figure A-2). When \( \tau \) was 3.465 s, all signals from PAA were positive. This \( \tau \) corresponds to a \( T_1 \) of 5 s therefore a repetition delay of 25 s is needed between scans to produce a quantitative \(^1\text{H} \) NMR spectrum of PAA.

![Figure A-2. \(^1\text{H} \) NMR spectra of PAA10k in dioxane-\( d_8 \) using the inversion recovery pulse sequence. The different spectra correspond to different \( \tau \) lengths: 0.693 s (black), 1.386 s (red) and 3.465 s (blue).](image)
7.3 Error in Molar Mass Determined by NMR with an Insufficient Repetition Delay

The importance of establishing quantitative conditions in NMR is often underestimated. The additional error arising with the use of typical but unquantitative conditions was estimated. Determining the $M_n$ with a repetition delay of 8 s and 25 s by $^1$H NMR spectroscopy was used to show the potential error caused by not having a repetition delay greater than $5T_1$. From Table A-1 it is shown that there was around 5 % difference in the determined $M_n$. The S5 signal was used for the end group signal since it was not overlapping and was clearly assigned as an end group signal (see section 3.1). The number of scans for quantitative conditions was 128 scans to identify any low intensity signals. For unquantitative conditions 16 scans were used as this is more commonly used to determine $M_n$. In both cases the RSD on the end group signal was 0.01 % determined from Equation (4). Therefore the number of scans should not influence the values determined only the length of the repetition delay.

Table A-1. Comparison of short and long repetition delays on determining the $M_n$ of PAA2k by $^1$H NMR spectroscopy. All other conditions for obtaining the spectra were identical except the number of scans was 16 and 128 when 8 s and 25 s were used respectively.

<table>
<thead>
<tr>
<th>Repetition delay (s)</th>
<th>8</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_n$ (g mol$^{-1}$)</td>
<td>1877</td>
<td>1965</td>
</tr>
</tbody>
</table>
7.4 Calibration Curves

The linearity of CE-CC to quantify homopolymers was determined by constructing a calibration curve of polymer concentration against the peak area. The equations and regression coefficients are shown in Table 12. The calibration curves are shown in Figure A-3.

![Calibration curves](image)

**Figure A-3.** Calibration curves of the concentration of homopolymer against the corrected peak area: a) PAA homopolymers (PAA2k, green and PAA10k, red), b) PAPTAC homopolymers (PAPTAC2k, blue and PAPTAC5k orange).

The LOD was estimated by creating a calibration curve of polymer concentration against SNR. The LOD was extrapolated from the curve when SNR equalled 3. The SNR was calculated in a similar manner as in section 3.3.1 except the noise was not divided by 2.5. The LOD values are shown in Table 12. The calibration curves are shown in Figure A-4.
Figure A-4. Calibration curves of the concentration of homopolymer against the SNR. a) PAA homopolymers: PAA2k (black, $R^2 0.94$) and PAA10k (purple, $R^2 0.93$). b) PAPTAC homopolymers: PAPTAC2k (pink, $R^2 0.989$) and PAPTAC5k (dark blue, $R^2 0.998$).

7.5 Theoretical Percentage of ‘Dead’ Chains

The percentage of dead chains ($De$) can be determined using Equation (35) which uses the values in Table A-2.

Table A-2. Amount of initiator and MADIX agent used in polymerisation

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Amount of initiator (mmol)</th>
<th>Amount of MADIX agent (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA</td>
<td>0.16</td>
<td>3.90</td>
</tr>
<tr>
<td>PAPTAC</td>
<td>0.19</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Note that the initiator decomposes to produce two radicals and so can create two possible chains.

$$De(\text{PAA}) = \frac{(2 \times 0.16)}{(2 \times 0.16) + 3.90} \times 100 = 7.64 \%$$

$$De(\text{PAPTAC}) = \frac{(2 \times 0.19)}{(2 \times 0.19) + 1.15} \times 100 = 24.8 \%$$
Theoretical weight of unreacted homopolymer based on the fraction of dead chains was calculated using the theoretical molar mass of the homopolymer and block copolymers. This assumes all non dead chains are converting into a block copolymer.

weight of $De$(PAA2kPAM10k) = \frac{7.64 \times 2000 \times 100}{(100 - 7.64) \times 12000 + 7.64 \times 2000} = 1.36 \%$

weight of $De$(PAA10kPAM10k) = \frac{7.64 \times 10000 \times 100}{(100 - 7.64) \times 20000 + 7.64 \times 10000} = 3.97 \%$

weight of $De$(PAPTAC2kPNIPAM8k) = \frac{24.8 \times 2000 \times 100}{(100 - 24.8) \times 10000 + 24.8 \times 2000} = 6.20 \%$

weight of $De$(PAPTAC5kPNIPAM5k) = \frac{24.8 \times 5000 \times 100}{(100 - 24.8) \times 10000 + 24.8 \times 5000} = 14.2 \%$

7.6 Derivatives Determined in section 5.2

Derivative of electrophoretic mobility ($\mu$) with respect to migration time ($t_m$):

$$\mu = \frac{l_\text{d}l_t}{V} \left( \frac{1}{t_m} - \frac{1}{t_{\text{EOF}}} \right)$$

$$\frac{d\mu}{dt_m} = -\frac{l_\text{d}l_t V}{V^2 t_m^2}$$

$$\frac{d\mu}{dt_m} = -\frac{l_\text{d}l_t}{V} \frac{1}{t_m^2}$$

$$\frac{d\mu}{dt_m} \propto \frac{1}{t_m^2}$$
Derivative of chemical charge density ($\xi$) with respect to electrophoretic mobility ($\mu$).

