Characterisation of Vinylphosphonic Acid-based Block Copolymers

Master of Research

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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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Michael Ian Martin Horgan

May 2017
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To my fellow Masters peeps, play-nice. You’ve got a long way ahead of you, so enjoy the fun times and always remember for **Matting** is a nightma_______________re. So get it done early!
And to my Family and friends, I thank all you efforts putting up with me these past few years. It has been a wild ride but I wouldn’t’ve done it with anyone else.
And finally but by no means the least important.

To Chrisanne,

Mo maighdeann-ròin caen-duhb coimeas
Luthien thu bòidheach.
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(2) $\Delta V = \text{NEP} + \text{NEOF}$ ..................................................... 15
(3) $\mu \propto \text{CHARGE/FRICITION} = qf$ .................................... 15
(4) $1/tm = 1/\text{tep} + 1/tEOF$ ...................................................... 17
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<td>$\bar{M}_{n,\text{NMR}}$</td>
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</tr>
<tr>
<td>$\bar{M}_{n,\text{th}}$</td>
<td>Theoretical molar mass</td>
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<td>Temperature difference across the capillary</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees celcius</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Electrophoretic mobility</td>
</tr>
<tr>
<td>$\mu_0$</td>
<td>Electrophoretic mobility of charged homopolymer in the critical conditions</td>
</tr>
<tr>
<td>$\mu_{5\text{mM}}$</td>
<td>Electrophoretic mobility at ionic strength of 5 mM</td>
</tr>
<tr>
<td>$\mu_{\text{BC}}$</td>
<td>Electrophoretic mobility of the block copolymers</td>
</tr>
<tr>
<td>$\mu_{\text{EOF}}$</td>
<td>Electrophoretic mobility of the EOF marker</td>
</tr>
<tr>
<td>$\mu_{\text{Homo}}$</td>
<td>Electrophoretic mobility of the parent homopolymers</td>
</tr>
<tr>
<td>$\mu_{\text{Impurity}}$</td>
<td>Electrophoretic mobility of impurities</td>
</tr>
<tr>
<td>$\mu_{\text{rel}}$</td>
<td>Relative electrophoretic mobility</td>
</tr>
<tr>
<td>$^{31}$P NMR</td>
<td>Phosphorus-31 Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>AA</td>
<td>Acrylic acid</td>
</tr>
<tr>
<td>AM</td>
<td>Acrylamide</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom-transfer radical polymerisation</td>
</tr>
<tr>
<td>BGE</td>
<td>Background electrolyte</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>$C$</td>
<td>Composition</td>
</tr>
<tr>
<td>$c$</td>
<td>Concentration</td>
</tr>
<tr>
<td><strong>CE</strong></td>
<td>Capillary electrophoresis</td>
</tr>
<tr>
<td><strong>CE-CC</strong></td>
<td>Capillary electrophoresis in the critical conditions</td>
</tr>
<tr>
<td><strong>CE-MS</strong></td>
<td>Capillary electrophoresis coupled mass spectroscopy</td>
</tr>
<tr>
<td>$C_n$</td>
<td>Number-average composition</td>
</tr>
<tr>
<td>$C_w$</td>
<td>Weight-average composition</td>
</tr>
<tr>
<td>$D$</td>
<td>Dispersity</td>
</tr>
<tr>
<td>$D(\mu)$</td>
<td>Dispersity of $\mu$</td>
</tr>
<tr>
<td>$D(C)$</td>
<td>Dispersity of compositions</td>
</tr>
<tr>
<td>$D(W(\mu))$</td>
<td>Dispersity of $\mu$</td>
</tr>
<tr>
<td>DHBC</td>
<td>Double hydrophilic block copolymer</td>
</tr>
<tr>
<td>$d_i$</td>
<td>Inner diameter of the capillary</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>$d_o$</td>
<td>Outer diameter of the capillary</td>
</tr>
<tr>
<td>$E$</td>
<td>Electric field strength</td>
</tr>
<tr>
<td>EOF</td>
<td>Electroosmotic flow</td>
</tr>
<tr>
<td>$f$</td>
<td>Friction of analyte in solution</td>
</tr>
<tr>
<td>$g$</td>
<td>Grams</td>
</tr>
<tr>
<td><strong>HPLC</strong></td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>$I$</td>
<td>Ionic strength</td>
</tr>
<tr>
<td>$L$</td>
<td>Litres</td>
</tr>
<tr>
<td><strong>LC</strong></td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td><strong>LC-CC</strong></td>
<td>Liquid chromatography in the critical conditions</td>
</tr>
<tr>
<td><strong>LC-LCD</strong></td>
<td>Liquid chromatography under limiting conditions of desorption</td>
</tr>
<tr>
<td>$l_d$</td>
<td>Length of capillary to detector</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>$l_t$</td>
<td>Total length of capillary</td>
</tr>
<tr>
<td>m</td>
<td>Metres</td>
</tr>
<tr>
<td>$M$</td>
<td>Molar mass</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.d.</td>
<td>Not determined</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NB</td>
<td>Sodium borate</td>
</tr>
<tr>
<td>$n_C$</td>
<td>Number of charged monomer units</td>
</tr>
<tr>
<td>NMP</td>
<td>Nitroxide-mediated radical polymerisation</td>
</tr>
<tr>
<td>$n_U$</td>
<td>Number of uncharged monomer units</td>
</tr>
<tr>
<td>NVP</td>
<td>$N$-vinyl pyrrolidone</td>
</tr>
<tr>
<td>P(AM-b-VPA)</td>
<td>Poly(acrylamide-\textit{block}-vinylphosphonic acid)</td>
</tr>
<tr>
<td>P(EG-b-VPA)</td>
<td>Poly(ethylene glycol-\textit{block}-vinylphosphonic acid)</td>
</tr>
<tr>
<td>P(VP-b-VPA)</td>
<td>Poly(vinyl pyrrolidone-\textit{block}-vinylphosphonic acid)</td>
</tr>
<tr>
<td>PAA</td>
<td>Poly(acrylic acid)</td>
</tr>
<tr>
<td>PAM</td>
<td>Polyacrylamide</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PEO</td>
<td>Poly(ethylene oxide)</td>
</tr>
<tr>
<td>PES</td>
<td>Poly(ether sulfone)</td>
</tr>
<tr>
<td>pH</td>
<td>Potential Hydrogen</td>
</tr>
<tr>
<td>PVP</td>
<td>Poly($N$-vinyl pyrrolidone)</td>
</tr>
<tr>
<td>PVPA</td>
<td>Poly(vinyl phosphonic acid)</td>
</tr>
</tbody>
</table>
\( q \)  
Charge of analyte

**RAFT/MADIX**  
Reversible addition-fragmentation chain-transfer/Macromolecular design via interchange of xanthates

**RDRP**  
Reversible-deactivation radical polymerisation

**RP**  
Radical polymerisation

**RSD**  
Relative standard deviation

**S**  
Slope

**SEC**  
Size exclusion chromatography

**SNR**  
Signal-to-Noise ratio

**t**  
Time

\( t_{\text{EOF}} \)  
Migration time of EOF marker

\( t_m \)  
Migration time

**UV**  
Ultraviolet

**V**  
Voltage

\( v_{\text{An}} \)  
Electrophoretic velocity of anions

\( v_{\text{app}} \)  
Apparent velocity

\( v_{\text{Cat}} \)  
Electrophoretic velocity of cations

\( v_{\text{EOF}} \)  
Electroosmotic velocity

\( v_{\text{ep}} \)  
Electrophoretic velocity

**VPA**  
Vinylphosphonic acid

\( w(\mu) \)  
Weight distribution function of electrophoretic mobility

\( W(\mu) \)  
Plot of the weight distribution function against electrophoretic mobility

\( \alpha \)  
Rescaling factor

\( \xi \)  
Chemical charge density
Block copolymers incorporate the properties of multiple homopolymers into discrete chemical domains. A wide variety of physical and electrochemical behaviours are exhibited by these ‘smart’ polymers. Changes in environment effect the confirmation and structures of these materials, such stimuli-responsivity includes pH, temperature or ion concentration. This potential for controlled behaviour in solution and surface chemistry has enabled the production and investigation of block copolymers in many fields. Notably these materials are applied as flocculants and facilitate ion transport in water filtration systems without the need for power generators. Therefore block copolymers present a valuable construction material for use at industrial facilities. Where each block exhibits like-attractions with hydrogen-bonding environments, these species of copolymers are called double hydrophilic block copolymers (DHBC). These particular groups of block copolymers are of primary interest in the development of PEM batteries and drug delivery systems. DHBCs with vinylphosphonic acid (VPA) moieties display strong research potency in these investigations.

Elucidation of the chemical structure for such materials has been attempted using a number of analytical techniques. However, at current there is no established method that evaluates the purity of the block copolymers in terms of the parent polymers and the distribution of compositions. Techniques for the analysis of these properties in polymers generally involve separation chemistry. Size exclusion chromatography (SEC) is the most common method within research and industry for determining these properties. This method separates analyte in solution by hydrodynamic volume, polymer volumes are highly dependent on molar mass as well as composition and branching, as such incomplete separation by one of these factors occurs in SEC leading to errors in molecular weight calculations. Furthermore ‘smart’ polymers that exhibit charges are susceptible to aggregation and adsorption onto the stationary phase. To evaluate DHBCs with charged and neutral blocks alternative methods were investigated in this work.

Block copolymers of PVPA have been produced by RAFT/MADIX polymerization, this synthetic scheme for aqueous production of high charge polymers can result in parent homopolymers remaining in sample. In order to assess the purity of PVPA-based DHBCs made via RAFT/MADIX this project presents a method for their quantification via free solution CE-CC. Free solution capillary electrophoresis (CE) is a separation method for the characterization of ions based on their charge to friction ratio. Sample analyte is placed in a fluid medium with buffer and subjected to an electric field. This causes ionization of analyte components while migration is initiated by attraction of these ions to the poles of the electric field. It has previously been observed that hydrodynamic volume in
approaches a maximum with an increase in size of polyelectrolytes. Above a certain threshold these
polymers exhibit negligible increases in volume; in these cases separation by electrostatic friction
outweighs molar mass dependent hydrodynamic friction at these chain lengths. This state of CE
separation for large polyelectrolytes is termed the ‘critical conditions’ (CE-CC) where polymers can
be characterised in terms of their structure or end-groups. The charged-uncharged DHBCs were
examined in this work by CE-CC and the complete separation and detection of the parent
homopolymers was achieved.

Polymers constructed from these charged species, though valuable for industrial and bio-
technologies, can be difficult to synthesize with narrow chemical distributions and few techniques
are available that can accurately quantify all species present in these samples. In the separation of
block copolymers by CE we observed that short chain di-block copolymers of charged PVPA and
neutral block species PAM, PEG and PVP displayed large distributions in block structure as well as a
number of impurities. For RAFT/MADIX synthesised PVPA-based DHBCs the dispersity of different
structures was found to be dependent on the degree of polymerisation of the first charged block
species more so than the second neutral block species. The heterogeneity of these distributions was
examined using CE-CC and related to the compositions of the block copolymers using a novel
conversion of electrophoretic mobility to distribution of charge density. This work builds a robust
and efficient method for assessing the structures and species of interesting DHBCs by separation in
CE.

To summarise, the purity and heterogeneities of structures in smart charged-uncharged DHBCs were
investigated by CE-CC. Properties such as structural distribution, purity and charge density were
assessed within a single separation using this technique. Further investigation of other difficult to
characterise copolymers especially smart DHBCs by CE-CC could be used to study the synthesis of
these materials and aid the development of more complex copolymers for commercial application.
Chapter 1 – INTRODUCTION

1.1 DEFINING BLOCK COPOLYMERS: APPLICATIONS, SYNTHESIS AND STRUCTURES

Polymers hold a crucial position in the construction of many products within the petrochemical and biomedical industries. These materials provide cost efficient means for building everyday appliances and benefit from their novel properties. Included are natural, synthetic polymers such as rubber for textiles, high and low density polyethylenes for packaging and other charged multi-unit co- and tri-polymers for biomedical applications. The development and analysis of new polymer constructs in the sciences has permeated these industries with various applications for these materials and continues to do so. Current technologies in the collection and adaption of solar energy, analytical separation chemistry and chromatographic techniques are further developed by the study of polymers within these fields[1]. Leading this investigation are multi-unit polymer chains or copolymers[2]. Various structural confirmations have been achieved with copolymers ranging from long chain-like or branched structures to highly conforming small repeat chains. Investigations into controlled polymerisation of these and other polymers has not produced comprehensive models for the determination of their properties and sample purity, as of yet much data is missing from the literature in regards to copolymer characterisation. There are a number of techniques available for the determination of copolymers but methodologies with greater sensitivity are needed for new intricate polymer structures being developed[3].

Copolymer molecules are constructed from differing repeating units, while homopolymers consist of only a single species of these units. The large speciation of these repeating units can be useful in generating copolymers with novel properties. Typical compositional structures produced from copolymerisation are statistical and block copolymers (BC)[4]. The former are copolymers in which distributions describe the composition and position of subunits within the polymer chain. These chains are produced through step-addition and chain-growth reactions, such as radical polymerisation and polycondensation, for any integer number of subunits (monomers; e.g. A and B) (Figure 1b). Common species such as vinylic copolymers are typically produced through radical addition of the subunits which can lead to chain-transfer of the radical groups as well as side-reactions with the medium resulting in chain termination. These chains are also formed with random
and sometimes uneven distributions of composition due to the kinetic growth of the polymers. Polycondensation is also commonly used for production of synthetic fibers such as polyesters and nylon. These copolymers can be made with very high molecular weights as well as controlled compositions. The latter group of copolymers, BC, are macromolecular chains composed of blocks of one subunit species, each of these chemically attached to a neighbouring block of differing subunits (-AAAA-BBBB-) (Figure 1c). Depending on the desired constituent monomers, there are many controlled copolymerisation techniques available to produce BCs with well-defined polymer architectures as well as narrow distributions of compositions[5, 6]. BCs produced via RAFT/MADIX polymerisation can express highly controlled chain structures as well as greater specificity in the distribution of subunits compared to statistical copolymers. The polymer industry is highly dominated by the production of simple polymers and homopolymer mixes such as polyethylene and polypropylene[7]. However, shifts in manufacturing copolymer technologies highlight the need for better characterisation and analysis of such materials.

![Figure 1](image.png)

**Figure 1.** (a): Homopolymer (b): Statistical Copolymer, where species A and B exhibit a statistical ratio composition presence (c): Block Copolymer, each species is present in discrete "blocks" therefore composition is dependent on the Degree of Polymerisation for block.

### 1.1.1 Applications

#### 1.1.1.1 Smart Copolymers

BCs incorporate the properties of multiple polymers within a single material, depending on the arrangement and choice of blocks the molecules can express stimulus-responsive behaviour. These BCs referred to as ‘smart’ polymers include pH, thermokinetic and chemical-responsiveness often expressed in one block and adhesive properties in another. This ability to deliver condition dependent work on micro-, macroscopic sites is desirable in biomedical imaging[8], drug delivery[9-11] and film membranes. The microphase separation of smart BCs allows for colloidal structures to form in solution, many of these express self-assembly and have been investigated as micellar components in the fields mentioned above.
Due to the stability of these colloids many have been used as ion carriers in mineral extraction[12], flocculants[13], paints including nanolithographic applications[14] and removable coatings[15]. Particle aggregation and block rearrangement occurs in the formation of smart BCs, generating many polyelectrolytic superstructures useful as frameworks for sol-gel assisted synthesis[16]. The monomeric units that compose these smart polymers express a formal charge that reacts to changes in the chemical environment of the polymer. Structural or chemical coordination responses are typical of micelle-like smart BC superstructures, these can be used to alter the permeability at the micelle-solution interface[17]. The behaviour of these smart BCs in solution as well as their interaction with different substrates, both bioactive and inorganic, has advanced research into nanotechnologies for the medical and electrical industries[18, 19].

1.1.1.2 Industry, Research and Development

The production of polymers by radical polymerisation is well understood in terms of kinetics and mechanism of action in a few cases[20] but not in others[21, 22]. By the 1990s this technology had matured both within the academic and industrial fields. Millions of tons of vinyl copolymers are produced with high reproducibility as well as great tolerances for a variety of functional groups, protic polymerisation media and the ease of implementation all contributed strongly to this method’s adoption[2]. However, difficulties with the control of the average molecular weight of the chains had been present for decades in conventional radical polymerisation techniques. The pre-existing methods of polymerisation relied heavily on living ionic radicals to propagate chain growth but were severely limited in compatible functional groups and reaction conditions. Alternative dormant-active equilibrium states for controlling chain growth was the focus following years of research into living anionic polymerisation. Work by Szwarc established the ‘killing’ mechanism for some polymers by electron withdrawing elements such as oxygen and hydrogen and thus developed the use of anionic polymerisation in non-acidic solvents to maintain a ‘living’ polymer[23]. The first external chain radical control agents were proposed as early as the 1970s in Tatemoto et al.[24], followed by Otsu et al.[25] looking at perfluoroalkyliodides and thiuram disulfide iniferers respectively. These along with others attempted reversible deactivation of the living radical polymer chain as a means to control monomer to polymer conversion. The success of these methods led to the development of other controlled radical polymerisation (CRP) techniques. The robustness of implementing these CRPs as well as the variety of chemical
structures and architectures in macromolecular design have revolutionised modern polymer science[26, 27].

Some of the earliest uses of block copolymers were as styrene-based elastomers in synthetic rubbers and thermoplastics[28]. The ability to tailor the microstructure of these chains was an essential step forward in CRP generated copolymers. However, the primary focus of research for these materials in the last decade has been their application as smart macromolecules in nanotechnologies. Tuning the length and properties of each block in such copolymers has allowed for unique architectures to be developed from these materials. They exhibit novel behaviours in solution and semi-permeability as solid networks, the alignment of the chains influences the micro-phase separation and is important when controlling the morphologies in a block copolymer product[29]. A recent example of advantages these materials have is seen in self-healing thermoplastics, in which a sample of ABA structured block copolymer is capable of realignment with neighbouring polymer chains after physical fracture to the macrostructure[30]. The polymers of interest for this work function in a similar fashion when used as supramolecular micelles or charged nano-particles. Tools for the synthesis of these materials are improving everyday as many are being implemented into the design of cutting-edge technologies, however these properties make characterisation increasingly more difficult and new strategies are necessary to assure their product quality.

1.1.2 Synthesis

1.1.2.1 Techniques: Advantages and Difficulties

Block copolymers are typically constructed by chemically attaching multiple homopolymer blocks end to end forming a linear composite polymer, though that is not the only architecture possible[31]. This involves reacting one or more homopolymer species with monomers of another species to produce each block in sequence. As mentioned in Section 1.1.1.2 radical polymerisation techniques have displayed robustness in the number of functional groups and solvents they can be used with; however, conventional methods for block copolymer production are highly susceptible to irreversible chain termination. This occurs where the propagating radical of the initial homopolymer is either neutralised or transferred away from the leading edge, halting the growth of the chain. The rate of these unfavourable kinetic events is high in radical polymerisation for short timescales, reducing the likeliness of producing a block copolymer. As discussed in brief techniques have been developed that increase or control the ‘livingness’ of these initial homopolymers. By
extending the half-life of the living radical with a protective molecule or modifying the end functional groups the propagative centre of the homopolymer can be maintained for longer even indefinitely. These techniques also called Reversible-Deactivation Radical Polymerisation (RDRP) produce polymers using an initiator and a chain transfer agent to protect the reactive end-group of the growing polymer. RDRP techniques supersede this final step by transfer or deactivation of the radical group which can then be reactivated in the presence of another chain species to produce a block copolymer[32]. Common RDRPs include Atom Transfer Radical Polymerisation (ATRP), Nitroxide Mediated Polymerisation (NMP) and Reversible Addition-Fragmentation Chain Transfer/Macromolecular Design via Interchange of Xanthates (RAFT/MADIX). In radical polymerisation the propagating edge of the growing chain undergoes irreversible termination thus ‘killing’ the polymer. Alternatively different propagating species such as polyanions are capable of producing narrow molar weight distributions without the loss of the propagating group from chain transfer or irreversible chain termination. Living Anionic Polymerisation (LAP) is one of these techniques as well as important in the production of industrial polystyrenes and their copolymers[33]. However drawbacks such as the selectivity of monomer species and the solvents needed for initiators limit the potential polymer compositions that can be achieved by these techniques.

1.1.2.2 Reversible Addition-Fragmentation Chain-Transfer/Macromolecular Design via Interchange of Xanthates Polymerisation (RAFT/MADIX)

For the construction of double hydrophilic block copolymers (DHBCs) the preferred synthesis is generally via RAFT/MADIX[34]. The other RDRP techniques listed have advantages where stereochemistry or more reactive functional groups must be maintained, however these require high temperature and/or environmentally-unfriendly reaction conditions such as toxic solvents. This is not the case for RAFT/MADIX which is often employed at room temperature in aqueous conditions[35]. The typical reaction scheme is similar to conventional radical polymerisation where chain growth is initiated by a radical introduced at the start of the reaction. Chain growth of the nascent homopolymer or first ‘block’ species continue as the propagating radical end group(s) pick up free unreacted monomer (M1). Radical polymerisation is generally performed at high temperature but RAFT polymerisation can be performed at room temperature[36]. The technique adds an additional step to radical polymerisation where the radical end groups of the completed first block react with and are capped by the Z-group of the RAFT control agent. Typically, a thio-carbonylthio
group is used that stabilizes the radical end of the polymer, before bond rearrangement causes decomposition releasing another initiator (R●). This process allows for polymers of controlled length to be generated that are still living. Copolymerisation is initiated with a different monomer species (M₂), following the same steps as previous at the end terminal of the first block and producing a block copolymer (BC).