$$\xi = \frac{\alpha \mu}{\mu(\alpha - 1) + \mu_0}$$

$$\frac{d\xi}{d\mu} = \frac{\alpha \mu (\alpha - 1) - \alpha \mu (\alpha - 1) + \alpha \mu_0}{[\mu(\alpha - 1) + \mu_0]^2}$$

$$\frac{d\xi}{d\mu} = \frac{\alpha \mu_0}{[\mu(\alpha - 1) + \mu_0]^2}$$

Derivative of electrophoretic mobility ($\mu$) with respect to chemical charge density ($\xi$).

$$\xi = \frac{\alpha \mu}{\mu(\alpha - 1) + \mu_0}$$

$$\alpha \mu = \xi [\mu(\alpha - 1) + \mu_0]$$

$$\alpha \mu = \xi \mu (\alpha - 1) + \xi \mu_0$$

$$\mu [\alpha - \xi (\alpha - 1)] = \xi \mu_0$$

$$\mu = \frac{\xi \mu_0}{\alpha - \xi (\alpha - 1)}$$

$$\frac{d\mu}{d\xi} = \frac{\mu_0 [\alpha - \xi (\alpha - 1)] - [-\xi \mu_0 (\alpha - 1)]}{[\alpha - \xi (\alpha - 1)]^2}$$

$$\frac{d\mu}{d\xi} = \frac{\alpha \mu_0 - \xi \mu_0 (\alpha - 1) + \xi \mu_0 (\alpha - 1)}{[\alpha - \xi (\alpha - 1)]^2}$$

$$\frac{d\mu}{d\xi} = \frac{\alpha \mu_0}{[\alpha - \xi (\alpha - 1)]^2}$$
7.7 Moments in a distribution

7.7.1 Molar mass distribution

Moments are determined as stated in reference [194]

Table A-3. Moments in a weight-molar mass distribution where \( w(M_i) \) is the total weight of chains with a molar mass \( M \) of \( i \).

<table>
<thead>
<tr>
<th>Moment</th>
<th>Integral</th>
<th>Discrete form</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>( \int w(M) M^{-1} dM )</td>
<td>( \sum_i w(M_i) M_i^{-1} (M_{i+1} - M_i) )</td>
</tr>
<tr>
<td>0</td>
<td>( \int w(M) dM )</td>
<td>( \sum_i w(M_i) (M_{i+1} - M_i) )</td>
</tr>
<tr>
<td>1</td>
<td>( \int w(M) M dM )</td>
<td>( \sum_i w(M_i) M_i (M_{i+1} - M_i) )</td>
</tr>
<tr>
<td>2</td>
<td>( \int w(M) M^2 dM )</td>
<td>( \sum_i w(M_i) M_i^2 (M_{i+1} - M_i) )</td>
</tr>
<tr>
<td>3</td>
<td>( \int w(M) M^3 dM )</td>
<td>( \sum_i w(M_i) M_i^3 (M_{i+1} - M_i) )</td>
</tr>
</tbody>
</table>

7.7.2 Electrophoretic mobility distribution

Table A-4. Moments in a weight-electrophoretic mobility distribution where \( w(\mu_z) \) is the total weight of chains with an electrophoretic mobility \( \mu \) of \( z \).

<table>
<thead>
<tr>
<th>Moment</th>
<th>Integral</th>
<th>Discrete form</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>( \int w(\mu) \mu^{-1} d\mu )</td>
<td>( \sum_z w(\mu_z) \mu_z^{-1} (\mu_{z+1} - \mu_z) )</td>
</tr>
<tr>
<td>0</td>
<td>( \int w(\mu) d\mu )</td>
<td>( \sum_z w(\mu_z) (\mu_{z+1} - \mu_z) )</td>
</tr>
<tr>
<td>1</td>
<td>( \int w(\mu) \mu d\mu )</td>
<td>( \sum_z w(\mu_z) \mu_z (\mu_{z+1} - \mu_z) )</td>
</tr>
<tr>
<td>2</td>
<td>( \int w(\mu) \mu^2 d\mu )</td>
<td>( \sum_z w(\mu_z) \mu_z^2 (\mu_{z+1} - \mu_z) )</td>
</tr>
<tr>
<td>3</td>
<td>( \int w(\mu) \mu^3 d\mu )</td>
<td>( \sum_z w(\mu_z) \mu_z^3 (\mu_{z+1} - \mu_z) )</td>
</tr>
</tbody>
</table>
### 7.7.3 Composition distribution

**Table A-5.** Moments in a weight-composition distribution where \( w(C_k) \) is the total weight of chains with a composition \( (C) \) of \( k \).

<table>
<thead>
<tr>
<th>Moment</th>
<th>Integral</th>
<th>Discrete form</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>( \int w(C) \mu^{-1} dC )</td>
<td>( \sum_k w(C_k) C_k^{-1} (C_{k+1} - C_k) )</td>
</tr>
<tr>
<td>0</td>
<td>( \int w(C) dC )</td>
<td>( \sum_k w(C_k) (C_{k+1} - C_k) )</td>
</tr>
<tr>
<td>1</td>
<td>( \int w(C) \mu dC )</td>
<td>( \sum_k w(C_k) C_k (C_{k+1} - C_k) )</td>
</tr>
<tr>
<td>2</td>
<td>( \int w(C) \mu^2 dC )</td>
<td>( \sum_k w(C_k) C_k^2 (C_{k+1} - C_k) )</td>
</tr>
<tr>
<td>3</td>
<td>( \int w(C) \mu^3 dC )</td>
<td>( \sum_k w(C_k) C_k^3 (C_{k+1} - C_k) )</td>
</tr>
</tbody>
</table>
REFERENCES


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