The homopolymers that are successfully capped by the transfer agent are called ‘living’ while those that are not are termed ‘dead’ chains (Figure 2). Due to the reactivity of the radical end group there are several kinetic events that result in early end-chain irreversible termination[37]. These ‘dead’ homopolymer chains, often called unreacted or residual homopolymer, remain after further copolymerisation resulting in an impure BC sample. The presence of these unreacted homopolymer chains is not limited to the first block (B₁) in BCs synthesized via RAFT/MADIX. Where external initiators are utilised to produce the second block (B₂) or solvent-monomer transfer results in an initiator, secondary propagation can occur with M₂ species forming residual homopolymer instead of a second block in the BC. A standard method for the quantification and identification of these homopolymers that remain after copolymerisation has not yet been established.

**Figure 2.** A diagram of the pathways for synthesis of some homopolymers and Block copolymer by RAFT/MADIX[38].

DHBCs previously investigated for quantification of unreacted homopolymer content include poly(acrylic acid-b-acrylamide) (P(AA-b-AM), poly(AA-b-ethylene oxide) (P(AA-b-EO), poly(acrylamido-N-propyltrimethylammonium chloride-b-N-isopropylacrylamide) (P(APTAC-b-NIPAM) and P(APTAC-b-AA)[39, 40]. These polymer species exhibit novel functionality in one block while in solution, such as thermoresponsiveness in PNIPAM[41] or...
pH responsiveness in PAA blocks[42, 43]. These properties are made compatible with substrates by the addition of some adhesive polymer in the second block, such as PAM which is well known for its adsorptive properties and ability to form gels used in flocculants, water treatment[44] and soil erosion[45]. Continuing this investigation of charged smart DHBCs a similar group of polymers have been investigated in this work, these are poly(vinylphosphonic acid-b-acrylamide) (P(VPA-b-AM) and poly(N-vinylpyrrolidone-b-VPA) (P(VP-b-VPA)) (Figure 3). VPA based DHBCs have found interest in research as fuel cell components[46] for their ability to form proton conducting polymer electrolyte membranes[47] and interface between organic and inorganic environments.[48] The application of organophosphorus and vinylphosphonic acid polymers has shown robust functionality in metal-polymer adsorption[49] this is demonstrated in the formation of PEGylated iron oxide nanoparticles[50]. Analysis of these compounds is important for their development within the environmental and electrical industries among others. As an expansion on the techniques and materials previously investigated, these DHBCs are suitable candidates for this study.
Figure 3. Chemical Structures of subject polymers A) P(VPA-\textit{b}-AM), B) P(VP-\textit{b}-VPA), C) P(VPA-\textit{b}-EO). The thiocarbonylthio chain transfer agent is seen capping the end of the block with chain length denoted \textit{m}.

1.1.3 Structure

1.1.3.1 Composition

Small chemical species or particles with discrete compounds forming the micro and macro infrastructure of a material are often much easier to characterise than polymers. The number of different compounds and architectures in the former can range from hundreds to thousands, whereas polymer samples exhibit a mixture of chemical architectures that range into the millions[51]. This makes separation of each individual component a tedious and highly impractical task given the many similar but slightly different species formed in polymerisation. In synthetic block copolymers material is distributed according to the total chain length of the polymer and the chain length of each block species present in the
polymer. This attributes a distribution of compositions per block copolymer sample. Determining these properties is often complicated by different end-groups and branching in the polymer backbone leading to many different structures even where composition is known due to differences in branch chain length and position on backbone. Some examples include polyacrylates with star, hyperbranched, randomly branched and linear chain architectures[52, 53]. As such separation techniques often output very broad peaks of polymers even taking band broadening into consideration. Further research combining the composition distributions of copolymers with the branching distributions for non-linear samples is necessary to build a holistic model of polymer structure and composition.

1.1.3.2 Purity

As discussed in ‘Synthesis’, in addition to composition distributions block copolymers also exhibit a mixture of different structures and chemical species. This influences the purity of these samples which is important in determining the properties of the block copolymer. The number of different species present in the product is dependent on what components are introduced at each step of the copolymerisation reaction. For block copolymers where a homopolymer (A) is reacted with monomer of a different species (B), several events throughout the synthetic process can result in both the homopolymers of A and B as well as the monomers remaining in the final product. This becomes a problem where high purity samples with strong yields are necessary, but separation techniques are unable to distinguish these components. Interestingly there are events where an impure sample with residual homopolymer can increase the stability of close packed block copolymer systems[54], this can also help support and binding of the block copolymer to a homopolymer base functionalised in membrane fibres[55]. However, properties such as micelle formation for use in PEM batteries and ion reservoirs can be compromised by homopolymer impurities.

1.2 HOMOPOLYMER QUANTIFICATION

1.2.1 Role of Homopolymer in Production and Purity of Block Copolymers

Homopolymer impurities can remain after synthesis of the block copolymer. Among these impurities distributions in the composition, species and transfer agents show a variety of homopolymers can be present in block copolymer samples. Because these species affect the phase separation in the block copolymer[56], quantification of these impurities is an important step to elucidating their relationship to polymer-polymer interactions and structure.
1.2.2 Current Homopolymer Quantification Methods: Potential and Limitations

1.2.2.1 Size-Exclusion Chromatography (SEC)

The presence of residual homopolymer within block copolymer samples has been detected but not yet quantified for polymers synthesised via a range of RDRP techniques [56-58]. Typically, such polymers are characterised in terms of their molar mass distribution by Size Exclusion Chromatography (SEC), also named Gel Permeation Chromatography (GPC) [3, 59]. Rarely in SEC is the homopolymer content separated from the block copolymer with enough resolution to perform quantification [40]. This has been demonstrated with the parent homopolymers of the triblock copolymer poly(styrene-\textit{b}-ethylene oxide-\textit{b}-styrene) (P(S-\textit{b}-EO-\textit{b}-S)) [60]. Analyte in SEC is separated according to its hydrodynamic volume and not molar mass [61], this can explain the lack in detection of the residual homopolymers from the block copolymers given the relative similarity in their hydrodynamic volumes.

1.2.2.1.1 Polymer characterisation with SEC

In the analysis of synthetic polymers SEC is one of the most widely used chromatographic methods. The complete distributions of molar masses for a block copolymer can be obtained given several factors such as a quantitative sample recovery and no degradation of sample, the correct calibration of the system and separations of all sample as well as system peaks. This involves running a known standard through an appropriate column (stationary phase) and solvent (mobile phase). Injected polymer samples penetrate the porous structure of the stationary phase whilst under the influence of the solvent flow potential drawing material towards the detector. The molecular hydrodynamic volume of the polymer chains affects the penetration and overall time they spend in the column, species with greater hydrodynamic volume become too large to enter the micropores [3]. Smaller polymer chains elute more readily into these microporous structures resulting in the elution order following largest to smallest hydrodynamic volumes [62]. In coupled SEC-NMR instruments fractions of block copolymer samples eluting from the liquid chromatography separation have been individually collected and transported to a sample holder for spectroscopic analysis [63, 64]. Complementary spectroscopic detectors have been utilised in conjunction with liquid chromatography techniques performed in SEC mode for the combined compositional and molar mass-based analysis of polymers. Coupling of multiple separation and detector instruments are a powerful tool for the characterisation of copolymers for both their
composition and architecture, however analysis of these materials is typically conducted in unidimensional SEC.

1.2.2.1.2 SEC for homopolymer quantification in DHBCs

DHBCs are often difficult to characterise by SEC as they require highly polar solvents and eluent systems to perform the separation. This is particularly troublesome for DHBCs with charged functional groups, as these polymers can undergo aggregation[65] and ‘ion exchange’[66-68] introducing errors in the accuracy of molar mass measurements[69]. Due to the strong solid phase interactions with cationic polymers, recovery becomes a difficult task requiring high salt conditions or co-eluents that risk degradation of the column[70, 71]. Anionic polymers such as sodium polystyrene sulfonate (NaPSS) and polysaccharides do not fair much better in these separation conditions[72]. The phosphonated polymers of interest in this work have also been investigated for their homopolymer content by SEC though quantification of these species can be difficult due to elution volume overlap[73]. Developments into aqueous SEC columns under low salt conditions has been researched for use on cationic polymers[74], however achieving separation conditions that satisfy all possible homopolymer residues in a DHBC sample is very challenging.

1.2.2.1.3 Disadvantages to SEC for Block copolymers

A major drawback in the application of SEC based separations is the incomplete separation of the copolymer by its composition. Separation in SEC is dependent on the hydrodynamic volume of the analyte as well as composition, any values determined from this technique must be deconvoluted from the effect of hydrodynamic volume on retention time. The problem of convoluted separation mechanism that select for molar mass and composition in the analysis of copolymers is present throughout all the methods listed. Capillary Electrophoresis in the Critical Conditions (CE-CC) on the other hand does not suffer from this effect. The limitations affecting characterisation can be overcome by hyphenating multiple separation techniques. Typical two Dimensional (2D) analysis involves separation schemes that are significantly dissimilar in each dimension. Coupled LAC-SEC and LCCC-SEC have been used to separate complex mixtures of block copolymers[75, 76]. Increasing the dimensionality of the separation allows concurrent sample aliquots to be further separated by a different property, this increases the theoretical plate count and reduces the effect of overlapping separation mechanisms in one dimension. These instrumental setups are costly in terms of time, optimising mobile phase and solvent systems as well as selecting and running a well-known standard that is appropriate for each dimension. Some of these issues can be
abated by coupling of a spectroscopic detector. However these instrument installations add considerably to the costs of equipment and maintenance[77].

1.2.2.2 Other Liquid Chromatography (LC) Modes of Separation

Other forms of chromatographic separations exist that allow for different molecular features of the block copolymers to be analysed. These include Liquid Chromatography at the critical point of adsorption (LC-CC) and Liquid Chromatography under Limiting Conditions of Desorption (LC-LCD). Both techniques have been used to separate block copolymers with some success[60, 78].

1.2.2.2.1 Critical Conditions (CC)

Liquid Chromatography at the critical point of adsorption or simply Liquid Chromatography under Critical Conditions (LC-CC) is a separation technique for the separation of analyte independent of molar mass[79]. This separation mechanism is achieved in conditions for solvent and stationary phase interactions that are found to occur between the conditions for separation by SEC and Liquid Adsorption Chromatography (LAC). It has been shown that adjusting the polarity of the mobile phase and stationary phase will shift the thermodynamics of the analyte in the column towards strong or weak interaction with each phase. A partition coefficient can be used to describe these different modes of separation, analyte can favour stationary phase interaction (LAC) or lean towards interaction-free (SEC) elution through the column micropores[80]. LC-CC exists where these modes meet as demonstrated below in Figure 4.

![Figure 4](image)

**Figure 4.** The trends of elution order as per the logarithmic change in molar mass for macromolecules depending on chromatographic mode.

The lack of interaction between the stationary phase and the analyte as well as the distribution of pore sizes dictates the order of elution in SEC as largest to smallest.
Conversely in LAC the degree of analyte/polymer-stationary phase interactions increases with size causing larger molecules to be retained on the column for longer than smaller molecules. This results in an inverse elution order to SEC. The critical conditions in Liquid Chromatography (LC-CC) are met where neither of these separation mechanisms are dominant in a system. For any homopolymeric species in these conditions, separation is not molar mass dependent. By finding the CC of one block species (equivalent homopolymer) in a DHBC, LC separation of the copolymer chains based on molar mass of the second block species (not in CC) can be achieved[81]. However the conditions for LC-CC often suffer from poor recovery[82, 83] especially where mixtures of solvents are required in the mobile phase that lead to preferential solvation for some analytes and stationary phase adsorption for others[84]. The analysis of polymer compositions and detecting the presence of homopolymer within DHBC samples becomes challenging via LC-CC.

It must be noted that other techniques such as Temperature-Gradient Chromatography are capable of achieving separation in CC. Determining the point of CC by temperature is often easier than by selection of solvents[85].

1.2.2.2 Limiting Conditions of Desorption (LCD)

In theory a combination of solvent species and hyphenated chromatography techniques would be required to retrieve ‘accurate’ molecular weight and composition distributions. For block copolymer samples this presents even more difficulties, as each block sequence will experience similar mobile-stationary phase interactions. Liquid Chromatography under Limiting Conditions of Desorption (LC-LCD) was developed similar to adsorption-desorption chromatographic techniques to separate macromolecules with more than one monomeric component[86]. This method has shown to be capable of separating the parent homopolymer content from PEG, PS and PMMA based block copolymers[81, 87-89]. LC-LCD fractionates polymers by combining adsorption chromatographic columns with solvent gradients or co-eluent mobile phases. This is achieved by running a solvent that first promotes desorption of all polymer analyte (exclusion), then a second solvent that promotes adsorption of the target block species onto the column (retention), this acts as a solvent ‘barrier’ of partial adsorption. Controlling for the strength of the adsorption-desorption eluents in the mobile phase impacts which separating mechanism is dominant. Under the correct conditions partial adsorption of one block species will cause all macro-block copolymers with the selected species to elute from the column independent of molar mass separated only by degree of adsorbing species present (composition).
LC-LCD has demonstrated good recoverability in comparison to LC-CC for a number of commercial block copolymer samples[90], however the choice of solvent systems can limit the barrier efficiency significantly depending on block composition and chain length[91]. For charged and highly polar DHBCs this presents potential hurdles in selecting appropriate solvents without destruction of the column and maintaining the exclusion-retention barrier efficiency. As of yet no literature has applied this technique to characterise these macro-polymers.

1.2.2.3 Capillary Electrophoresis (CE)

1.2.2.3.1 Free Solution CE

As an alternative for the quantification of residual homopolymer content in charged and highly polar DHBCs, free solution Capillary Electrophoresis (CE) has shown great potential in this field[40]. Compared to the previous techniques of separation, CE does not suffer from the same pitfalls in terms of sample preparation especially for difficult to separate species and expense running experiments. HPLC columns are costly and prone to contamination from highly adhesive species, whereas CE provides robust direct detection of analyte using relatively inexpensive capillaries at much higher resolutions, undesired components that remain after separation can be flushed from the capillary for future use[92-95]. In free solution CE molecules migrate in a fluid medium across a silica capillary under the influence of an electric field. CE does not use a stationary phase unlike gel electrophoresis and electrokinetic chromatography, this allows suspended analyte to freely move through the capillary. Injected sample volumes are typically small but vial stocks are produced in bulk for separation, in conjunction with the high voltages utilized and small analyte consumption often provide less interstitial and intermolecular friction resulting in fast separations[96, 97].

The movement of all species within the capillary is influenced by Brownian motion and the flow of the medium towards the end of the capillary. This fluid is a buffered saline solution with pKₐ usually selected to similar properties as physiological buffers in proteomics and genomics studies but can be much higher in carbohydrate and synthetic polymer analysis (typically sodium borate [NB]) for charged species to migrate through the capillary. Because there is no stationary phase the velocity of these polyelectrolytes is directly proportional to the electric field strength (E), while the electrophoretic mobility (µ) describes the proportionality between charge and friction see Eq. (1). The net apparent velocity of analyte in the CE is therefore the total sum of all velocities acting upon the analyte, where $v_{EOF}$ is the
velocity of the medium and $v_{ep}$ is the velocity of the analyte under an electric field see Eq. (2).

\[ v_{ep} = \mu E \]  

(1)

\[ v_{app} = v_{ep} + v_{EOF} \]  

(2)

Electrophoretic mobility therefore determines the separation of ions in CE[98]. For any charged species this constant is dependent on the ratio of its charge to the frictional force shown in Eq. (3). The frictional force retarding the ions movement impacts the apparent velocity $v_{app}$ of analyte inversely proportional to $\mu$.

\[ \mu \propto \frac{\text{Charge}}{\text{Friction}} = \frac{q}{f} \]  

(3)

Both hydrodynamic and electrostatic interactions playing a significant role in the mobility of ions in CE[99]. The Electroosmotic Flow (EOF) is the potential that drives the mobile phase or separation medium, silanol groups (Si-OH) at the inner surface-solution interface of the fused silica capillary undergo ionization. Counterions from the buffer solution (cations) condense at this interface generating an electric double layer. The diffuse cations from the buffer migrate towards the cathode under the influence of the electric field, this force entrains the solution and hydrated analyte resulting in an EOF[100]. Cations or anions are attracted to either one of the ends of the electric potential (anode or cathode) while neutral species migrate with the EOF. The net effect of the capillary potential and all directions of flow is summarized in Figure 5.
Figure 5. Migration velocity vectors of ions in free solution CE. A vectors schematic is shown describing the motion of all species in the capillary: the apparent velocities of Cations \( v_{\text{app-cat}} \) and Anions \( v_{\text{app-an}} \) as well as the EOF and neutral species velocity \( v_{\text{EOF}} \). The flow profile is represented on the right (flat bar).

Demonstrated above are the net velocities for the ions in a CE separation, usually the EOF is of significant enough force to drive anions toward the detector at the cathode-end of the capillary. In cases where this is not sufficient changes to the migration speed of analyte can be done by altering the electric field strength, pH of buffer or injecting sample by pressure-mobilization, though the last can cause band-broadening to peaks. Corrections to peak asymmetry from pressure-driven injections is possible with sample propagation at the inlet plug\[^{101}\]. Unlike in liquid chromatography such as HPLC which has pressure-driven mobile phase, CE is electroosmotically driven as such the flow profile of the former is laminar while the latter has a flat front\[^{102}\]. Band-broadening thus does not typically occur from the EOF, the number of theoretical plates in CE often lies in the tens of thousands to hundreds of thousands even without significant optimization.

Diagrammatic profiles of a CE experimental commonly appear in literature as migration time \( t_m \) plots (electropherograms). Many factors can affect the apparent \( t_m \) of an analyte between experimental runs and thus reducing the repeatability as well as reproducibility of \( t_m \) measurements with this technique. The primary factor influencing sample repeat studies in CE is irreproducible flow-rates\[^{103}\]. Changes to the EOF (flow-rate) will influence systematically the migration time of all analytes in the capillary, which can
lead to drifting of baselines and analyte migration times. As such converting $t_m$ into electrophoretic mobility ($\mu$) better describes the migration velocity of the analyte. Migration time is inversely proportionate to apparent velocity which allows us to express Eq. (2) in the form bellow:

$$\frac{1}{t_m} = \frac{1}{t_{ep}} + \frac{1}{t_{EOF}}$$ \hspace{1cm} (4)

where the migration time of the EOF marker ($t_{EOF}$) and electrophoretic migration time of analyte ($t_{ep}$) are expressed accordingly. From the above Eq. (4) the electrophoretic velocity can be expressed as the product of the inverse $t_{ep}$ and the length of the capillary to the detection window ($l_d$):

$$v_{ep} = \frac{l_d}{t_{ep}}$$ \hspace{1cm} (5)

Calculating the electric field strength ($E$) is possible by measuring the applied voltage ($\Delta V$) and the total capillary length ($l_{total}$):

$$E = \frac{\Delta V}{l_{total}}$$ \hspace{1cm} (6)

The effective field strength ($E_{eff}$) has been found to approach $E$ as the concentration of the background electrolyte (BGE) decreases approaching zero. Conversely increases in electrodispersion and counter-ion hydrated radius have been shown to decrease $E_{eff}$ along with increases in the solvate viscosity ($\eta$)[104]. Though these concerns must still be taken into consideration during wet-lab setup of a CE experiment, modern instrument software and detector suites have incorporated protocols for the accurate determination of $E$. Rearranging Eq. (1) and combining with Eq. (5) and (6) the electrophoretic mobility can be calculated as the distance travelled across the capillary relative to the difference in electrophoretic velocities for the analyte to the EOF. This is shown below:

$$\mu = \frac{l_d l_{total} \Delta V}{\Delta V \left( \frac{1}{t_m} - \frac{1}{t_{EOF}} \right)}$$ \hspace{1cm} (7)

An issue this equation brings up is the relative velocity and hence time analyte with opposite charge signs spend in the detector window at the cathode end of the capillary. Solving this equation for anionic polymers introduces negative mobilities (relative to the mobility of the EOF, $\mu_{EOF} = 0$), this is unlike chromatography where the detector is post-column thus all species will have the same velocity. The greater the magnitude of charge-to-
friction ratio the more pronounced the effect is, it is particularly apparent for anionic polymers such as the ones used in this study (Figure 3) as they will move much slower through the detector window than cationic polymers.

1.2.2.3.2 Joule Heating

Despite the potential benefits CE-CC has for the analysis of DHBCs there remains a number of factors that hinder efficiency in the separation of our polyelectrolytes. Given the intense electric field applied to the silica-based capillary, with potentials hundreds of times greater in magnitude than typically used for other electric field separations, resistive heating (Joule heating) is one such issue that may arise. Buffer that passes through the capillary will experience resistance to electroconductivity by the electric field resulting in heating profiles appearing across the inner wall. The temperature profile gradient seen in the cross-section of a capillary is radial (Figure 6).

![Image of Joule Heating](image_url)

**Figure 6.** Cross section of silica capillary highlighting the temperature gradient of Joule heating profile (red dashed line), the effective electrophoretic mobility profile of ion analyte (blue circles) caused by the temperature profile (dark blue) and the apparent migration profile (Bold light blue) [102, 105].

These temperature bands have the effect of decreasing the specific viscosity of the buffer solution radially in the width cross section. This is known to change the profile of the EOF causing band broadening in terms of lower peak height and wider migration bands[106]. Measuring of these Joule heating profiles has been a difficult task outside of numerical
simulations due mostly to the instrument setup. However Raman spectroscopic and NMR thermometry have been used previously to estimate these radial gradients[107].

A calculation of the temperature gradient is possible where the thermal conductivities of the fused silica capillary wall and polyimide inner coating are known. This expectant radial temperature difference in the bulk electrolyte ($\Delta T_{\text{Radial}}$) can be calculated as follows[108]:

$$\Delta T_{\text{Radial}} = \frac{1}{4\pi\lambda} \cdot \frac{P}{l_{\text{total}}} \quad (8)$$

Here $\lambda$ represents the thermal conductivity of electrolyte, while the power $P$ is the product of the current inside the capillary and the applied voltage. For dilute aqueous electrolytes such as sodium borate (NB) at concentration $<500$ mM the $\lambda \approx 0.605 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}$. The second equation relates the temperature difference from the electrolyte-wall interface across the capillary coating and fused silica outer wall ($\Delta T_{\text{Across Wall}}$), shown below:

$$\Delta T_{\text{Across Wall}} = \frac{1}{2\pi\lambda_{\text{Wall}}} \cdot \ln\left(\frac{d_o}{d_i}\right) \cdot \frac{P}{l_{\text{total}}} \quad (9)$$

This function shows the relationship of the inner diameter of the capillary wall ($d_i$) to the outer diameter of the capillary wall ($d_o$) given the thermal conductivity of the wall ($\lambda_{\text{Wall}}$). The double layer wall does not have a linear conductivity gradient given the different chemical compositions in each layer, as such the difference in the temperature gradient across the entire wall must be adjusted by each layers respective thermal gradient. The geometry of the capillary wall is visualised in Figure 7, here we see the internal radius is considerably smaller than the total cross-section radius.
Figure 7. A) Schematic cross-sectional diagram of capillary and the T gradients. B) Schematic of dimensions and temperature profile for fused-silica capillary; \( l_{\text{total}} \) 32.2 cm, \( d_i \) 74.0 µm, \( d_o \) 362.8 µm, phosphate buffer BGE 10.0 mM at pH 7.21, software controlled \( T = 25 \) °C for a Power per unit length \( \frac{P}{l} \) of 1.00 W·m\(^{-1} \) [108].

The apparent temperature profile in the capillary is shown above, using the on-board thermometers in the CE instrument to measure the temperature at the air and cartridge interface can lead to errors in the estimation temperature. The EOF is known to be sensitive to changes in temperature thus applying measurement of the \( \Delta \mu_{\text{EOF}} \) as weighted averages of the \( T_{\text{Axis}} \), \( T_{\text{Wall}} \) and \( T_{\text{Mean}} \) in the forms: \( \frac{2}{3} T_{\text{Axis}}, \frac{1}{3} T_{\text{Wall}} \), the mean occurring at a distance \( r_f \sqrt{3} \) from the central axis. These can be substituted into Eq. (9) to provide accurate temperature profiles of the BGE.

1.2.2.3.3 Separation and Detection of Natural and Synthetic Polymers

A variety of natural compounds from small oligomers to large polymeric structures can be separated in CE-CC. This method has been used in the characterisation of natural compounds from polysaccharides[93, 94, 109, 110] to proteins and peptides[111, 112], and synthetic polymers such as PNaA[113, 114] in some cases revealing multiple chain architectures not observed before. An example of CE’s robustness lies in food monitoring, such as the quantification of ethanol in fermentation of carbohydrates[94] and nutrient content within breakfast cereals[95]. More importantly for the purposes of this study it has previously been demonstrated that DHBCs of P(AA-b-AM), P(AA-b-EO), P(APTAC-b-NIPAM), P(APTAC-b-AA) as well as copolymers of poly(diallyldimethyl ammonium
Other bio-polymers with disperse block sequences have been separated in free solution CE by attaching neutral protein tags (uncharged block) onto the ends of DNA chains. Neutral tags change the charge to friction ratio of the polymer allowing specific sequences of DNA to be separated from the bulk genetic material[116]. Sequences of free unlabelled DNA (homopolymer) migrated faster than to labelled or block copolymerised DNA (copolymer) due to frictional retarding[117].

The choice of detection instrument needed for polymeric samples are many and various modes of detection are available for CE, though the number of detectors found in LC is comparatively greater including light scattering, viscometry and refractometry. For many of the polymers in the studies mentioned a UV detector was utilized to resolve a chromophore group within or repeated throughout the polymer chains. In the case of detecting polymers without a chromophore group buffer-polymer complexes often occur that allow for detection of a moiety in the chain. These salt-polymer complexes can be repeat sequences along the polymer backbone or a single UV absorbing unit that identifies a polymer. Alternatively laser-induced fluorescence spectroscopic detectors have been used for the quantification of small bio-polymers such as microRNAs[118], and like other separation techniques conductivity detectors are a common instrument for pH and charged molecule monitoring. CE can be used to measure much smaller quantities of analyte than other chromatography techniques, typically in the nano-litre range. However criticism of CE as a low sensitivity technique has led to improvements in sample stacking methods to increase bulk sample concentration in the capillary[119]. Low resolution or wavelength absorbing samples can thus be better detected.

1.2.2.3.4 CE in the Critical Conditions (CE-CC)

CE is capable of separating oligo-electrolytes by molar mass, with high resolution up to a degree of polymerization (DP) of 10 units[120]. The separation in CE of large macromolecular polyelectrolytes such as polyacrylates (PAA) chains become independent of molar mass at high DP[121]. That is the electrophoretic mobility of any polyelectrolyte increases with DP of charged units, before dropping to a plateau at constant mobility see Figure 8 [122, 123]. This state is analogous to LC-CC separating analyte independent of molar mass and is referred to as CE in the “critical conditions”. As counterions corrugate around the polyelectrolyte chains while in the diffuse bulk of the capillary the polymer chains conformation changes to a coiled state[124]. This results from the dominant intermolecular
electrostatic friction overcoming the hydrodynamic friction between polyelectrolytes and background electrolyte (BGE)[125]. Like LC-CC this mode of separation is capable of differentiating complex polymer samples by the distribution of compositions, such as chitosan (by degree of acetylation)[109, 126] and gellan gum (by degree of acylation and double-helix conformation)[110]. The ‘critical conditions’ achieved in CE are much easier to obtain than those in LC, given that polymer samples only need to be of sufficient size to enter this state of separation. Analyte mobility is dependent on friction and charge, one or both factors being potential contributors by the end group speciation.

![Diagram of polymer conformations](image)

**Figure 8.** Generalised trend for the conformations and mobilities of a polyelectrolyte with increasing DP.

Often is the case that filtering of polymer solutions especially complex mixtures of polymeric material can lead to component loss and potentially the degradation of the macromolecules. For Liquid Chromatography-based techniques filtering is almost always necessary to reduce the complexity of the sample and remove particulates that damage the columns but this step can be detrimental to analysis[127], unlike in CE-CC no filtering is necessary given the lack of a stationary phase. Typical capillary diameters of 50-75 µm are sufficient to prevent solid particles from entering the capillary while any smaller in size will easily pass through the CE without causing damage.

Synthetic polymers exhibit multi-dimensional properties such as differences in end group, composition and molar mass. Within this project a method was developed for the complete characterisation of RAFT/MADIX constructed complex charged DHBCs using CE-CC. The polymers studied contain poly(vinylphosphonic acid) (PVPA) anionic blocks as well
as poly(N-vinylpyrrolidone) (PVP), poly(acrylamide) (PAM) and poly(ethylene glycol) (PEG) neutral blocks species. Using this method a distribution of the electrophoretic mobilities and homopolymer purities were determined, these were later converted into a composition distribution for the block copolymers.

1.3 COMPOSITION IN BLOCK COPOLYMERS

1.3.1 Role of Composition on Copolymer Properties

The complexity of copolymers increases with the addition of more subunit species in the chemical composition. The ratio of subunit species within a single chain must be measured to determine the weight-distribution of compositions \((W(C))\) within the copolymers, comparatively homopolymers are constituted from a single species and thus have uniform \(W(C)\).

Properties of a copolymer are highly influenced by the distribution of the compositions as well as the intermolecular architectures. This has been demonstrated where tensile strength in graft copolymers can be altered considerably by introducing a bimodal \(W(C)\) for polymers of different lengths compared to unimodal samples[128]. Controlling for the content of adhesive species in a copolymer can also alter other mechanical properties in gel media where swelling and elasticity play a vital role in the integrity of the material or bioaccessibility[129]. Long term application of these copolymers in drug carriers and biomedical applications is affected by the composition-dependent properties such as tensile strength, viscosity and gelation, biodegradability and bioreducibility of PEO-based BCs[130, 131]. Monitoring of monomer content in block copolymerisation reactions becomes that much more difficult without techniques capable of discriminating the distribution of compositions produced. Current techniques and number averages for these distributions may not be suitable for tuning the production of these polymers.

1.3.2 Effects of RAFT Agent on Polymerisation

As discussed copolymer properties are highly dependent on the composition given as a distribution of ratios between each block species present. In diblock copolymers this ratio of block species affect the phase separation for each block[132]. Periodic nanostructures are a typical feature of this microphase separation (Figure 9), each set of structures has unique ion binding and adhesive properties[133]. These composition distributions also have an effect on the materials self-assembly into particle or film-like architectures[134, 135]. End groups of block copolymers impact thermoresponsivity[136], an important trait in designing smart
DHBCs for micellar architectures especially in drug delivery. The response from heating or cooling of some sol-gel BCs can greatly change the permeability and behaviours of these micelle-like assemblies[137]. For living DHBCs polymerised by RAFT/MADIX, such as the materials investigated in this study, end groups composed of the fragmented chain transfer agent (CTA) remain covalently bonded. Chain growth, stability and intermolecular assembly are affected by the composition of these species[138]. The effectiveness of the CTA in controlling the polymerisation is important for future synthetic studies as well as quality control.

Figure 9. Schematics of thermodynamically stable diblock copolymer phases. Each of the blocks (shown in simplified form at top) self-organise into their own 3D domains.[29]

Separating polymers by end group has been demonstrated in LC-CC by substituting charged benzaldehyde species onto a reactive end-group functionalised PEG homopolymer. The addition of a 4-carboxybenzaldehyde group at either one or both terminal ends changes the elution time of the polymer[139]. Because of end-to-end and other termination events during synthesis, copolymers and homopolymers will form either with or without a CTA at
the terminal ends of the chain[40]. Differences in end groups adds to the factors affecting composition distribution and must be determined in separation. Similar to the example in LC-CC separating polymers by their end group in CE-CC has been achieved with polyacrylates where substitution of some bulky moiety at a reactive site yields a change in the mobility of the polymer[113].

1.3.3 Methods for Determination of Compositions

1.3.3.1 Average Compositions

Literature composition values for copolymers are often stated as an average, this is due to the lack of suitable techniques capable of determining the distributions. These techniques often ‘look’ at the entire polymer sample. Some repeat functional groups in polymers are visible in the infrared (IR) and near IR spectrum, this has allowed composition averages to be determined by IR spectroscopy for industrial materials such as polyolefins[140], poly(styrene-b-isobutylene-b-styrene)[141] and poly(acrylonitrile-co-acrylamide)[142]. Also interesting is the use of IR spectroscopy on treated konjac polysaccharides to determine the average degree of acetylation (DA) in the samples and relating this to nutrient and health content[143]. The most commonly used technique for average polymer composition studies is Nuclear Magnetic Resonance (NMR) spectroscopy. Bioactive and RAFT polymerised copolymers have been assessed for their microstructure and average composition by NMR[144-146]. In addition time-dependent sample compositions have been achieved with both spectroscopic techniques for the formation of industrial copolymers[147]. Most samples tested by these techniques have differing signals for each monomer subunit, this allows quantification of the relative ratio for each component in the copolymer. However without differing signals corresponding to each monomeric unit in the polymer an average composition cannot be determined by spectroscopic analysis alone.

Precise measurements of the molar mass within a polymer sample have been achieved with Mass Spectroscopy (MS) in relation to individual chains[148]. In theory this allows the ratio of monomer units to be calculated relative to each resolvable chain in the sample. However in practice not all macromolecules species will ionise evenly, leading to inaccurate compositions. Combining multiple MS, ionisation steps like Matrix Assisted Desorption/Ionisation (MADLI) and spectroscopic (NMR) detectors has yielded molar mass determination of the block length for some mPEG-b-PS synthetic BCs[149]. A similar methodology was employed in determination of the sizes of blocks for PS and
polymethacrylate (PMAA) based copolymers, by transforming the different blocks into two distinct or one single homopolymer that can be easily optimised for[150, 151]. However this hides the homopolymer content present, reducing the accuracy of the molar mass and block ratios. Other challenges in measuring the composition in copolymers by MS are the bias in ionisation of monomer species and lower molar mass biases[121, 152, 153]. These potential drawbacks may be avoided if a separation technique were utilised to reduce the complexity of analyte entering the detector.

1.3.3.2 Distributions of Compositions
Composition averages for copolymers are not suitable to fully determine the properties of some materials while others can be assessed. However without a distribution of compositions available for the user of a product the number of synthesised molecules and speciation, whether narrow or broad, cannot be determined by the average. This is a significant factor affecting copolymer synthesis and selection. As such obtaining a distribution separation of the copolymeric sample is needed. As outlined in the previous section, spectroscopic analysis of entire copolymer samples is unsuitable to give a distribution of the composition, methodologies have been developed that couple some separation technique with a spectroscopic detector. Analyte is fractionated and sent sequentially through a detector such as IR or NMR. Some separation techniques that have utilised this method for copolymer analysis include Size Exclusion Chromatography (SEC)[154], Liquid Chromatography in the Critical Conditions (LC-CC)[155-158], Temperature Rising Elution Fractionation (TREF)[159-161], High Temperature Liquid Chromatography (HT LC)[80, 162, 163] and Thermal-Field Flow Fractionation (ThF3)[164-166]. However due to the incomplete separations of analyte by composition in most of these separation steps, spectroscopic detectors will still produce averages of heterogeneous samples. Thus the distributions of compositions are not typically investigated.

1.3.3.3 Characterisation by the Distribution of Electrophoretic Mobilities
Capillary Electrophoresis in the Critical Conditions (CE-CC) has been discussed earlier in relation to the separation of polymers independent of their molar mass. Here the mechanism of separation is by electrophoretic mobility ($\mu$) which is influenced by charge composition of the analyte. For block copolymers (BCs) where one block is composed of charged subunits and another block from neutral subunits, the additional friction ($f$) of the neutral block changes the BC mobility such that homopolymers of either block will express
different mobilities and separate from the BC in CE. This is apparent as all neutral species migrate with the EOF while charged species will be drawn towards either the anode or cathode depending on net surface charge (see Figure 5).

Typically chromatographic techniques record separations by the detector response $R(t)$ as a function of time ($t$). For SEC a weight fraction is calculated from the elution volume and detector response in the ordinate of the elugram[167]. This allows a distribution of analyte to be determined from a peak. For CE-CC these detector outputs correspond to the UV absorbance and migration time ($t_m$). Like SEC data both the coordinates are converted into distributions of analyte fractions as the electrophoretic mobility ($\mu$) and the weight fraction of $\mu$ ($w(\mu)$) respectively. Conversion of the detector response is necessary as the UV absorbance or $UV(t_m)$ is a function of $t_m$ and not $\mu$. The relationship between the two values is hyperbolic, so correcting the UV absorbance is achieved by multiplying by $t_m$ as per Eq. (10). Plotting without the correction in the electrophoretic mobility distribution will cause skewing to occur in the detector ordinate due to this non-linear conversion of the horizontal. Thus the UV signal must be corrected in order to retrieve accurate data.

The UV signal is dependent on the amount of analyte passing through the detector window over time. As the transformation of $t_m$ into $\mu$ is non-linear and the relative UV absorbance of analyte is stronger for slowly moving particles, the peak areas for material moving before and after the EOF will be skewed. To correct for this the UV absorbance is scaled by $t_m$ which gives the weight distribution of $\mu$ ($w(\mu)$) as follows:

$$w(\mu) = UV \text{ absorbance} \cdot t_m$$ (10)

These values plotted against $\mu$ provide us with a continuous distribution of mobilities useful in determining the properties of polymer samples [167]. The dispersity of a polymer is usually related to its molar mass distribution ($D$), often given as the ratio of weight-average molar mass ($\bar{M}_w$) to number-average molar mass ($\bar{M}_n$)[168]. Though this measurement is ubiquitous within both academia and industry for the determination of a polymers molar mass distribution, another molar mass value ratio used for the dispersity polymers is described: $z$-average molar mass ($\bar{M}_z$) to $\bar{M}_w$. Due to the value being more sensitive to higher molar mass polymers it is not as readily utilised.

Separation in CE-CC is dependent on the charge composition and not molar mass ($M$). To quantify the heterogeneity of compositions for DHBCs by CE a different dispersity
calculation must be used based on the distribution of $w(\mu)$. This value can be calculated in
relation to the moments of $\mu$, analogous to $M_w/M_n$ this dispersity (called here $D$) is the ratio
of weight-average $\mu$ ($\mu_w$) and moment-average $\mu$ ($\mu_m$). Similarly in analogy to $M_z/M_w$ a $D$
with $z$-average $\mu$ ($\mu_z$) and $\mu_w$ can be calculated. Combining these terms into the equations
below:

$$D(w(\mu), 1, 0) = \frac{\mu_w}{\mu_m} = \frac{\int w(\mu)\mu d\mu[\int w(\mu)\mu^{-1}d\mu]}{\int w(\mu)d\mu^2} \quad (11)$$

$$D(w(\mu), 2, 0) = \frac{\mu_z}{\mu_w} = \frac{\int w(\mu)\mu^2 d\mu[\int w(\mu)d\mu]}{\int w(\mu)d\mu^2} \quad (12)$$

The data obtained from a CE experiment relates discretely to the mobility of analyte
within a separation, these moments are thus expressed in the form below as they are
calculated from the software: \[169\]

$$\mu_m = \frac{\sum w(\mu_i)(\mu_{i+1}-\mu_i)}{\sum w(\mu_i)(\mu_{i+1}-\mu_i)} \quad (13)$$

$$\mu_w = \frac{\sum w(\mu_i)\mu_i(\mu_{i+1}-\mu_i)}{\sum w(\mu_i)(\mu_{i+1}-\mu_i)} \quad (14)$$

$$\mu_z = \frac{\sum w(\mu_i)\mu_i^2(\mu_{i+1}-\mu_i)}{\sum w(\mu_i)(\mu_{i+1}-\mu_i)} \quad (15)$$

1.3.3.3.1 Rescaling Factor $\alpha$

The relationship between the charge composition of a polyelectrolyte and its $\mu$ moment
is monotonic where increases in the magnitude of the polymers net charge correlate with
increases in subunit count. Even without assuming the charge sign is homogenous across the
subunit species, this relationship holds true. For DHBCs with one charged and one neutral
block this may only hold true at any given DP in the neutral block. The effective number of
subunits for the total BC has been related to $\mu$ in the following: \[170\]

$$\mu_{BC} = \mu_0 \frac{n_c}{n_c+\alpha n_u} \quad (16)$$

where $\mu_{BC}$ is the electrophoretic mobility of some block copolymer, the number of
charged ($n_c$) and uncharged ($n_u$) subunits are related by $\mu_0$ which represents the constant
electrophoretic mobility of the charged homopolymer(s). To account for the difference in
hydrodynamic friction between the subunit species $\alpha$ is used as the rescaling factor. At the
CC the polyelectrolyte homopolymer has a constant mobility \((\mu_0)\). Therefore changes in the charge to friction ratio are caused by the additional electrostatic friction from the uncharged block \((B_u)\), this \(\Delta\mu_{BC}\) is represented by Eq. (16)[171]. It is important to understand the rescaling factor is dependent on the relative flexibility of the uncharged subunits and the size of these unit species as they relate to the total hydrodynamic volume of the BC under critical conditions. Greater flexibility from the \(B_u\) results in more movement in the buffer solution and an increase in the friction to the entire BC[172]. \(\alpha\) is shown to change with chemical species in both the charged \((B_c)\) and uncharged \((B_u)\) blocks, this is because both species contribute to the total BC friction. Additionally the solvent-polymer interaction affects \(\alpha\) based on the ionic strength and solution temperature[171]. To determine the value at a given set of \(\mu_{BC}\), Eq. (16) is rearranged so that \(\alpha\) is the slope from the following function:

\[
\frac{\mu_0}{\mu_{BC}} - 1 = \alpha \frac{n_{u}}{n_{c}}
\]  

(17)

While this can be used to establish the relationship between charge to friction ratios for BCs with different \(B_u\) content, the effect each subunit has on \(\mu_{BC}\) is not equal. Surface and end-groups contribute much greater to the overall mobility of a polyelectrolyte than core-units being less exposed to the counter-ions in the solvent[173]. Therefore \(\alpha\) must be calculated for each experimental run due to the shifts in the specific run conditions as well as between BC samples depending on the specific block length.

1.3.3.3.2 Charge Composition

In literature the charge composition for a sample is given as the chemical charge density \((\xi)\). In the context of polyelectrolyte chemistry this defines the ratio of charges per subunit species to the total number of subunits in the polymer (see Eq. (18)). For BCs where each species is discretely ordered into their respective blocks, this charge density describes the distribution of compositions for the copolymer.

\[
\xi = \frac{n_{c}}{n_{c} + n_{u}} = \frac{1}{1 + \frac{n_{u}}{n_{c}}}
\]  

(18)

It has been shown that the charge density can be related to the electrophoretic mobility by combining Eq. (17) with the rearranged form of Eq. (18)[39] shown below in Eq. (19). Having both the rescaling factor \((\alpha)\) and the charge density \((\xi)\) written as coordinates relating to the electrophoretic mobility \((\mu)\) of the block copolymer in the critical conditions provides a means to determine the dispersity of compositions for the \(W(C)\) of the block copolymers.
\[ \zeta = \frac{\alpha \mu_{BC}}{\mu(\alpha - 1) + \mu_0} \]  \hspace{1cm} (19)

### 1.3.3.3 Ionic Strength Dependence of \( \mu \)

The ionic strength (\( I \)) of the surrounding buffer solution influences the velocity of the EOF in CE. Increasing \( I \) results in a greater viscosity (\( \eta \)) to the background solution and lower \( \nu_{EOF} \) and \( \nu_{BC} \). Counter-ion condensation around analyte affects the mobility of species travelling through the capillary, the resolution of disperse samples in the separation is dependent on both \( I \) and the degree of adsorption of counter-ion to the surface of the analyte. In the case of Poly(styrene-b-ethyl acrylate) (P(S-b-EA)) latexes, BGE and pH were dependent factors in the resolution of homopolymers[174]. Lower resolution of analyte can increase the convolution of neighbouring peaks, potentially affecting the measurement of dispersity as a sample, as such BGE composition and \( I \) must be taken into account.

The speciation of counter-ions in the buffer solution influence the zeta potential, if there is only one species present in the counter-ions the concentration of ions is equivalent to the \( I \) of the solution. Ionic strength dependence of \( \mu \) has been documented for different electrolytic species, such as small ions, nanoparticles and polyelectrolytes. Each group exhibits different surface areas and counter-ion condensation rates. At the minimum concentration of solute in the critical conditions the mobility \( \mu_{ep} \) is found to be equal to a decreasing function of \( I \) for all analyte[175]. A slope plot can be used to compare the relative change in \( \mu_{ep} \) per unit of \( I \) between these differing particle groups(Figure 10).

![Figure 10. Slope plot with \( S \) values as a function of the effective \( \mu \) at \( I = 5 \) mM. Different particles are highlighted for each coordinate.[175]](image_url)

\[ \frac{\mu}{\mu_{5mM}} = -S \log(I) + R \]  \hspace{1cm} (20)
The slope \( S \) is the change in relative electrophoretic mobility \( \frac{\mu}{\mu^{5\text{mM}}} \) by ionic strength as defined in the equation above. This is plotted against the mobility of analyte at a set ionic strength \( I = 5 \text{ mM} \) \( \mu^{5\text{mM}} \). These slope plots reveal the differences in \( \Delta\mu_{ep} \) of the analyte depending on \( I \). For polyelectrolytes where the charge density \( \zeta \) and hydrodynamic volume change significantly compared to small ions. This is reflected in the above slope plot, the order of dependence on ionic strength is: nanoparticle > polyelectrolyte > small ions.

Advances in slope plot analysis for the identification of electrolyte species has been made by incorporating hydrodynamic volume into an xyz (triplet variables) 3D visual plane. Demonstrate that prediction of the \( \mu_{ep} \) for any given polyelectrolyte is difficult even with known \( \zeta \) and \( S \) data. Instead comparing a standard polymer with known slope plot triplets is useful in predicting the change in mobility as well as polyelectrolyte composition for copolymers[176].

1.4 AIMS AND OBJECTIVES OF STUDY

It is the aim of this work to develop a standard method of characterisation for various VPA-based smart block copolymers. Smart copolymers are of interest in the synthesis of nanotechnologies and novel drug delivery systems, phosphonic acid-based DHBCs have been utilised as smart copolymers in several biomimetic and nano-electric cell technologies for their high charge utility and novel micro-, macrostructures. Despite the emphasis placed upon these materials as potential synthetic technologies of the future, they remain relatively untouched in terms of characterisation. This work has analysed a number of these materials by CE-CC. These include P(VPA-\(b\)-AM), P(VP-\(b\)-VPA), P(VPA-\(b\)-EO). For anionic DHBCs characterisation is a challenging task as these materials are generally unsuitable for most common polymer analytical techniques. In collaboration with the synthetic studies of these materials this paper hopes to complement further research into construction and application of ‘smart’ neutral-charged DHBCs.

Building upon previous work in the characterisation of DHBCs, a primary focus of this study is the analysis of sample purities. This involves the identification of parent homopolymer species present in the block copolymers as well as developing accurate methods for the quantification of these components. To this end the block copolymer samples were separated in CE-CC, desired product and homopolymer impurities migrated though the
capillary at different rates allowing visualisation and calculations to be performed on the sample components. A detailed quantification of the homopolymer content in a block copolymer is not generally performed in the literature, yet structural properties of the copolymer are highly influenced by the ratio of these components in the final product.

The second key focus of this work looks at characterising the composition of the synthesized block copolymers. Having separated the different species according to their charge composition, CE-CC also separates the many different structures of both homopolymers and block copolymers present. This provides the $W(\mu)$ which was used to calculate the dispersity of compositions for the block copolymer content. By optimising the method used on phosphonic acid based block copolymers a new method was achieved for the characterisation of DHBCs in terms of purity and compositional distributions not yet achieved in literature.
Chapter 2 – MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Capillary Electrophoresis

Water used for CE was Milli-Q quality (Merck Millipore, Bedford, MA, USA). Buffer solutions were prepared from the following stocks: boric acid (≥ 98.0%) purchased from BDH Chemical Co. AnalAr, Merck Pty Limited and Sodium hydroxide pellets (NaOH, > 99.0%) purchased from Sigma-Aldrich, Inc. Chemical Co. Dimethyl sulfoxide (DMSO, ≥ 99.5%) also purchased from Sigma-Aldrich was obtained from WSU Parramatta Laboratories and used as an EOF marker for some polyelectrolyte separations, while separations of neutral polymers used marker Methyl green manufactured and supplied by Gorge T. Gurr. High sensitivity fused-silica capillaries \((d_i = 50.0 \, \mu m, \, d_o = 363.0 \, \mu m)\) purchased from Polymicro (Phoenix, AZ, USA) were used for all separations. Syringes (20.0 mL) were obtained from BD Plastipak and poly(ether sulfone) (PES) membrane filters (0.22 \, \mu m) bought from Merck Millipore for filtering of buffer solutions into sample vials.

2.1.2 Polymer Samples

All PAM-\(b\)-PVPA block copolymers were synthesised and purified by Géraldine Layrac using supplies at the Institute Charles Gerhardt Montpellier (MACS, Montpellier, France). All PVP-\(b\)-PVPA and PEG-\(b\)-PVPA samples were synthesised by PhD candidate Lucie Seiler at the University Paul Sabatier (IMRCP Laboratory, Toulouse, France). Homopolymer samples: PVP K30 was supplied from BASF, PAM (AnalAr) purchased from BDH Chemical Co., Merck Pty Limited. Monomers Vinylphosphonic acid (VPA) supplied from BASF, Acrylamide (AM) supplied from SNF Australia and \(N\)-vinyl pyrrolidone (NVP) supplied from Acros Organics were used in the synthesis of homo- and block copolymers as well as CE injections. All block copolymers were prepared by the Reversible Addition-Fragmentation Chain Transfer/Macromolecular Design via Interchange of Xanthates (RAFT/MADIX) technique using the carboxyl-functionalised xanthates \(O\)-ethyl-\(S\)-carboxylethylthiodithiocarbonate (X1) and \(O\)-ethyl-\(S\)-(1-methoxycarbonyl)ethylthiodithiocarbonate (XA1) as transfer agents respectively. The complete list of polymer and monomer samples is found in Table 1:

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Table 1. List of all polymeric and monomeric samples analysed by CE in this work. The theoretical molar mass ($M_{n,th}$) for all samples both polymer and non-polymer samples is listed and experimentally determined molar mass values: NMR spectroscopy ($M_{n,NMR}$) and SEC-MALS ($M_{n,MALS}$), have been provided by manufacturers for some polymer samples.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Polymer/ Description</th>
<th>$M_{n,th}$ (g.mol⁻¹)</th>
<th>$M_{n,NMR}$ (g.mol⁻¹)</th>
<th>$M_{n,MALS}$ (g.mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
<td>115</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide</td>
<td>155</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Monomers**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>VPA</td>
<td>Vinylphosphonic acid</td>
</tr>
<tr>
<td>NVP</td>
<td>N-Vinylpyrrolidone</td>
</tr>
<tr>
<td>AM</td>
<td>Acrylamide</td>
</tr>
</tbody>
</table>

**Homopolymers**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PVPA-XA1 0.5K</td>
<td>Poly(vinylphosphonic acid)</td>
</tr>
<tr>
<td>PVPA-XA1 2K</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PEG-X1 1K</td>
<td>Polyacrylamide</td>
</tr>
<tr>
<td>PAM-XA1</td>
<td>Poly(N-vinyl pyrrolidone)</td>
</tr>
<tr>
<td>PVP K30</td>
<td></td>
</tr>
</tbody>
</table>

**Block Copolymers**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>PAM(5K)-b-PVPA(0.5K)</td>
<td></td>
</tr>
<tr>
<td>PAM(5K)-b-PVPA(1.5K)</td>
<td>Poly(acrylamide-b-vinylphosphonic acid)</td>
</tr>
<tr>
<td>PAM(5K)-b-PVPA(2.5K)</td>
<td></td>
</tr>
<tr>
<td>PAM(10K)-b-</td>
<td></td>
</tr>
<tr>
<td>Block Copolymer</td>
<td>Polymerisation</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>PVPA(1.5K)</td>
<td>Poly(vinylpyrrolidone-(b)-vinylphosphonic acid)</td>
</tr>
<tr>
<td>PVP_{72-b-PVPA_5}</td>
<td>8000-(b)-541</td>
</tr>
<tr>
<td>PEG_{45-b-PVPA_5}</td>
<td>2550 (1982-(b)-568)</td>
</tr>
<tr>
<td>PEG_{45-b-PVPA_10}</td>
<td>3750 (2614-(b)-1136)</td>
</tr>
<tr>
<td>PEG_{112-b-PVPA_6}</td>
<td>Poly(ethylene glycol-(b)-vinylphosphonic acid)</td>
</tr>
<tr>
<td>PEG_{112-b-PVPA_8}</td>
<td>6500 (5168-(b)-682)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.a. = not applicable
n.d. = not determined

*Molar mass stated by the supplier

*aValue determined for PVPA block (PVPA-X1, where X1 = MADIX transfer agent) using \(^{31}\)P NMR

### 2.1.2.1 Synthetic Procedure

The synthetic scheme for all homopolymer samples is described in [177]. Expanding upon Section 1.1.2.2, the general synthetic procedure for homopolymers and their conversion into block copolymers by RAFT/MADIX followed a three-step process in the following order: synthesis of MADIX transfer agent, first block species macro-initiator polymerized using MADIX agent, and second block species copolymerised with first block homopolymer. Each of the intermediate materials was carried over to form the respective blocks for the completed BCs.

### 2.1.2.2 MADIX Transfer Agent and Homopolymerisation

Different MADIX transfer agents were synthesized for homopolymerisation of neutral species of PEG and PAM/PVP-based block copolymers, as such different protocols were utilised for end-functionalisation of PEG-blocks compared to all other neutral-blocks in...
homopolymer synthesis. The difference in the polymerisation mechanism for each species is illustrated in Figure 11. The general synthetic protocol for all block species in this work involves the functionalisation of M₁ monomers with the transfer agent to form an adduct for further polymerisation, for example PAM-XA1 was formed as follows. A solution of XA1 (4.77 g) in ethanol (35.5 mL) was degassed with argon at ambient temperature. 4,4’-azobis(4-cyanovaleric acid) (ACP) (1 g) in 10 mL distilled water was added to the xanthate solution, this mixture was thermostated at 70 °C before M₁ (220 mL of 50% w/v AM solution) monomers were dropwise added to the mixture at a rate of 2 mL·min⁻¹. The reaction was kept at 70 °C for 2 h before vacuum evaporation filtration of converted homopolymers[178].

Figure 11. Synthetic procedure for the polymerisation of PVPA homopolymers and P(EG-b-VPA) diblock copolymers[50].

In the case of PEG-based BCs, homopolymers of M₁ (PEG) were purchased with experimental $\bar{M}_n = 2012$ g·mol⁻¹ from the manufacturer. Functionalisation of these polymer chains was achieved by bromine nucleophilic substitution of the hydroxyl groups at the terminal ends of the polymer (PEG-Br)(Figure 11). A mixture of PEG-OH (20 g, 0.01 mol), Et₃N (1.77 g, 0.03 mol), CH₂Cl₂ (50 mL) was degassed with argon for 10 min before dropwise addition of 2-bromopropionyl bromide at 0 °C. The reaction mixture was warmed with stirring for 20 h. The product was filtered from salt precipitates, and the aqueous-organic phase was separated with 1,2-dichloromethane (CH₂Cl₂) and water aliquots. Further filtration and drying of the organic phase resulted in a powder product of PEG-Br. Anhydrous CH₂Cl₂ (100 mL), ethyl potassium salt of xanthate X₁ (3.75 g) and 16.7 g of PEG-Br were added to a flask and left to react at room temperature (RT) for 17 h. The KBr salt precipitate was filtered under reduced pressure to remove solvent and after precipitation by filtering aliquots of pentane (3 x 200 mL) the PEG-X₁ product (B₁) was obtained as a beige powder[50].
2.1.2.3 Block Copolymerisation

Homopolymer from the previous step (2.1.2.2) is extracted and purified from their reaction pots, and used as a macro-RAFT agent for copolymerisation with monomer of the second block species. The final step in the RAFT/MADIX copolymerisation involves introducing this second block species to the B1-end functionalised homopolymers. A typical procedure for this synthetic reaction is given in the example of PAM-b-PVPA as follows. PAM_{n-XA1} (B1 at 58.80 g, 36% w/v aqueous solution), VPA monomer (M2 at 6.70 g) and 2,2'-azobis(isobutyramidine) dihydrochloride (AIBA at 0.40 g) was placed in a Schlenk flask and degassed by bubbling argon through the solution for 30 min with heating to 65 °C. Magnetic rods stirred the solution while being thermostated in an oil bath for 8 h. After this period, the flask was removed from the heating bath and the reaction halted. Polymers were separated from the mixture by precipitation from methanol, the sample was dried under vacuum and collected as PAM-b-PVPA with a VPA monomer to PVPA block copolymer conversion degree of 36%.

A similar synthetic procedure was undertaken for all other polymeric samples in this work, some samples like PEG and PVP-based homopolymers were purchased presynthesized from manufacturer and underwent end-functionalisation with the MADIX transfer agent before moving onto block copolymerisation.

2.2 METHODS

2.2.1 Capillary Electrophoresis (CE)

Separations for all PAM-based copolymers and PVPA homopolymer samples were performed by Master Candidate Michael Horgan, Bachelor Honours Student Melissa Meinel and Bachelor Student Katie Tran. For PVP and PEG-based copolymer samples separations were performed by Melissa Meinel and Lucie Seiler respectively. A wide variety of DHBCs were examined in this work using several capillaries and chemical standards for optimisation of the CE experiments.

2.2.1.1 Free Solution CE Procedure

Preconditioning of the capillary was conducted with a 10 min flush of 1 M NaOH, continued by a 5 min flush of each solution in the following order: 0.1 M NaOH, Milli-Q water and sodium borate (NB) buffer. To clean the fused-silica capillaries between each sample injection the following the protocol was applied: 2 min flush with 1 M NaOH, then 5 min flush with NB buffer. After each experimental series (completed sample separations) the
capillary was post-conditioned with a final cleaning and drying step: 1 min flush with 1 M NaOH, then 4 min flush with 0.1 M NaOH, then 10 min flush with Milli-Q water and aired dried for 10 (no injection only empty vial).

All BC samples were injected and separated in an Agilent CE 7100 (Agilent Technologies Waldbronn, Germany) using a Diode Array Detector (DAD) set to monitor 195 nm signals with a 10 nm bandwidth. Unless stated otherwise all electropherograms are displayed at 195 nm. Preliminary CE experiments were carries out in a capillary with total length \( l_t \) of 90.0 cm (81.5 cm length to detection window \( l_d \)) for PVPA homopolymer samples. Calculations and measurements for other homopolymers and BCs were performed on adjusted capillaries with lengths \( l_t \) of 99.5 cm \((l_d = 91.0 \text{ cm})\) and 100.0 cm \((l_d = 91.5 \text{ cm})\). Vials prior to separation were thermostated at 25 °C and samples injected hydrodynamically by applying 30 mbar of pressure for 5 s. Sample injections were followed immediately by buffer solution injection in the same manner. Standard separation conditions were achieved for all samples: electric field strength \( E \) of 30 kV with a cassette air temperature of 25 °C. Sample injection concentrations were 5 g·L\(^{-1}\) unless specified otherwise. The raw data for separations was acquired using the Agilent packaged software Chemstation A.10.01, while plots, transformations, corrections, and post processing were carried out using Origin Pro 9.1 (OriginLab).

### 2.2.1.2 Buffer Preparation

A Stock buffer solution (100 mL) of Sodium borate (NB) buffer was prepared at a concentration of 500 mM by dissolving 3.09 g (0.05 mol) boric acid in 75 mL Milli-Q water. This solution was titrated to pH 9.2 with 1 M NaOH and diluted with Milli-Q water to a final volume of 100.0 mL. Each of the desired solution concentrations were prepared from the stock to a concentration of NBx, where x = concentration in mM. Buffer solutions were sonicated for 5 min and filtered into CE vials using 0.22 µm PES membrane filter syringes, ready for injection.

### 2.2.1.3 Sample Preparation

Polymer samples were prepared from each of the materials listed in Table 1 by dissolving approximately 5.0 mg of sample in 1 mL of Milli-Q water. The desired injection concentration of sample, if different from the initial concentration of 5 g·L\(^{-1}\), was obtained by serial dilution. These solutions were injected and analysed in CE using different NB buffer concentrations: 25, 110, 200 and 300 mM. All samples were loaded into CE vials (total volume 500 µL) ready for injection. Before starting sample separations an injection of
oligo(acrylic acid) (oligoAA) with an average degree of polymerisation of 3 subunits (AA3) was measured in each buffer standard, the same injection of AA3 was then performed after separation of polymer samples and electropherograms of each compared. This AA3 sample was prepared similarly to the polymer samples described earlier. Perturbances in the baseline as well as any non-repeatable behaviour in the CE separations such as significant heating issues or loss in UV detector sensitivity is determined via the injection of this standard.

2.2.1.3.1 Homopolymer Calibration Curve

A calibration curve was constructed from RAFT polymerised PVPA homopolymers with $M_n,th$ of 500 and 2000 g·mol$^{-1}$. These were used to quantify the residual homopolymer content in the BC samples. The standard concentrations used were as follows: 0.5, 1, 3, and 5 g·L$^{-1}$ dissolved in Milli-Q water. Where needed 1 and 5 µL of DMSO was added to the 400 and 450 µL volume respectively to mark the EOF of the separation. In some cases, 30 µL of methyl green was added to a total sample volume of 420 µL to mark a known electrophoretic migration time of the charged molecule where DMSO is not applicable. A buffer of Sodium borate with concentration of 200 mM (NB200) and pH 9.2 was utilised for separations as per procedures outlined in previous CE of polyelectrolytes[113].

Similarly, a calibration curve of PEG homopolymers was constructed with $M_n,th$ of 2000 and 5000 g·mol$^{-1}$ respectively. The standard concentrations used are as follows: 0.1, 0.5, 1, 3, and 5 g·L$^{-1}$ dissolved in Milli-Q water injected with buffer concentration NB200. Similar injection conditions as above were used.

2.2.1.3.2 Block Copolymer Samples

The separation conditions for all DHBCs were as follows: Polymer sample concentrations were made up to 1 and 5 g·L$^{-1}$ in Milli-Q water with buffer concentrations of NB110 and NB200, polymer concentrations of 5 g·L$^{-1}$ were also used with buffers NB25 and NB300 for all PAM and PVP containing BCs. PEG-$_b$-PVPA samples were only made to a concentration of 5 g·L$^{-1}$ in Milli-Q water and injected with buffer concentration NB200. In order to mark the homopolymer impurity peaks in the DHBC separations, samples were spiked with 5% and 2% (w/v) PAM and PVP respectively.

2.2.2 Data Treatment and Analysis

All experimental data collected from Chemstation A.10.01 software was transferred into Origin Pro 9 for treatment and analysis. Data processing was conducted by Master Candidate Michael Horgan and Bachelor Honours Student Melissa Meinel. Migration time
(\(t_m\)) and UV absorbance were plotted using the software’s built-in functions and graphing tools. For conversion of \(t_m\) and UV absorbance into electrophoretic mobility distributions a standard template using Eq. (8 and 9) to calculate \(\mu\) and \(w(\mu)\) respectively was utilized. This file has been developed by the Macromolecular Characterisation team at Western Sydney University.

Converting electropherograms of block copolymer separations into a form that can be analysed in terms of the distribution of compositions and purities is the primary task of data treatment in this work. Some amount of post-signal processing is necessary to recover accurate results from the raw data, as such baseline treatment as well as peak-picking were optimised for analysis of the samples.
Chapter 3 – Determination of Block Copolymer Purity

In order to better the development of DHBCs and BCs in general we need to establish the purities of these materials as produced through RAFT/MADIX block copolymerisation. Understanding the products of such techniques is a step towards improving the synthetic process. This chapter will focus on characterising the homopolymer precursors as well as residual content in the BCs of interest. The heterogeneity of compositions and quantification of residual homopolymers in PVPA-based DHBCs are assessed by CE-CC.

3.1 Characterising Homopolymers

CE in these ‘Critical Conditions’ (CE-CC) was performed on both charged and uncharged homopolymers of the DHBCs of interest (Figure 3) to identify the species present in the samples as well as quantify the residual impurities in the BCs. Separation of polymers in CE is related to their charge density, since the BCs of interest contain blocks with charged monomer subunits the relationship between charge and \( \mu \) directly infers the degree of charged monomer conversion. Throughout this section CE-CC is optimised for the separation of homopolymers and their respective block copolymers.

3.1.1 Free Solution CE-CC: Assessing the Electrophoretic Mobility of Homopolymers

Homopolymers of charged PVPA, neutral PEG, PAM and PVP were separated by CE-CC in this work. These species were compared and the separation conditions optimised according to the effective capillary length, background electrolyte (BGE) and sample concentrations. A comparison of different chain lengths for PVPA and PEG homopolymers was conducted showing the difference in electrophoretic mobilities (\( \mu \)) with monomer conversion, the critical conditions for each of these homopolymer sizes was compared and components quantified for their distributions.

3.1.1.1 Separation and Identification of PVPA Homopolymers

From the electropherogram in Figure 12-(A) the separation of PVPA at 500 g·mol\(^{-1}\) (PVPA 0.5K) and 1950 g·mol\(^{-1}\) (PVPA 2K) is found around migration times of 28.6 and 21.9 min respectively. A number of other signals are present in the sample that appear before and
after the migration time of the EOF marker ($t_{EOF} = 7.9 \pm 0.06 \text{ min}$) including a large narrow signal that appears just after the migration time of homopolymer ($t_{Homo}$) suggesting a highly negatively charged species with low hydrodynamic friction. The small spikes that appear before the EOF are particles with very high mobility. These peaks are too small to be quantified and are either contamination, salts from the buffer that have aggregated, or injected air bubbles, this is likely to occur where solutions are not appropriately filtered or buffers sonicated before injection. For the small peaks between $t_{EOF}$ and $t_{Homo}$ most appear to be signal noise, but some of these also align with corresponding peaks in both 0.5K and 2K samples. These small repeated peaks after the EOF are possibly oligomers of VPA with increasing DP moving towards the homopolymer peak. As expected the peaks are more pronounced in the higher molar weight homopolymers given the kinetic increase in side reactions, chain termination events and loss of monomer during polymerisation. These impurities (called here OligoVPAs) were not quantified as the peak areas were too small to measure by standard concentration curves. Area calculations of these small peaks around $\mu$ of $1.5 - 3 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$ were performed and found to be less than 0.01 % w/w of the total area under the curve of the sample, suggesting quantities much less than 0.5 g·L$^{-1}$ present. What can be stated is the presence of impurities within the samples, both at high and low peaks which this work examines more precisely by the samples electrophoretic mobilities ($\mu$).
Figure 12. (A) Electropherogram of PVPA by free solution CE-CC in NB200 buffer with high sensitivity capillary ($l_s = 90, l_d = 81.5$ cm). Sample was dissolved in Milli-Q water to 3 g·L$^{-1}$. 5 μL added DMSO to the samples volume was used to visualise the EOF, seen in electropherogram around 8 min. (A) PVPA homopolymer appears around 26 ± 3 min. (B) An impurity appears around 8 min. Dashed lines indicate sample repeat experiments. (B) $W(\mu)$ of...
electropherogram corresponding to (A) where the x-axis is presented as the absolute magnitude of electrophoretic mobility.

Relative $t_m$ does not provide strongly repeatable separations as such the raw electropherograms were transformed as per Section 1.3.3.3 into $W(\mu)$. The homopolymer peak maxima were compared between 0.5K and 2K samples, a slight shift towards lower $\mu$ was observed for an increase in $\bar{M}_{n,\text{th}}$ of PVPA. Different injection concentrations of the homopolymer were examined and large tailing resulting in a triangular shape peak in the 2K samples at high concentration ($\geq 3 \text{ g} \cdot \text{L}^{-1}$) occurred in Figure 13-(B) suggesting significant electro-migration dispersion (EMD) affecting the analyte. A comparison of the $W(\mu)$ found no difference between the end-points of $\mu_{\text{Homo}}^{0.5K}$ and $\mu_{\text{Homo}}^{2K}$ peaks after increasing the concentration of sample. It can be inferred the homopolymers are sufficiently large enough at 2000 g mol$^{-1}$ to be in the critical conditions for these experiments. This validates the respective block copolymers separations being in the critical conditions as the chain length is much greater in those samples. However, the broadness of the analyte peak caused by tailing is likely to have skewed the position of $\mu$ at the mean and median weight fractions of homopolymer mobilities, area calculations can be affected that lead to imprecise standard concentration curves. Different buffer solutions were then tested on homopolymer and block copolymer samples to find optimal separation conditions as well as reduce viscosity and joule heating effects. These optimisations are further expanded upon in Section 4.1.3.1. Using the best conditions in these experiments an optimised separation protocol was developed for the analysis of the corresponding block copolymer samples.

![Graph](image)

**Figure 13.** CE separation of PVPA homopolymers at different concentrations in NB200 buffer in fused silica capillary ($l_t = 90.0 \text{ cm}$, $l_d = 81.5 \text{ cm}$). Graphs show the absolute mobility in the $W(\mu)$ of PVPA with $\bar{M}_{n,\text{th}}$ of (A) 525 g mol$^{-1}$ and (B) 1950 g mol$^{-1}$. 

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In all homopolymer separations there appeared a large sharp peak around \( \mu = 4.1 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1} \), this species has high mobility and given the narrow, triangular peak shape is likely very mobile. Initially assigned as an impurity, the peak was found to correlate with a separate injection of VPA monomers and has been assigned this species (Figure 14). Interestingly this result shows that the absolute value of \( \mu \) for homopolymers of VPA is a decreasing function of DP approaching the critical conditions. Unlike another anionic species, the absolute value of \( \mu \) for homopolymers of AA is an increasing function of DP. These results for predicting the \( \mu \) of PVPAs of different length appear to be inversely proportional to the model presented by Hervé Cottet and Pierre Gareil [99]. This may be explained by the high charge capacity of the phosphonic acid \((\text{PO(OH)}_3)\) functional group, greater concentrations of the neighbouring \(\text{PO(OH)}_3\) groups along a higher DP and more rigid polymer backbone result in greater electrostatic friction between chains. Thus a decrease in the mobility of the homopolymer is thought to correspond with lower DP under a critical threshold.

Figure 14. Comparison separations of PVPA 2K homopolymer (Red) and VPA monomer (Pink dashed line) both injected with NB200 buffer in fused-silica capillary \((l_c = 90.0 \text{ cm})\).
It should be noted that the injection of VPA monomers in the above graph was overloaded at a concentration of 1.5 g·L\(^{-1}\), further injections at different concentrations are necessary to quantify this species in the homopolymers.

### 3.1.1.1 Injection Parameters and Interpreting Peaks in \(W(\mu)\)

Samples were injected into the capillary at concentrations from 0.5 to 5 g·L\(^{-1}\) as shown in Figure 13. For PVPA 0.5K samples the peak shape and area increased linearly with concentration. The peak maximum (\(\mu_{\text{max}}\)) did not deviate by more than 1 % standard deviation (SD) until concentration > 3 g·L\(^{-1}\). This high concentration also coincided with a shift in peak shape no longer following the linear increase in area. Similar observations were made of the PVPA 2K samples for concentrations above 1 g·L\(^{-1}\). The comparison of peak areas and \(\mu_{\text{max}}\) for each concentration are displayed in Table 2. It is noted that the change in peak area is dissimilar between high concentrations of PVPA 0.5K and 2K samples, at 5 g·L\(^{-1}\) the former appears to overload the detector while the latter becomes heavily shifted likely due to EMD. This is an expected result as the longer PVPA chains will experience greater counter-ion condensation and take on a coiled conformation, whereas the shorter chains remain as rod-like oligoelectrolytes in the BGE. A number of sharp peaks appeared overlapping the main PVPA 0.5K peak. These peaks likely correspond to different DPs and tacticity’s of oligoVPA similar to the peaks found in separation of oligoAAs by CE[121]. In the larger 2K sample these peaks are barely visible > 3 g·L\(^{-1}\) sample concentration, implying a lower weight fraction of these chains in the sample and DP of the homopolymer closer to the target concentration assuming theoretical \(\bar{M}_{\text{n,th}}\). If EMD is considered these oligoVPA peaks may not be well resolved from homopolymer peak, thus assumptions about the average and distribution of molar masses may be even less accurate.

#### Table 2. Comparison of the homopolymer peak measurements in Electropherogram (\(S(t_m)\)) and Electrophoretic mobility distribution (\(W(\mu)\)) plots. *n.d. = not determined due to lacking resolvable homopolymer peak.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Concentration (g·L(^{-1}))</th>
<th>Peak Maximum (n = 3)</th>
<th>Peak Areas (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu_{\text{max}}) (x10(^{-8}))</td>
<td>RSD (%)</td>
<td>(W(\mu)) (x10(^{-7}))</td>
</tr>
<tr>
<td>PVPA</td>
<td>0.5</td>
<td>3.67</td>
<td>0.3</td>
</tr>
</tbody>
</table>

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From these concentrations of PVPA a calibration curve can be generated for the quantification of residual homopolymers that separate from a block copolymer sample. This also provides a visual determination of the effect EMD and overloading has on the homopolymer peak at different sample concentrations in CE.

The presence of short chain oligomers as well as different migrating species around the PVPA peak show a disperse arrangement of polymers, oligomers and monomers within the homopolymer samples. Though the weight percentage for these impurities were not determined, the $M_n$ values provided by the manufacturers, theoretical and experimentally determined, are likely inaccurate. The $M_{n,NMR}$ determined by $^{31}$P NMR spectroscopy which was capable of distinguishing quantitative chemical shifts of the VPA and VPA-X1 functionalised monomers, however chemical shifts for PVPA of different DP overlapped and distinct oligoVPA chemical shifts were not observed[177]. Thus $M_{n,NMR}$ of the PVPA homopolymer samples is considered in this work to be overestimated from the true $M_n$. Alternatively the value determined by SEC-MALS $M_{n,MALS} = 1420\ \text{g}\cdot\text{mol}^{-1}$ was much higher than both $M_{n,th}$ and $M_{n,NMR}$, which is not congruent with either measurements by NMR or the distribution of peaks in CE observed in this work.

### 3.1.1.1.2 Assessing the critical conditions in the CE of homopolymers

The critical conditions in CE are defined in Section 1.2.2.3.4 as the point by which separation of analyte occurs independent of molar mass. In this work the limit or minimum DP in a polyelectrolyte determines at which point these critical conditions are met. Using the $M_n$ of homopolymers provided in Table 1 a threshold can be determined from the CE separations where $\mu$ of the PVPA peak changes little with DP. To evaluate the point at critical conditions, we observe a behavior of $\mu$ as a function of $M_n$.
conditions with the limited homopolymer samples available an arbitrary threshold of 5 %
decrease in magnitude of $\mu$ was established. From Table 2 a shift in the PVPA peak of 4.9 %
was observed at injection concentrations of 3 g·L$^{-1}$ between $\mu_{\text{max}}$ of $3.71 \times 10^{-8}$ m$^2$·V$^{-1}$·s$^{-1}$
(PVPA 0.5K) and $3.53 \times 10^{-8}$ m$^2$·V$^{-1}$·s$^{-1}$ (PVPA 2K). This concentration was selected to avoid
significant bias by EMD and retrieve the strongest possible peak for the homopolymer, a
comparison $W(\mu)$ for each homopolymer is provided in Figure 12-(B). Accordingly a
threshold for the critical conditions was evaluated at a DP > 16 monomer units. This value is
very close to those obtained from PAA homopolymers similarly produced by RAFT/MADIX
polymerisation[39].

This DP threshold can also be described in terms of the minimum chain length at the
‘critical conditions’ ($L_n^{CC}$) otherwise considered where molar mass is negligible. The chain
length as VPA subunits calculated from the theoretical molar mass, $\bar{M}_{n,\text{th}} = 16$ subunits, was
compared to the chain lengths calculated from $\bar{M}_{n,\text{NMR}} = 18$ subunits and $\bar{M}_{n,\text{MALS}} = 25$
subunits respectively. Some impurities were detected by CE that may cause significant
heterogeneity in the distribution of hydrodynamic volumes as such the MALS determined $\bar{M}_n$
may not provide accurate measurements to calculate $L_n^{CC}$ for the PVPA homopolymers[69].
Similarly as discussed in Section 3.1.1.1.1 detected oligoVPAs suggest a heterogeneous
homopolymer which could cause overshoot in $\bar{M}_n$ determined by $^{31}$P NMR, though a
quantitative measurement of total polymerised VPA may be obtained by this technique,
meaning a closer estimation of $\bar{M}_n$ than obtained by SEC-MALS. Regardless of which values
are taken all block copolymer samples in this work are sufficiently larger in chain length than
the minimum needed for separation in the critical conditions.

The bulky MADIX end-group (invisible to the $^{31}$P NMR detector) attached to the
polymer affects both the molar mass and the hydrodynamic friction of the chain in solution,
thus the true point at which critical conditions are met is likely a few monomer units more
than the value given above. Future research combining separation techniques with a molar
mass detection method such as coupled CE-MS[121] may provide useful information about
the ‘critical conditions’ and possible pathways to better characterisation of unknown
polyelectrolyte samples. This is outside the scope of this work and estimates are only used
here to support measurements of block copolymers by CE-CC.
3.1.1.2 Characterisation of Neutral Homopolymers

Unlike the charged PVPA homopolymers neutral species typically migrate through the capillary with the EOF and thus do not separate from each other. However some homopolymer species with no net charge are capable of forming complexes or pseudo-ions in the buffer solution[120]. The following neutral homopolymers were separated in CE-CC and measurements of the electrophoretic mobilities were conducted on some of these materials.

3.1.1.2.1 Poly(ethylene glycol) (PEG) homopolymers

PEG polymers synthesised by polycondensation of ethylene glycol monomers were purchased and end-functionalised using the MADIX transfer agent XA1 by PhD student Lucie Seiler (Figure 11). A similar polymer with the same chain composition is poly(ethylene oxide) (PEO). Of the two polymer species PEO generally has a narrow distribution of compositions compared to PEG which can express much broader distributions. To improve the RAFT polymerisation technique of these and other hydrophilic homopolymers as well as related block copolymers, PEG was characterised in CE-CC as shown in Figure 16 below.

![Figure 16](image_url)

**Figure 15.** Distribution of PEG samples by free solution CE-CC in NB200 buffer with high sensitivity capillary ($l_t = 100$, $l_d = 91.5$ cm). Sample was dissolved in Milli-Q water to 1 g·L$^{-1}$.
and 5 g·L\(^{-1}\). A third injection of PEG with equal components \(N\)-hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide at 5 g·L\(^{-1}\) was run at the same conditions as the previous injections. An impurity appears at 1.05 x 10\(^{-8}\) m\(^2\)·V\(^{-1}\)·s\(^{-1}\). Dashed lines indicate sample repeat injections. Sample \(W(\mu)\) have been stacked to better visualise the position of peaks.

From the above \(W(\mu)\) graph the CE of neutral homopolymer PEG at 1000 g·mol\(^{-1}\) (PEG 1K) at a concentration of 5 g·L\(^{-1}\) was performed. The peaks observed at \(\mu\) of 3.2 x 10\(^{-9}\) m\(^2\)·V\(^{-1}\)·s\(^{-1}\) and 1.1 x 10\(^{-8}\) m\(^2\)·V\(^{-1}\)·s\(^{-1}\) were visible in all sample injections and repeat injections produced near identical peaks. At different injection concentrations the peak with lower \(\mu\) changed in \(w(\mu)\) (Figure 16) while the higher \(\mu\) peak did not change significantly (Figure 15). The coupling agents NHS and EDC-HCl were used in the functionalisation of PEG homopolymers with MADIX agent by a similar method to grafting peptides onto chitosan[112], the polymer has no charged functional groups but several peaks appear in the \(W(\mu)\) around the \(\mu\) of the EOF marker. To confirm the position of the homopolymer peak and identify any remaining coupling agents a third sample injection spiked with NHS and EDC was injected into the capillary. An additional peak appears roughly at around the EOF and a very strong peak appears before the EOF at an absolute \(\mu\) of 1.4 x 10\(^{-8}\) m\(^2\)·V\(^{-1}\)·s\(^{-1}\). Considering the \(pK_a = 7.8\) for NHS[179] this molecule will be strongly negatively charged in the buffer solution and does not correspond to either peaks. It is more likely that the high mobility peak corresponds to the positively charged EDC-HCl as it appears in CE by Taylor and Thevarajah (2015). However the peak at the EOF corresponds to a net neutral species such as the EDC-PEG homopolymer intermediate before the functionalisation with the MADIX agent. If this is true then the amount of functionalised homopolymer can be quantified by CE, quantitative compositional analysis such as NMR may confirm the presence of these intermediates. Given the lack of this peak in the functionalised PEG homopolymers it is likely that all PEG chains were converted into PEG-XA. Interestingly another peak with higher mobility than the others is apparent in all PEG sample injections at \(\mu\) of 1.05 x 10\(^{-8}\) m\(^2\)·V\(^{-1}\)·s\(^{-1}\). This peak area is repeatable between separations at different sample concentrations suggesting a strongly UV absorbing species at 195 nm wavelength but not concentration dependent. The peak has been assigned as an impurity from the synthesis of PEG.
Figure 16. $W(\mu)$ of PEG homopolymers at different injection concentrations in NB200 buffer in a fused silica capillary ($l_t = 100.0$ cm, $l_d = 92.5$ cm).

The peak for PEG homopolymers occurs shortly after the migration time of the EOF which indicates a slightly negative net charge of the polymers as they migrate through the capillary. Due to the strong hydrophilicity of the polymer it can be understood that hydrated positively charged ions are attracted to the surface of the polymer, especially the (CH$_2$ – O – CH$_2$) ether functional group in the chain[180]. As the surface becomes coated in a layer of sodium ions and other cations, the borate anions will form a diffuse bilayer around the polymer chains.

Table 3. Table of electrophoretic mobilities for PEG 1000 g·mol$^{-1}$ (PEG 1K) samples at different concentrations. Measurements were taken from $W(\mu)$ for $\mu_{Homo}$ peak seen in Figure 16.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Concentration (g·L$^{-1}$)</th>
<th>Peak Maximum ($n = 4$)</th>
<th>Peak Areas ($n = 4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\mu_{max}$ (x10$^{-9}$)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>
PEG peaks display a slanted triangular peak shape in $W(\mu)$, this may be the result of a left shoulder peak most visible in the $2 - 3 \text{ g}\cdot\text{L}^{-1}$ concentration range. These two peaks may represent different DP populations of the homopolymer. There are too few repeat injections ($n = 4$) to support this assertion outright and further injections of different DP PEG samples would be necessary to determine the mobility of each of these peaks. Confounding variables may also have an effect on the separation such as baseline shift between injections may change the peak profile. Regardless of the separation each displayed high repeatability with area calculations $\pm 2.3 \times 10^{-3}$ standard error. Alongside this trend the position of the $\mu_{\text{max}}$ is observed to increase with sample concentration until steadying $> 4 \text{ g}\cdot\text{L}^{-1}$. This corresponds with the change in start and end points at the base of the peak (width at full height) (Figure 16) reaching a constant at high concentration, this implies a reproducible and narrow $W(\mu)$. The distribution of $\mu$ for each of these polymers is assessed in detail in Chapter 4.

### 3.1.1.2.2 Polyacrylamide (PAM) homopolymers

Polyacrylamide (PAM) is a major component in the design and engineering of water-treatment facilities. Its robust physical properties give PAM excellent flocculant and filtration ability depending on the product it is geared towards[181]. This includes ionic[182], non-ionic[183], and Mannich-modified[184] varieties of PAM used in stabilizers or colloidal systems. These functions are highly desired in the synthesis of neutral-anionic block copolymers and previous work has investigated PAA-based BCs containing the non-ionic form of PAM by CE-CC[39]. In Sutton (2014) separation of PAA-$b$-PAM samples demonstrated possible detection of the PAM homopolymers however in most cases it was not characterised as the analyte peak heavily overlapped with the neutral EOF marker DMSO. PAM is ubiquitous within the polymer manufacturing industry and an increasing body of research involving the species in block copolymer development may see it entering new markets, however a similar lack of characterisation of this species with other homopolymers found in such products needs to be addressed.
In this work marker was not added to some sample repeats allowing PAM homopolymers to be characterised. CE separations of PAM-based neutral-anionic block copolymers were undertaken in the critical conditions, the homopolymer content corresponding to the uncharged PAM block species was detected in all samples (Figure 17). The PAM peaks occurred at a repeatable $\mu_{\text{max}} = 0.13 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ in NB200 buffer, however more than 50% of the right-half peak appeared convoluted with the start of the block copolymer peak in PAM(10K)-b-PVPA(1.5K) samples. It is noted that the position of the EOF was either taken in these samples at the apex of the water peak or mobility of DMSO in a blank directly preceding sample injection. The position of homopolymer peak $\mu_{\text{max}}^{\text{PAM}}$ was demonstrated to change with the concentration of the background electrolyte (BGE), as shown in Figure 17 (A) – (D) lower buffer concentration correlated with an increase in mobility. This trend continued until reaching a NB buffer concentration of 25 mM by which point the resolution of the PAM peak was very low due to large peak overlap with the block copolymer peak. The separations at this concentration were also performed with DMSO as such no separation of homopolymer and EOF marker was obtained.

![Graph A](image1.png)

![Graph B](image2.png)
Figure 17. $W(\mu)$ of residual PAM homopolymers from P(AM-b-VPA) block copolymer samples in NB: 25, 110, 200 and 300 mM. All separations are without DMSO except graph (A). CE performed in high sensitivity fused-silica capillary ($l_t = 99.5$ cm, $l_d = 81.5$ cm) at a sample concentration of $5 \text{ g} \cdot \text{L}^{-1}$. Full width at half $\mu_{\text{max}}$ (FWHM) is presented graphically for each resolved PAM homopolymer peak.

The PAM homopolymers used in the synthesis of the corresponding block copolymers have no net charge, despite this, the homopolymers clearly show a slightly negative mobilities. This characteristic has implications on the charge densities of the corresponding block copolymers in NB, as well as determining the distribution of their compositions by CE-CC. Separation conditions varied with buffer concentration, it was found that NB300 (without DMSO) provided the best resolution of PAM peak as well as the most stable baseline for area calculations. Future quantifications of the residual PAM homopolymer content by CE should be considered using the conditions established in this work.

3.1.1.2.3 Poly(N-vinylpyrrolidone) (PVP) homopolymers

The last neutral homopolymer and corresponding block species examined in this work is PVP. A highly adaptive non-toxic polymer, this material is used in the pharmaceutical and cosmetic industries for its strong adhesive properties[185]. At current PVP is used in many applications especially for its biocompatibility, potential application as smart complexes for fuel cells and drug delivery systems make the polymer a valuable research material[186]. PVP has found use in analytical CE similar to PAMs as matrix-like networks (gel) for DNA sequencing, however little characterisation of the species in copolymers involving the critical conditions[187] and no studies in CE are available in the literature. The material provides a good template for synthesis of smart DHBCs, as such some samples have been included in this methodology for analysis.
Homopolymers of PVP were characterised from the corresponding PVP(8K)-b-PVPA(0.5K) block copolymer samples in CE-CC. The same buffer concentrations were applied here as in Section 3.1.1.2.2 and the electrophoretic mobility distributions were compared in Figure 18. A range of mobilities around the EOF were observed for each PVP homopolymer peak typically from a $\mu$ of $0.05 - 0.1 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$, keeping in mind mobilities that come after the EOF marker are only negative when using the EOF as a reference point where no net charge is expressed by analyte. Since all polymer samples except PVP exhibit mobilities in this ‘negative’ region the $W(\mu)$ throughout this work has used the absolute value $\mu$ as the x-axis except in Figure 18 where this is used to illustrate the peak of the PVP homopolymer in relation to its net charge and mobility. The $\mu$ for the homopolymer peak decreased with lower buffer concentration. As can be observed from the graph, the full width of the peak at half maximum became increasingly wider with a decrease in maximum $w(\mu)$. This indicates a partial anionic net charge of the chains in low NB solution. However peaks appeared more than 80% overlapping so a comparison of the shift in $\mu$ with buffer concentration cannot be made with confidence from these results. Future investigations of lower buffer concentrations including standard concentration curves in these solutions will allow quantification of PVP homopolymers in the block copolymer samples.
Figure 18. $W(\mu)$ of PVP(8K)-b-PVPA(0.5K) block copolymer in different buffer concentrations, using high sensitivity fused-silica capillary ($l_t = 99.5$ cm, $l_d = 90.5$ cm). The above graph shows a section of the separation, focusing on PVP homopolymers. Full width at half $\mu$ max (FWHM) is presented graphically for each homopolymer peak. X-axis shows the actual sign of $\mu$ in relation to the EOF marker.

As with the other neutral homopolymer species the mobilities of the homopolymer peak occur shortly after the EOF for all samples. This result can be understood by the complexation of the BGE ions with the polymer chains producing partially negative net charges across all samples. Noted previously the charge composition distributions in the polymer chains play a significant role in determining the apparent electrophoretic mobilities of polyelectrolytes and DHBCs in CE-CC. When converting the electropherograms into distributions of compositions this aspect must be kept in mind for distinguishing truly neutral blocks from charged blocks.

P(AM-b-PVPA) samples were spiked with both PVP and PVPA homopolymers to identify the peaks in the separation of the block copolymers (Figure 19). The UV absorbance increased by 350 Wt% after the spike in the PVP peak but less than 6 weight% (Wt%) in the
PVPA peak. For the neutral homopolymer this change in $w(\mu)$ can be plotted as a 1 point calibration curve of concentration.

**Figure 19.** $W(\mu)$ of PVP(8K)-$b$-PVPA(0.5K) in NB200 buffer solution. Graph (A) shows block copolymer spiked with 2% (w/v) PVP K30, graph (B) shows the same concentration of block copolymer spiked with 5% (w/v) PVPA(0.5K).

### 3.1.1.3 Peak Broadening and Electrodispersion Correction

The fronting of the analyte as it passes through the capillary takes on an almost flat flow profile ([Figure 6](#)). Under these conditions the peak maximum corresponds with the greatest weight fraction of polyelectrolytes and can be taken as the electrophoretic mobility of the peak. However as mentioned earlier the peak shapes can be significantly distorted by differential diffusion of solute and background electrolyte (BGE) in the case of peak broadening by EMD. This occurs where counterions exhibit large differences in electrical conductivity ($\Delta\sigma$) at the solute peak[188]. The phenomenon, electro-migration dispersion (EMD), produces asymmetrical almost triangular shaped peaks as is demonstrated in the overlay of monomer and PVPA homopolymer separations in **Figure 14**: the monomer peak takes on this shape in both separations however the more concentrated pure monomer injection displays significant distortion in peak width as well as a greatly shifted $\mu_{max}$. Weight fractions of analyte moving across the horizontal of the peak are non-linear where EMD is apparent, this is the result of stable and unstable boundaries at either end of the peak profile. Differences in the conductivities of analyte and BGE result in a difference in $\mu$ for each ion species at the sample peak, these cause stacking of the sample ions at the tail end of the peak (stable boundary) whereas those ions at the front do not experience this effect and result in a more spread sample peak (unstable boundary)[189].
Several strategies are available for reducing the effects of EMD on the separation profiles for polyelectrolytes. Dilution of sample to reduce the stacking effects or introducing BGE counter-ions with similar $\mu$ to the analyte are potential pathways. Comparing the mobilities of the homopolymers examined in this work with the mobility of the complexing ions (borate) in the BGE, it becomes clear that some species will be more affected by EMD than others. The $\mu$ of borate is known to migrate at $1.719 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$ [190], this mobility is closer to the mobilities of the neutral homopolymer species than to the charged PVPA homopolymer. However to some extent the both will experience EMD, though PVPA and VPA ($\mu > 3 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$) are the furthest away from the mobility of borate and experience the greatest effects of EMD. An unexpected benefit of the borate buffer is that the block copolymers examined in this work appear to have the same $\mu_{\text{max}}$ as he buffer ions. As such no correction is necessary for block copolymer peaks. As mentioned earlier highly dilute samples are not typically affected by this type of distortion due to the lack of analyte molecules necessary for generating the boundaries. This is further benefitted by the fact that sample plugs are already very dilute and polyelectrolytes of sufficient size are visible even at these concentrations. Lowering the sample concentration will never the less result in decreased sensitivity of all species particularly any homopolymers present that are already highly dilute. Changing the composition of the BGE to express similar $\mu$ to analyte has its own difficulties as few species have identical $\mu$ and selecting for very disperse polymer samples would be tedious.

The band broadening that occurs from EMD affects the migration of analyte on one side of the peak, while the other is normally broadened by diffusion only. As such a relationship governs the distance between the apex of the stable boundary and the ‘actual’ electrophoretic mobility of the analyte peak graphically represented by the red line in Figure 19.
Figure 20. Illustration of EMD affected peak shapes in free solution CE. Dashed line represents the broadening due to diffusion, donated as distance $d$. Delta represents the broadening due to EMD. Bold red line indicates true $\mu$ of pure analyte[39, 191].

This correction to $\mu_{\text{max}}$ was applied to all homopolymer samples and the results compiled in Table 4. All corrected values except PEG had RSDs less than half that of the average untreated homopolymer peak value. In homopolymer peaks for PVPA 0.5K and PVPA 2K samples the standard deviation was the greatest at over $0.2 \times 10^{-8}$ m$^2$·V$^{-1}$·s$^{-1}$, this SD was much greater for 2K samples than 0.5K samples observed in $W(\mu)$ the effect of EMD is greatest at 5 g·L$^{-1}$. It is noted that EMD corrected $\mu_{\text{max}}$ for both samples was near identical (0.5K: 3.748, 2K: 3.753 x $10^{-8}$ m$^2$·V$^{-1}$·s$^{-1}$) even with the samples at different molar masses, which is indicative of the homopolymers being within the threshold of the critical conditions. Similarly PEG 1K samples also showed a change in mobility after correction, though the difference in RSDs is minimal the difference in SD after is less than half the SD before correction. In context the relative difference in mobility was less than 1% of the change in mobility observed for PVPA samples thus a quantitative increase of 45 % in repeatability is observed after correction for EMD. This is the result of the analyte peak having very narrow FWHM at low mobilities, changes in the mobility from EMD distortion appear magnified compared to broad peaks with high mobilities(Figure 15). PEG also migrates close but not at the EOF marker, this indicates that in the NB200 buffer solution the polymers have very low charge densities.

Table 4. Difference in $\mu$ for all homopolymer species analysed at NB200 buffer concentration. n represents the total number of sample injections (both repeats and different injection concentrations).
<table>
<thead>
<tr>
<th>CODE</th>
<th>Before Correction</th>
<th>After Correction</th>
<th>samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_{\text{max}} \left(10^{-8}\right)$</td>
<td>$\mu_{\text{max}} \left(10^{-8}\right)$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$(w(\mu)_{\text{max}})$</td>
<td>(EMD corrected)</td>
<td>RSD %</td>
</tr>
<tr>
<td>PVPA</td>
<td>3.625</td>
<td>3.748</td>
<td>0.9</td>
</tr>
<tr>
<td>0.5K+2K</td>
<td>3.9</td>
<td>0.9</td>
<td>24</td>
</tr>
<tr>
<td>PEG 1K</td>
<td>0.312</td>
<td>0.245</td>
<td>5.3</td>
</tr>
<tr>
<td>PAM</td>
<td>0.129</td>
<td>0.135</td>
<td>0.4</td>
</tr>
<tr>
<td>PVP</td>
<td>0.063</td>
<td>0.031</td>
<td>0.9</td>
</tr>
<tr>
<td>VPA</td>
<td>4.072</td>
<td>4.205</td>
<td>0.6</td>
</tr>
</tbody>
</table>

For the PAM homopolymers the change in $\mu$ is reduced between samples and replicates after correction of the peak maximum. This trend is also observed to be true for $W(\mu)$ of the homopolymers at different NB buffer concentrations. Corrected peaks were observed to be closer to the $w(\mu)_{\text{max}}$ ($\mu_{\text{max}}$ taken at maximum $w(\mu)$) with increasing buffer concentration, this correlated with a decrease in peak width at half maximum. The higher buffer concentration may cause greater ion condensation and narrower peaks in the PAM samples, depending on the complexation of borate with polymer this may reduce the effects of EMD in other species. The PVP homopolymers displayed a similar trend after correction in different NB buffer concentrations, however fewer samples were available to make comparisons. In all cases the correction of the $\mu_{\text{max}}$ for peaks occurring at different mobilities to the borate buffer ions is recommended. All further mobilities of the homopolymers or other species outside a $\mu$ of $1.7 \pm 1.5 \times 10^{-8}$ m$^2$V$^{-1}$s$^{-1}$ were corrected using this method.

3.1.1.4 Correcting Bias in Conversion of Electropherogram to Electrophoretic Mobility Distributions

It has been noted that the conversion of migration time to electrophoretic mobility appears to introduce a degree of error into the peak measurements, as this transformation compresses the distribution scale seen in Figure 21-(B).
Figure 21. Comparison from (A) migration time \((t_m)\) to (B) electrophoretic mobility \((\mu)\) distributions. Each peak has an area = 1, but increases in full width at half maximum (FWHM) by a factor of 0.25 in migration time.

From the above, artificial peaks (Gaussian curves) were used to represent the spreading of the analyte peak shape in the electropherogram. The actual peaks produced by CE are not typically symmetrically Gaussian except in cases with disperse analyte of similar mobilities like the block copolymers of interest. More common are sharp triangular peak shapes often observed in cases of electro-migration dispersion (EMD)[192]. It has been demonstrated that in ideal conditions a Gaussian fitting can accurately estimate the peak shape whereas other formulas are useful for determining the degree of distortion in the asymmetrical ‘triangular’ peaks[193]. Thus the Gaussian modelled peaks here can be used to observe the distortion that occurs in peak height as well as width before and after transformation to electrophoretic mobility.

At high mobility the transformation of UV absorbance (y-axis) to \(w(\mu)\) is parabolic with \(t_m\) (see Appendix 1). The blue peak in Figure 21 has an 80% decrease in area relative to all peaks after conversion from (A) electropherogram to (B) \(W(\mu)\). In samples with peaks below the baseline, due to UV drift or loss in sensitivity, this conversion can ‘squash’ the peak and increase the error in area calculations. This bias is addressed in Section 3.2.3.1.

3.1.2 Homopolymer Separation in CE-CC: Conclusions

A method for the separation and analysis of some charged and uncharged homopolymers was developed using CE-CC. These samples were characterised in terms of their electrophoretic mobility as well as assessing purity and speciation of the separated analytes. concentration of 3-5 g·L⁻¹ of sample in solution was needed to resolve the peaks of interest with high reproducibility \((n = 16, \text{ operators } = 4, \text{ RSD } < 12\%)\).
Apart from the analyte homopolymer peaks some other species appeared throughout all samples, included is the presence of side products and monomer reagents that remained in samples that had been purified prior to separation. These peaks were highly reproducible but were not quantified in this work. Future concentration calibration curves of these materials can be determined using the separation protocols established in this section. The characterisation of these homopolymers as reagents and residual content in the formation of BCs will improve our understanding of RAFT polymerisation for DHBCs.

3.2 PURITY OF BLOCK COPOLYMERS

Previous research into block copolymers has established that purity of these materials plays a significant role in determining their intermolecular structure, functionality and properties[57]. In Section 1.3.2 it was discussed how the phase separation and assembly of some BCs are dependent on the homopolymer content; however, the purities of such polymeric samples are rarely determined in the literature. Following on from Section 3.1 a standard method for assessing and quantifying these homopolymers in the corresponding block copolymer samples is undertaken. This work is an innovation in the characterisation of DHBCs by their polymer components particularly in terms of analysing homopolymer content.

3.2.1 Nomenclature of Homopolymers in BCs: A Treatise of Names

Throughout this work a series of naming conventions have been used to describe the various components of block copolymers and their constituent homopolymers. These find common usage as descriptors in the literature surrounding block copolymer analysis; however, standard nomenclature for the homopolymer content in these samples has not been established. As the presence of these compounds in block copolymer samples is not typically quantified, the terms used to describe them can vary depending on the synthesis, analysis and use of the polymers.

For the purposes of this work, a working definition of each type of homopolymer in a block copolymer sample has been devised from common terms in the literature[39]. These definitions relate in two key categories: the ‘Synthetic Perspective’ sees the homopolymers in terms of reaction schemes and theoretical products. In this regard homopolymers for each respective block species are called parent homopolymers and so diblock copolymeric species will have two parent homopolymers. Synthesis often looks to produce samples with the
highest purity and greatest yield, for the initial parent homopolymers that are not successfully converted into block copolymers these are termed residual homopolymers. The other category is the ‘Application Perspective’ which describes final block copolymer products by utility. In this case, any homopolymer impurities not desired in a sample are called unintended homopolymers.

3.2.2 Comparing Block Copolymers: Separating Unintended Homopolymers in PVPA-based BCs

Throughout this section PVPA block copolymers have been assessed of their separation in CE-CC using standard conditions established in the literature [40, 113]. These are further optimised per the conditions found in Section 3.1.2 for the resolution of homopolymer content in the respective BC samples.

3.2.2.1 P(AM-b-VPA) block copolymers

PAM is a well-known polymer that has characterised in the literature in terms of architecture and properties, impurities such as residual monomer content in PAM-based polymeric materials before and after degradation has been quantified by HPLC [194] though few papers have examined the residual homopolymers in block copolymers based on this material. These aspects of the polymer species make it an ideal candidate for research into charged DHBCs by CE-CC; where one block (PAM) is a neutral species with known characteristics the other charged block species (PVPA) is not as well understood in terms of synthesis and properties. This favours characterisation of the resulting block copolymers by the relationship of its charged components to its neutral components. Elucidating this relationship between the components as well as the parent homopolymer content from each block species provides a model for further research into different neutral blocks.

Block copolymers of P(AM-b-VPA) had been separated in CE-CC with complete resolutions of the DHBC and parent homopolymer content. The conditions used for this separation are seen in Section 2.2.1.1. For these conditions at a sample concentration of 1 g·L⁻¹ all block copolymers separated in under 25 min. The mobility of the block copolymers are displayed in Table 5. In Figure 22 the far left peak and far right peak are identified as the parent homopolymer species. PVPA homopolymers are charged in the solution and given the similar $\mu$ displayed in the PVPA 2K samples at 1 g·L⁻¹ (Figure 13) this species corresponds to the right peak at $\mu_{\text{Homo}}^{\text{PVPA}} = 3.2 \times 10^{-8} \text{m}^2\text{V}^{-1}\text{s}^{-1}$. Similarly, the small far left peak is found to correspond with the neutral parent homopolymer PAM at $\mu_{\text{Homo}}^{\text{PAM}} = 0.13 \times 10^{-8} \text{m}^2\text{V}^{-1}\text{s}^{-1}$.
Figure 22. $W(\mu)$ of PAM(5K)-b-PVPA(2.5K) block copolymer in NB200 buffer solution at a concentration of 1 g·L$^{-1}$. The block copolymer peaks demonstrate a bimodal distribution highlighted by a blue and red region with electrophoretic mobilities denoted by $\mu_{BC}$ respectively. The parent homopolymers for both neutral and charged species are marked by $\mu_{Homo}^{PAM}$ and $\mu_{Homo}^{PVPA}$.

The $\mu_{Homo}^{PVPA}$ peak area was considerably smaller than the peak area of $\mu_{BC}$, the relative difference in peak height is substantial enough that the presence of this peak would go unnoticed without a standard homopolymer to test for.

Table 5. Table of electrophoretic mobilities for P(AM-b-VPA) block copolymers at concentration 1 g·L$^{-1}$. Measurements were taken from $W(\mu)$ for $\mu_{BC}$ red highlighted distribution population in Figure 22.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Peak Maximum (n = 3)</th>
<th>Peak Areas (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_{max}$ (x10$^{-8}$)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>PAM(10K)-b-</td>
<td>1.22</td>
<td>1.14</td>
</tr>
</tbody>
</table>
For all block copolymers tested at a concentration of 1 g·L⁻¹ the presence of the neutral parent homopolymer (PAM) peak was found repeatable and strongly resolved at the EOF. This was less the case for the charged parent homopolymer (PVPA) peak. Both species were confirmed by first overlaying the homopolymer separation data with the block copolymer data, matching peaks by their electrophoretic mobility and then by spiking samples with the respective homopolymers. As indicated in Figure 22 the P(AM-b-VPA) block copolymers exhibited different electrophoretic mobilities to each of the parent homopolymers, this is explained in Section 1.2.2.3.1 as changes to the charge-to-friction ratio, such as additional hydrodynamic friction from the PAM block without the addition of charge, will lower the effective $\mu$ of the polyelectrolyte. Having successfully separated and confirmed the residual homopolymer content in the block copolymer samples, the conditions used here were applied for analysis of other block species.

### 3.2.2.2 P(VP-b-VPA) block copolymers

Separation of PVPA homopolymers from P(VP-b-VPA) block copolymers was carried out in CE-CC in the same conditions as tested on PAM-based block copolymer samples (Figure 23). One change was made to the separation protocol: samples containing the former PAM block species were found to produce reproducible and well resolved copolymer peaks, however PVPA homopolymer peaks were less visible at these concentrations. To improve the resolution of this peak all block copolymers including PAM-based samples were prepared to a concentration of 5 g·L⁻¹ for reinjection. In the separation of PVP(8K)-b-PVPA(0.5K) broad twin peaks were observed in the block copolymer distribution from $\mu$ in the range $0.25 - 2.45 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$ with peaks at $\mu$ of 0.93 and $1.05 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$. This $W(\mu)$ for P(VP-b-VPA) samples is similar to the bimodal block copolymer $W(\mu)$ seen in the separation of P(AM-b-VPA) polymers. These peaks correspond well with the block copolymer fraction of the sample.
Figure 23. $W(u)$ plot of PVPA(2K) (pink dashed line) and PVP(8K)-b-PVPA(0.5K) (blue), repeat injection of block copolymer (purple dashed line). Separations were performed in a high sensivity fused silica capillary with $l_d = 91$ cm (8.5 cm detection window) in NB200 buffer solution. Injections were all performed at sample concentrations of 5 g·L$^{-1}$.

PVP peaks were difficult to separate from the block copolymer content, at different buffer concentrations the homopolymer peak did not shift much however in NB25 buffer solution the $W(\mu)$ for the block copolymer peak was completely resolved from the homopolymer.

Table 6. Table of electrophoretic mobilities for P(VP-b-VPA) block copolymers at concentration 1 g·L$^{-1}$. Separations performed in NB200 buffer.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Peak Maximum $(n = 3)$</th>
<th>Peak Areas $(n = 3)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_{\text{max}}$ (x10$^{-8}$)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>PVP(8K)-b-PVPA(0.5K)</td>
<td>0.91</td>
<td>0.88</td>
</tr>
</tbody>
</table>
The ratio of charged to neutral block lengths is a dependent factor in determining the migration of neutral-anionic block copolymers. For all samples listed a total capillary length $l_t = 100$ cm was reasonable to separate all PVPA-based polyelectrolytes in the samples.

### 3.2.2.3 P(EG-b-VPA) block copolymers

P(EG-$b$-VPA) block copolymers were separated in CE-CC under standard conditions. The first set of CE separations did not resolve any peaks and often resulted in highly noisy baselines (Figure 24). SNR values were obtained by measuring the average peak height of noise at baseline as a percentage of sample peaks. For injections of PEG(5.5K)-$b$-PVPA(0.9K) the SNRs were > 23% at the block copolymer peaks and > 30% at the respective homopolymer peaks. These initial sample injections were performed in NB200 buffer at a 1 g·L$^{-1}$ concentration. A similar arrangement of block copolymer peaks was detected to those found in Sections 3.2.2.1 and 3.2.2.2, notably two populations of the block copolymers are identified (POP1 and POP2) each having lower electrophoretic mobilities than the respective peaks found in P(AM-$b$-VPA) and P(VP-$b$-VPA) separations. This is supported by observing a slightly lower PEG homopolymer velocity to the EOF (Figure 15), thus block copolymers containing this species will migrate after the EOF if there is no apparent polymer charge.

Table 7. Peak maximum for the block copolymer peak in P(EG-$b$-VPA) samples.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Peak Maximum (n = 3)</th>
<th>Peak Areas (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_{\text{max}}$ (x10$^{-8}$)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>PEG(5.5K)-$b$-PVPA(0.5K)</td>
<td>1.52</td>
<td>0.64</td>
</tr>
<tr>
<td>PEG(5.5K)-$b$-PVPA(1K)</td>
<td>1.96</td>
<td>2.30</td>
</tr>
</tbody>
</table>
Figure 24. \(W(\mu)\) of PEG(5.5K)-b-PVPA(0.5K) 1 g∙L\(^{-1}\) samples by CE-CC in NB200 buffer solution at 200 nm UV detection wavelength. Separation performed with high se fused silica capillary. Number of injections \(n = 3\). Baseline produced from blank sample.

The atypical baseline experienced in the separation above was found to be due to several factors affecting the CE efficiency these include: poorly filtered buffer solutions, insufficient calibration of CE and low sensitivity fused silica capillaries. Another issue brought up is the curvature of the baseline along the horizontal axis particularly after \(2.5 \times 10^{-8}\) m\(^2\)∙V\(^{-1}\)∙s\(^{-1}\). In all datasets this curvature has high repeatability implying no effect of buffer or medium on the UV absorbance. Instead it has been confirmed that a slightly above 0 average y-axis in the baseline of the electropherogram can exhibit large changes in the apparent baseline when this data is converted into the electrophoretic mobility distribution. At the y-axis intercept (\(\mu = 0\)) UV absorbance = 0.5 whereas in the weight distribution of mobilities at the intercept \(w(\mu) = 5\), an order of 10 difference in magnitude, moving along the x-axis this conversion factor is logarithmic. This offset was corrected by running a blank to produce a baseline (orange dashed line) for each sample injection. Injecting a blank for every consecutive sample can become tedious and increase post processing time. To improve the separation and detection of the block copolymers the samples were reinjected into a high
sensitivity fused silica capillary \((l_t = 100 \text{ cm}, l_d = 91.5 \text{ cm})\) at a concentration of \(5 \text{ g·L}^{-1}\) and UV absorbance signal was collected in the 195 and 290 nm wavelengths. These conditions produced highly repeatable and well resolved peaks for the block copolymer as well as elucidating the presence of both parent homopolymers (Figure 25) compared to the previous separation where neither were well resolved.

Figure 25. \(W(\mu)\) of PEG(5.5K)-b-PVPA(0.5K) \(5 \text{ g·L}^{-1}\) samples by CE-CC in NB200 buffer solution at 195 nm UV detection wavelength. Number of injections \(n = 3\). A high sensitivity capillary with \(l_d = 91.5 \text{ cm}\) (effective UV window length of 8.5 cm).

The RSDs for the \(W(\mu)\) of the homopolymer and block copolymer peaks is less than 0.5% and SNR values at the block copolymer peaks less the 0.02%. The parent homopolymers were well separated from the block copolymer analyte, small repeating peaks between mobilities of \(2.3 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}\) and \(3.4 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}\) were indicated as noise from the baseline, though given the consistent distribution between injections and polymer peaks may also be related to small oligoPEG-b-PVPA chains. It was noted that block copolymers appeared in two distinct peak populations (POP1 \(\mu_{\max}\) at \(0.7 \times 10^{-8}\) and POP2 \(\mu_{\max}\) at \(1.5 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}\)), for chains with larger PEG : PVPA block length ratios the
mobility of these polyelectrolytes is greater than those with smaller ratios. This results in a bimodal $W(\mu)$ thus different block copolymer compositions have been resolved in this separation.

### 3.2.2.4 Similarities Between BCs

It has been demonstrated that the residual homopolymer content in each of the DHBCs tested has been successfully separated by CE-CC. Other similarities are also apparent between separations of different block copolymer species. $W(\mu)$ plot of P(AM-b-VPA) in Figure 22-(B) shows the peak shape of the block copolymer takes on a bimodal distribution. Indeed this indicates that moving from the higher (left side) to lower (right side) electrophoretic mobility of the peak relates to the degree of PAM : PVPA block length conversion. Greater neutral polymer content to lower charged polymer content in a block copolymer will lead to a macromolecule with a lower net charge and/or higher hydrodynamic friction this results in a peak distribution closer to the EOF. The inverse also holds true for block copolymers with relative greater fractions of charged polymer content, these can be visualised in the figure below.
Throughout all DHBC separations a bimodal block copolymer peak or two distinct peaks were present in the plot distributions. This indicates that all samples had at least two significant composition populations of the block copolymers. These peaks are analysed in terms of their electrophoretic mobility and compositions in Section 4.1.

Another feature shared between all samples was a highly-repeated peak around $\mu$ of $1 \times 10^{-8}$ m$^2$·V$^{-1}$·s$^{-1}$. An overlay of several block copolymer and homopolymer injections all in NB200 buffer solution displays this peak between samples (Figure 27). As the peak occurs with similar area and $\mu_{\text{max}}$ values in both copolymer and homopolymer samples, it can only be concluded that the analyte species does not correspond with a copolymer. All polymer samples except PEG were synthesised via RAFT/MADIX polymerisation, the synthesis control molecule ‘MADIX agent’ is generated in situ during the polymerisation reaction in the
case of PVPA, PAM and PVP-based polymers (see Section 2.1.2.2) and caps the end of the nascent polymer chains (Figure 2). In the synthesis of PEG-based block copolymers, the corresponding homopolymer chains are functionalised by substitution of the hydroxyl-end group with the MADIX agent (Figure 11). It has been suggested that the peak highlighted below likely results from an impurity formed in the MADIX agent. This impurity appears to comigrate with the block copolymer particularly between the bimodal peaks. If the MADIX agent impurity were to form a complex with the block copolymer, each would simultaneously be detected by UV resulting in a greater absorbance signal and hence appear as a peak on top of the broad block copolymer peak. However the peak height indicates that only the impurity is detected at this region.

Figure 27. Stacked overlay of CE-CC separations of PAM, PVP and PEG-based block copolymers with PVPA 500 g·mol⁻¹ charged blocks, as well as homopolymers of PVPA and PEG (1000 g·mol⁻¹) all performed in NB200 buffer solution at concentration of 5 g·L⁻¹.
3.2.3 Quantification of Homopolymers by CE-CC: Establishing a Method of Analysis

It has been established in this work that parent homopolymers can be separated from their corresponding PVPA-based block copolymers in CE-CC. Having these species separated allows for their quantification in a block copolymer sample. This section builds upon previous research into the quantification of both cationic and anionic residual homopolymer species in free solution CE-CC[39]. Several anionic-neutral block copolymers were characterised by this method and optimisations were performed to improve the separation and detection of the homopolymer peaks for quantification.

A common strategy for the quantification of an analyte in solution is to generate a calibration curve of standard concentrations for the target species. As explained in Section 1.2 residual homopolymers have been assessed by different chromatographic techniques but their ability to separate these species from the desired block copolymers is limited and so standard methods for their quantification have not been well developed in the literature. In this work analyte peaks of each homopolymer are resolved enough to perform calculations based on the integral areas. For polymers with well-defined peaks in a CE separation the relationship between concentration and area under curve is described below:

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

(21)

where the actual concentration of the homopolymer \( (c) \) is the product of the injection concentration \( (c_{i\text{nj}}) \) and the relative fraction of homopolymer peak area to total peak area of all polymers that are composed of the analyte monomer species. Short chain oligomer species for PVPA polymers either did not appear at all or were not strongly resolved from baseline. Upon close inspection some small peaks appear at a higher electrophoretic mobility than the large homopolymer peak, which may account for the highly convoluted block copolymer peak that appears around \( \mu = 0.2 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1} \) in Figure 27. This peak likely has a lower charge density than the neighbouring block copolymers and given the proximity of this peak to the EOF is significantly lower in PVPA chain length than the rest of the block copolymer. It can be stated then that short chain oligomers with functionalised MADIX agent end groups are present in the parent homopolymer samples used in the synthesis of P(AM-b-VPA) block copolymers, however quantifying this content in the homopolymer samples may be difficult at such low concentrations. As such all area calculations have been conducted without measurement of oligomers.
### 3.2.3.1 Homopolymer Calibration Curve

To quantify the concentration of parent homopolymers in the samples calibration curves for PVPA and PEG were produced. The linearity of the calibration curves was tested and regression coefficients ($R^2$) were compiled in the following Table 8. It was demonstrated that homopolymers with different end groups, i.e. different theoretical molar masses, displayed dissimilar calibration curves. Matching the correct calibration curve to each of the block copolymers was conducted to prevent bias in purity measurements. Each precursor homopolymer calibration curve should be matched to their corresponding block copolymer with equivalent species block lengths, however the samples provided in this work did not match exact theoretical molar masses to the respective block chain weights. This does not necessarily invalidate the purity measurements determined in this work given the error in molar mass measurements as well as the expected average chain lengths likely to have formed the completed block copolymer, leaving the bulk content of homopolymer outside this weight distribution. To better facilitate purity measurements in the future by linear calibration fits more samples with differing molar masses need to be investigated.

Slight changes in the mobility of the homopolymers with an increase in molar mass have already been observed relating to the point at which the sample is under ‘critical conditions’ (see Section 1.2.2.3.4), the ∆Area between each of the homopolymer samples of different molar mass was compared with the SNR and RSDs for those calibration curves to determine whether significant changes in the molar mass affected the calculated purities.

**Table 8.** Linearity of the concentration calibration curves, limit of detection (LOD) and limit of quantification (LOQ) for CE-CC of PVPA and PEG homopolymers prior to baseline correction of the weight distribution ($w(\mu)$). Where $c$ is the concentration of homopolymer, $o$ and $n$ represent the start and end points on the analyte peak collectively representing the $y(c)$ function as an area value. Data below is before systematic baseline correction.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration range (g L$^{-1}$)</th>
<th>Equation: $\int_{o}^{n} W(\mu) d\mu$</th>
<th>$R^2$</th>
<th>Number of points</th>
<th>LOD (g L$^{-1}$)</th>
<th>LOQ (g L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVPA (0.5K)</td>
<td>0.5 – 5.0</td>
<td>$= 2.031c + 2.154$</td>
<td>0.951</td>
<td>8</td>
<td>0.017</td>
<td>0.137</td>
</tr>
</tbody>
</table>
The LOD from the above table was calculated from the slope plot of the SNR at the analyte peak against the injection concentration of the homopolymers. The limit was determined as the concentration when the SNR = 3. Accordingly a limit of quantification (LOQ) at SNR = 10 was also determined at > 0.13 g·L\(^{-1}\) for all homopolymers. These values are useful in gauging the bulk preparation of standard polymer solutions. All linear fits of the homopolymer standards produced R\(^2\) values greater than 0.92 however some of these plots especially the lower molar mass homopolymers did not display linear regressions above a concentration 3 g·L\(^{-1}\). PVPA homopolymers at \(\bar{M}_{n,th}\) of 500 g·mol\(^{-1}\) did not produce reliable concentration curves often appearing skewed at high concentrations, this has been suggested as an issue with the viscosity of the sample overloading the detector.

The signal-to-noise ratio of the baseline was not uniform across \(W(\mu)\), at \(\mu_{BC}\) peaks (SNR < 0.5%), whereas at the \(\mu_{PVPA}^{\text{Homo}}\) peak the relative SNR > 10%. This can be explained as the transformation function of migration time to mobility compresses the signal in a non-linear fashion along the x-axis, as such the actual noise magnitude remains the same across the dataset but the frequency increases with \(\mu\). LOQ had an RSD of 3.5% between replicates, thus effects from the migration transformation do not impact the measurements strongly. It is recommended to avoid these issues in baseline fluctuations in cases of high LOQs by keeping a constant cassette and capillary temperature, one method is decreasing the background electrolyte concentration thus reducing the effect of Joule heating during separation. Some of these controls were implemented in this work though no quantitative measure of the change was made, further investigations of their effectiveness are necessary.
All $W(\mu)$ of the homopolymer and block copolymer samples were baseline corrected, this was a necessary step as explained in Section 3.1.1.4 baseline drift affects to transformation of UV signal especially for peaks at high mobility such as the PVPA homopolymers. For more information about the baseline correction process see Appendix 3. These corrections were applied to all samples in a uniform fashion, the change in the slope of the calibration curves was negligible across all samples but RSDs $> 20\%$ between $\bar{\mu}_m$ repeat injections dropped to $< 8\%$. After baseline correction of the $W(\mu)$, the calibration curve slopes appeared to increase with $\bar{M}_{n,th}$ of the homopolymers as seen in Figure 28. This trend also coincides with the LOD increasing with molar mass. It has not been fully established why this may occur as the homopolymers are expected to have increasing degree of complexation with the borate buffer ions in the background electrolyte (BGE) per increase in molar mass, more chances for ion complexation should increase the detector response of the polymers as demonstrated by the larger peak areas in PVPA samples: 2K area $> 1K$ area $> 0.5K$ area for concentration above 1 g·L$^{-1}$. However the LODs from lowest to highest occur in the reverse order which suggests that the increase in detector response is not significant enough to out-weight detection of a smaller homopolymer from the background signal.

![Figure 28](image)

**Figure 28.** Concentration calibration curves of PVPA homopolymers in $W(\mu)$ for (A) Untreated $W(\mu)$ and (B) corrected analyte peak area.

### 3.2.3.2 Unintended Homopolymer Fraction in BCs

Block copolymer synthesis often results in the formation of dead homopolymer chains or unreacted functional homopolymers that did not form a block copolymer. These impurities can be either detrimental to the quality of the product or beneficial depending on the properties and desired intermolecular architecture of the block copolymers[195, 196]. Quantifying these species by CE-CC is possible where the block copolymers and
homopolymers are well separated and a linear calibration curve of standard concentrations of the homopolymer is available. Both have been achieved as shown in Sections 3.2.2.4 and 3.2.3.1, the fraction of homopolymers can then be determined as a weight or molar percentage of the sample using the following equations:

\[
\% (w/w) = \frac{w_H}{w_S} \times 100
\]

(22)

\[
\% (mol/mol) = \frac{w_H/M_H}{w_H/M_H + w_B/M_B} \times 100
\]

(23)

Here \( w_H \) is the weight of homopolymer, \( w_B \) is the weight of block copolymer and \( w_S \) is the total weight of sample. \( M_H \) and \( M_B \) are the \( M_n \), th of each polymer respectively. Areas under the curve of the analyte peak can be plotted into the corrected standard concentration calibration curve in Figure 28-(B). Using this concentration in the above equations, an experimental fraction of homopolymers for each sample can be obtained. In all block copolymers, there was found a small fraction of PVPA homopolymers as well as some amount of the neutral block species. Multiple standard curves were produced for quantification of PVPA homopolymers of different chain lengths, as such each calculated concentration has been compared in Table 9.

Table 9. Quantification of residual PVPA homopolymers in block copolymer samples.

Purities in weight and molar fractions are given below. The average of concentration values from each standard curve were used in purity calculations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration ( C_{H}^{n,5K} ) (g·L(^{-1}))</th>
<th>RSD (%) (n = 3)</th>
<th>Fraction of residual homopolymer ( % (w/w) ) (n = 9)</th>
<th>Estimated fraction of block copolymer ( % (w/w) )</th>
<th>Estimated fraction of block copolymer ( % (mol/mol) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAM(5K)-b-PVPA(0.5K)</td>
<td>( C_{H}^{1K} = 0.052 ) ( C_{H}^{2K} = 0.054 )</td>
<td>3.8</td>
<td>1.17</td>
<td>10</td>
<td>99</td>
</tr>
<tr>
<td>PAM(5K)-b-PVPA(0.5K)</td>
<td>( C_{H}^{0.5K} = 0.270 )</td>
<td>22.0</td>
<td>6.25</td>
<td>23.4</td>
<td>94</td>
</tr>
<tr>
<td>Polymer Combination</td>
<td>CH&lt;sub&gt;0.5K&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;1K&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;2K&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVPA(1.5K) + PVPA(2.5K)</td>
<td>0.326</td>
<td>0.175</td>
<td>0.168</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVPA(1K) + PVPA(0.5K)</td>
<td>0.342</td>
<td>0.381</td>
<td>0.830</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM(5K)-b-PVPA(0.5K)</td>
<td>0.725</td>
<td>0.910</td>
<td>0.326</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM(10K)-b-PVPA(1.5K)</td>
<td>0.890</td>
<td>0.910</td>
<td>0.342</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG(2K)-b-PVPA(0.5K)</td>
<td>0.053</td>
<td>0.056</td>
<td>0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG(2.5K)-b-PVPA(1K)</td>
<td>0.0575</td>
<td>0.0575</td>
<td>0.063</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG(5K)-b-PVPA(0.5K)</td>
<td>0.089</td>
<td>0.103</td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVP(8K)-b-PVPA(0.5K)</td>
<td>&lt;0.05*</td>
<td>&lt;0.1</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Area of peak too small to see difference in concentration, reaching limit of quantification (LOQ).
$C_H$ is the concentration of homopolymer calculated from standard curves in Figure 28-(B), each concentration is given in three values for each standard curve with PVPA molar mass of: 0.5K, 1K, 2K.

In all cases the PVPA homopolymer peak was well separated from the block copolymer peaks producing specific purities at an average of 94 % w/w for PAM-$b$-PVPA samples and 99% w/w for PEG-$b$-PVPA samples. In general the larger PVPA block lengths resulted in an increase of detected parent homopolymer, as expected longer chains and synthesis time would contribute to a greater statistical occurrence of these species. However it was found that larger neutral species block lengths correlated with an increase in purity as seen in PEG-based samples. Influence from a longer neutral block may result in a more stable macroRAFT molecule, promoting initiation of monomer to block copolymer conversion. This result is similarly observed between PAM(5K)-$b$-PVPA(1.5K) and PAM(10K)-$b$-PVPA(1.5K) samples, however in both cases the pool of samples ($n = 2$) is not sufficient to draw conclusions. Further investigation is required involving different neutral block species to determine whether this observation is shared for all or some block species. For PVP-based block copolymers none of these relationships can be determined given only one sample was available during injections. However like other samples with large neutral blocks ($n_U > 5.5Kg\cdot mol^{-1}$) very little PVPA homopolymers were detected. The weight fraction of residual PVPA homopolymer is plotted against the ratio of charged to uncharged blocks in the copolymers in Figure 29. From this the increase in residual homopolymer is correlated with a change in the ratio of $n_C$ to $n_U$ block $\bar{M}_n$.

In the case of PEG-based block copolymers, the efficiency of covalent coupling during copolymerisation may be probed using this technique. From the graph below, < 2% (w/w) of PVPA homopolymer is detected from each sample, suggesting very high coupling during synthesis. Additional concentration curves of each monomer species would allow for both the degree of coupling and conversion rates to be determined from the synthetic scheme using only CE-CC.
Figure 29. Graph of the weight fraction of residual PVPA homopolymers against the ratio number-average molar masses for the charged to uncharged blocks in the copolymers.

The ‘specific’ purities in Table 9 refer to the fraction of residual PVPA homopolymers, however both parent homopolymers can be present in the block copolymer sample. These species have been observed and all neutral homopolymers have demonstrated good separations from the block copolymer peaks in the optimum conditions. A standard concentrations calibration curve of PEG1K (Table 8) has been used for fraction analysis of the neutral residual homopolymers. The fraction of PEG in all samples was less than 0.1% (w/w), detection of peak was close to the LOQ as such most samples were sufficiently pure of neutral homopolymers. This is likely the result of the copolymerisation scheme being significantly different from the other samples, PEG homopolymers were purchased preformed instead of polymerised in the lab from monomers. The resulting blocks are likely uniform and almost all chains appeared to copolymerise with VPA monomers.

3.2.4 Assessing Purity: Conclusions

Characterisation of block copolymers in terms of purity is important for understanding the synthetic process as well as determining the properties of a material. Unfortunately typical polymer characterisation techniques are unsuited for the assessment of purity in DHBCs due to many factors outlined in Section 1.2 In this chapter quantification of
homopolymers in different neutral-anionic DHBCs have been assessed and a standard methodology constructed for determining their purities in CE-CC. The method provides a fast and cost effective means of differentiating block copolymers from their parent homopolymers in several P(AM-b-VPA), P(EG-b-VPA) and P(VP-b-VPA) block copolymer samples with varying chain lengths.

Future investigations on the polymerisation mechanism by CE-CC are a potential pathway for further characterisation of these materials, assessing the effectiveness of the synthesis and improving the production of these species.
Chapter 4 – Assessing the Distribution of Compositions in Block Copolymers

In polymeric samples different chemical structures often exist as a distribution of compositions and architectures as explained in Section (1.1). This has been evidenced in the previous chapter as the purity of a homopolymer as well as the many different chain lengths present in the sample influences the number of chemical species in the corresponding block copolymer. These are observed in the CE separation as either clearly resolved or overlapping peaks, the width of these peaks, their $\mu$ as well as their $w(\mu)$ is indicative of the number of species with defined chemical compositions. Like other separation techniques the narrowness of these analyte peaks relates to a more narrow distribution of polymer structures, however the compositions can be very disperse resulting in broad peaks[197]. Throughout this chapter DHBCs and their corresponding homopolymers are assessed for their composition distribution by developing a method using CE-CC.

4.1 DISTRIBUTION OF MOBILITIES AND COMPOSITIONS

This section examines the conversion of data from a CE separation into a distribution of compositions and looks to produce a standard model for determining the dispersity values of many different block copolymer samples not only those tested in this work. As explained in Section (1.3.3.3) electropherograms can be converted into distributions of electrophoretic mobilities. This electrophoretic mobility ($\mu$) of a block copolymer analyte is then shown to be related to the ratio of block lengths and charge densities for each copolymer species present in the chain as demonstrated in Eq. (16). Thus compositions of the block copolymer can be obtained by measuring the distribution of their electrophoretic mobilities and relating to the charge densities of the blocks.
4.1.1 Obtaining Composition Distributions in CE: Theory and Practice

4.1.1.1 Comparing Electrophoretic Mobility Distributions of Different DHBC Block Lengths

From Figure 30 we can compare the analyte position and peak broadness in terms of the electrophoretic mobility distributions. The peak heights in this graph are proportional to the untreated electropherograms in Figure 21. The relative position of peaks better aligns between sample separations but the most noticeable difference is the broadness and shape of peaks: analyte peaks with \( \mu \) in the range \( 0.2 - 1 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1} \) and \( 1.1 - 2.4 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1} \) do not appear to have as strong tailing at the absolute higher mobilities (right side of peak). The block copolymer analyte peaks as described in Section (3.2.2.1) exhibit 2 major populations of polymer chains with similar compositional properties about the \( \mu_{\text{max}} \) of each peak respectively (here called pop1 and pop2). In the electropherogram these peaks do not share the same width at baseline for the sample PAM(5K)-b-PVPA(2.5K), whereas they do in the \( W(\mu) \) plot along the ordinate for up to 30% of the peak height from baseline. As expected this change in the peak shape accounts for the non-linear change in migration time to composition. In contrast the pop1 and pop2 peaks in the sample PAM(5K)-b-PVPA(0.5K) do not share the same peak width at baseline for either plots, likely due to the lower weight fraction of PVPA blocks within pop2 compared to that seen in sample PAM(5K)-b-PVPA(2.5K).
Dispersity values for the distribution of electrophoretic mobilities can be calculated based on the peaks produced in the above graph. The $\mu$ of the analyte separated by CE-CC and its dispersity of mobilities value or $D(\mu)$ is related mathematically to the charge density of the polymer by Equations (3, 11, 12). In this way an indirect measure of the chemical composition can be determined relating to the charged components of the block copolymers.

### 4.1.1.2 Weight Fraction and UV Signal in CE

The weight fraction of monomer units in a polymer is proportional to the UV absorbance of the analyte peak. Each subunit in the chain is, in ideal critical conditions, either directly UV active or visible by complexation with the BGE thus affecting the overall detector response depending on the average molar mass of the chains at a point in the distribution. When comparing homopolymers this proportionality can be directly calculated from the molar mass of the constituent monomer unit as all subunits in the polymer chain have the same molar mass. However this is not the case for the block copolymers examined in this work as the subunits have different molar masses between each monomer species. Assuming the molar absorptivities ($\varepsilon$) of each monomer species are the same a relationship

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**Figure 30.** $W(\mu)$ transformed graph of Figure 21.
can be determined describing the change in UV absorbance relative to the ratio of weight fractions for different monomer species present in the chain. Where each of the constituent monomer species have the same molar mass as well as equal \( \varepsilon \), the weight fraction can be calculated directly from UV absorbance. Though the spectroscopic detector is sensitive to the number of subunits in the polymer, for copolymers the ratio of one monomer species to another cannot be determined via this measurement alone.

### 4.1.1.3 Comparison of the Dispersity \( D(W(\mu)) \) of DHBCs

The distribution of electrophoretic mobilities relates to the composition of the block copolymers, each \( \mu \)-moment along the horizontal corresponds to a different charge-to-friction ratio as per Eq. (3). Thus assessing the broadness of the electrophoretic mobilities using the dispersity Eq. (11 and 12) will provide information about the heterogeneity of the distribution of compositions.

#### 4.1.1.3.1 \( D(W(\mu)) \) of P(AM-\( b \)-VPA) and P(VP-\( b \)-VPA) block copolymers

A stacked distribution of the different electrophoretic mobilities of PAM-based neutral-anionic block copolymers is shown in Figure 31. Separations of samples in this buffer concentration (NB110) demonstrate the highest resolution of different block copolymer peaks. These appear as peaks on-top of the broad block copolymer distribution from \( 0.25 – 0.8 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1} \). Given the proximity but separate mobility of the peak at \( 0.38 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1} \) to the neutral homopolymer peak it can be assumed the species migrating at this point are block copolymers with very low charge densities, possibly PAM(5K)-\( b \)-VPA(0.1K) (1 subunit of VPA monomer). The samples with the lowest charged block length also displays the highest weight fractions of these peaks (PAM(5K)-\( b \)-PVPA(0.5K)) and thus a higher concentration of chains at these mobilities. By counting the peaks in this sample different compositions of the block copolymers may be determined from the theoretical molar masses of the charged block lengths.

**Table 10.** Theoretical block copolymer compositions and electrophoretic mobility maximums of block copolymers in PAM(5K)-\( b \)-PVPA(0.5K) sample.

<table>
<thead>
<tr>
<th>Theoretical Composition</th>
<th>( \mu_{\text{max}} ) in ( 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1} )</th>
<th>Average Molecular Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

85
None of these individual block copolymer peaks are separated enough from the main block copolymer peak to perform area or $D(W(\mu))$ calculations with accuracy, though CE-CC demonstrates high precision with the degree of resolution for these peaks even between samples with different block lengths.
Figure 31. Stacked separations of P(AM-b-VPA) block copolymers of different $n_C : n_U$ length ratios in NB110 buffer solution. A – 1 VPA subunit, B – 2 VPA subunits, C – 3 VPA subunits, D – 4 VPA subunits and E – VPA subunits ≥ 5.

All PAM(5K)-based block copolymers produced the same arrangement of peaks as listed in Table 10, whereas the PAM(10K)-b-PVPA(1.5K) sample did not produce peak A and the shoulder at D was no longer visible. It also had narrower distribution at peak E compared to the distribution in PAM(5K)-b-PVPA(1.5K) sample. The greater neutral block length likely increases the friction of copolymers in solution resulting in a lower distribution of mobilities.

The distribution of all block copolymer species in the $W(\mu)$ were found to be dependent on the concentration of the buffer solution. This has been shown to influence the calculation of $D(W(\mu))$, higher NB concentration resulted in narrower peaks and less difference in the dispersity values between samples. No linear trend could be determined from $\Delta D(W(\mu))$ with concentration, as each block species interact with the BGE ions in a different fashion this likely resulted in non-linear change to the apparent mobility with regard to the block length.
and buffer concentration. What could be observed was a pivot point of dispersities around the NB200 buffer concentration shown in Figure 32. This may indicate that the least separation of different block copolymer chains occurred around this concentration and should be avoided in CE-CC for determining dispersities of these analytes. In general, values of both $D(w(\mu),1,0)$ and $D(w(\mu),2,0)$ had PAM(5K)-b-PVPA(0.5K) as highest and PAM(5K)-b-PVPA(2.5K) as the lowest dispersities. Given the difficulty in synthesis of the PVPA chains compared to the PAM chains it can be stated that the dispersity of the block copolymer is affected by the degree of polymerisation of the charged block more so than the uncharged block[177]. This is reflected in the dispersities of PAM(5K)-b-PVPA(1.5K) having a higher value on average in $D(w(\mu),1,0)$ than PAM(10K)-b-PVPA(1.5K). For $D(w(\mu),2,0)$ values of the polymers there is no trend relating to block length. It may be a consequence of this dispersity value in analogy to $\frac{M_x}{M_w}$ being more sensitive to high electrophoretic mobility. This may also explain why sample $D(w(\mu),1,0) > D(w(\mu),2,0)$ values, knowing that almost all block copolymer peaks appear closer to the EOF than to the residual charged homopolymer peak.

Figure 32. Dispersity values of PAM and PVP-based block copolymers in this work against NB buffer concentration[198].
PVP-based block copolymers did not demonstrate any trend or relationship of dispersity with buffer concentration or block length ratios. As with PAM-based block copolymers $D(w(\mu),1,0)$ values were higher than $D(w(\mu),2,0)$ dispersities, but the lack of separation of the block copolymer peak from the residual neutral homopolymer peak reduces the accuracy of all values. In both block copolymer species the impact of each block on the distribution of mobilities is not known and must be further investigated through the rescaling factor $\alpha$.

4.1.1.3.2 $D(W(\mu))$ of P(EG-b-VPA) block copolymers

Unlike the PAM or PVP-based block copolymers the PEG-based block copolymers were not separated using different buffer concentrations. All P(EG-b-VPA) samples were separated using NB200 buffer in a longer capillary than other samples ($l = 100.0$ cm), this could make it difficult to compare $W(\mu)$ between different block copolymer species due to the different CE runtimes. However, the apparent electrophoretic mobilities of residual PVPA homopolymers in the PEG, PAM and PVP-based block copolymers are approximately similar at $\mu = 3.7 \pm 0.15 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$. Therefore calculations of $D(W(\mu))$ have been carried out and compared with other samples for only the NB200 buffer dispersity values.

For PEG(2.5K)-b-PVPA(1K) and PEG(2.5K)-b-PVPA(0.5K) samples the two distinct populations of electrophoretic mobilities around $\mu_{\text{max}}$ of 0.75 and 1.85 $\times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ have been assigned to different composition groups of the block copolymers called POP1 and POP2. As was determined in Section 4.1.1.3.1 in NB110 buffer individual peaks correlating with block copolymer of P(AM-b-VPA) with 1, 2 and 3 charged monomer subunits were visible in the separation. Despite the high weight fractions of these peaks they were not resolved enough from the broad block copolymer peak to accurately quantify, this is not the case for the PEG-based samples shown in Figure 33. Block copolymer populations in PEG(2.5K)-b-PVPA(1K) sample are completely separated from each other as well as the MADIX impurity peak, unfortunately POP2 in PEG(2.5K)-b-PVPA(0.5K) overlapped with the MADIX impurity such that a similar peak shape occurred as was observed in PAM-b-PVPA samples in NB200 buffer (Figure 36).
### Table 11. Dispersity values obtained for PEG-based block copolymers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(D(w(\mu),1,0))</th>
<th>(D(w(\mu),2,0))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POP1</td>
<td>POP2</td>
</tr>
<tr>
<td>PEG(2.5K)-b-PVPA(0.5K)</td>
<td>1.004 ± 3x10^{-4}</td>
<td>1.19 ± 0.001</td>
</tr>
<tr>
<td>PEG(2.5K)-b-PVPA(1K)</td>
<td>1.006 ± 1x10^{-4}</td>
<td>1.03 ± 0.002</td>
</tr>
</tbody>
</table>

**Figure 33.** Baseline corrected \(W(\mu)\) of PEG(2.5K)-b-PVPA(1K) (blue) and PEG(2.5K)-b-PVPA(0.5K) (red) separated in CE-CC using NB200 buffer. Samples injected at concentration of 5 g·L⁻¹. Blank injection of DMSO taken as baseline.

Due to the significant change in mobility between each of the peak populations, it can be assumed the behaviour is similar to P(AM-b-VPA) block copolymers in that the neutral block species does not affect the mobility as strongly as the charged species. In this case the ratio of \(n_U : n_C\) is much smaller than the other block copolymers in this work due to the shorter PEG block, which further increases the effect of the charged block on the electrophoretic mobility. The \(D(w(\mu),1,0)\) and \(D(w(\mu),2,0)\) values for the block copolymers showed a lower dispersity in PEG(2.5K)-b-PVPA(1K) compared to PEG(2.5K)-b-
PVPA(0.5K) sample (Table 11). As expected the larger PVPA chain length resulted in a lower heterogeneity of mobilities, this also correlated with a greater weight fraction of block copolymers compared to the shorter PVPA block. This suggests a UV absorbance primarily dependent on the molar mass of the PVPA block, though given the limited number of samples evaluated in this work such a trend cannot be determined.

4.1.2 Determining Electrophoretic Mobilities in the Critical Conditions

Block copolymers separate in CE-CC by their charge-to-friction ratio (Eq. (3)) this relates directly with the distribution of charges in the polyelectrolyte otherwise called charge density. Converting electrophoretic mobility to a composition based on charge density requires the electrophoretic mobility of the homopolymer in the critical conditions to be known. This is needed to scale the upper limit of compositions the block copolymer can attain: in this work \( D(c) \) values range from 0 (neutral homopolymer) to 1 (fully charged homopolymer). As demonstrated in Section (3.1.1) the electrophoretic mobility for homopolymers both charged and uncharged is determined by measuring the apex of analyte peak \( (\mu_{\text{max}}) \), however peak shape can be distorted due to bias in mobility conversion, as outlined in Section (3.1.1.4), but more commonly is affected by distortions in CE separation such as solute-solvent effects and resistive heating in the capillary. These can lead to asymmetrical peaks or highly baseline-distorted distributions in free solution CE.

In order to assess the compositions of a block copolymer by CE-CC accurate electrophoretic mobilities must be obtained. Correcting for changes in baseline, dispersive and diffusive migration phenomena as well as temperature control is examined in this section.

4.1.2.1 Influence of Buffer Solution

The BGE composition has a definitive impact on the \( \mu \) of a polyelectrolyte in free solution CE. Factors like peak broadness and apparent electrophoretic mobility \( (\mu_{\text{app}}) \) are affected by changes in the BGE. The distribution of weight fractions for an analyte are determined by these factors and so dispersity measurements such as \( D(W(\mu)) \) and \( D(C) \) will change relative to the medium conditions. In this section the influence of buffer, BGE ionic strength and band broadening from resistive heating will be assessed for the homo- and block copolymers in this paper.
4.1.2.1.1 Slope Plot of Electrophoretic Mobilities

Buffer medium plays a significant role in determining the separation power and efficiency of separations of analyte in free solution CE. For polyelectrolytes, composition of the buffer solution (BGE) is an important aspect to consider in method development. Common BGE compositions tend to look for high buffering capacity and low specific conductivity ($\kappa$) to reduce the effects of differential co-ion migration as examined in Section (3.1.1.3) as well as selecting for the largest range of analytes especially where injecting ‘dirty’ samples[199]. However other criteria must be kept in mind such as the stability of BGE in terms of conductivity and analyte selectivity in the range of pH values chosen for separations. Under an electric field some of these species interact unfavourably with the capillary coating, the diameter of double electric layer at the wall of the fused silica capillary will decrease with an increased ionic strength of BGE[200]. This has effects on the EOF and separation efficiency of all species but particularly affects polyelectrolytes with high mobilities. In this work sodium borate buffer was implemented in all separations, this buffer was selected for its strong efficacy in separation of various synthetic polyelectrolytes including some of the polymers of interest[39, 120]. A detailed study of the effects of buffer species on separation of polyelectrolytes in CE-CC is outside the scope of this paper but may provide future research into buffer-polyelectrolyte interactions.

A relatively new practice in multiple-valent analyte characterisation in CE is the generation of the ‘slope plot’, explained in Section 1.3.3.3.3 changes in the electrophoretic mobility ($\mu_{ep}$) with ionic strength ($I$) of BGE are characteristic of solute nature and composition[175]. For the neutral-anionic block copolymers examined in this paper, determining $D(c)$ from the dispersities of electrophoretic mobility distributions is difficult if the contribution of either block species to $\mu_{ep}$ is unknown, if the electrophoretic behaviour of the polymer under different ionic strength conditions does not significantly change then an isotonic relationship between composition taken as charge density ($\xi$) and $\mu$ may not be achievable. This also relates to the structure of the polymers in solution: whether the polymers behave like polyelectrolytes or nano-particles shown in Figure 10, can be distinguished by the $\Delta\mu_{ep}$ in the slope plot.

Slope plots were generated by Bachelor Honours student Melissa Meinel for the PAM-$b$-PVPA and PVP-$b$-PVPA DHBCs listed in this study. For further details on the methodology see Meinel (2016)[198]. These plots were created by graphing the apparent electrophoretic mobility of the block copolymers at $I$, evaluated as buffer concentration, equal
to 5 mM ($\mu^{5mM}$) against the slope ($S$) value determined as the change in mobility with $I$. Experiments at this concentration did not return repeatable data as such the $\mu^{5mM}$ value was extrapolated from a slope of the moment-average electrophoretic mobility ($\bar{\mu}_m$) against $\log(I)$ shown in Figure 34.

**Figure 34.** Determination of $S$ value from the following slopes (A) $\bar{\mu}_m$ as a function $\log(I)$ where $I$ is taken as NB buffer concentration for PAM-$b$-PVPA samples and (B) the relative electrophoretic mobility ($\frac{\mu_m}{\mu_m^{5mM}}$) against $\log(I)$, including calculated $\mu_m^{5mM}$ value for PAM(5K)-$b$-PVPA(1.5K).

From **Figure 34-(A)** a trend is observed where $\mu_{BC}$ decreases with an increase in the concentration of the NB buffer. From this plot $\mu_m^{5mM}$ can be calculated from the linear fitting of the data points. It has been noted that the change in mobility with NB buffer concentration only appears to follow a linear relationship from NB25 to NB200, whereas all mobilities for PAM-$b$-PVPA samples do not follow this trend in NB300 buffer. Thus calculations of linear fit have excluded the NB300 samples. The shift in mobilities away from the expected values could be explained by a change in the charge density of the copolymers. This assumes that the high ionic strength of the buffer is capable of dissociating protons not only from the anionic block (PVPA) but also the neutral block (PAM). A change like this in the charge composition distribution would incur greater complexation of the neutral block with borate ions from the BGE. Alternatively the polymer-ion interaction may have reached a critical concentration threshold, by which point the polymer acts differently in solution. Chains may become less folded exposing more positions for complexation to occur resulting in higher ion condensation and observed increase in mobility. Though outside the scope of this paper studying the polymers interaction with different BGE ion species may elucidate the underlying mechanism for these changes in mobility.
Using the calculated $\mu^{5mM}_m$ for each block copolymer, a slope plot can be generated based on the relative mobility ($\frac{\mu_m}{\mu^{5mM}_m}$) where $S$ value is the slope of the linear fitting in Figure 34-(B). The order of block copolymer $\bar{\mu}_m$ and $S$ values from highest to lowest always followed the same trend for samples PAM(5K)-b-PVPA(2.5K) > PAM(5K)-b-PVPA(1.5K) > PAM(5K)-b-PVPA(0.5K). This was not the case for $S$ values of PAM(10K)-b-PVPA(1.5K) lying just after PAM(5K)-b-PVPA(2.5K). In the former samples each of the PAM blocks are of comparable length while the latter is considerably larger, the ratio of $n_u : n_c$ in these chains has a greater affect on $\alpha$ and $\bar{\mu}_m$. Due to the increased friction from the larger neutral block (PAM(10K)), the electrophoretic mobilities at all buffer concentrations are lower than the PAM(5K)-based DHBCs. This order is similarly predicted from the ratio of block lengths against the moment-average mobility in Figure 35. For comparison mobilities at each buffer concentration are represented to show the change in linearity with block ratio, a relative error <3.5% was determined for each data point demonstrating some overlap with sample block ratios $n_u : n_c$ above 0.9. To accurately elucidate this trend and relate it to the block composition of DHBCs, more experimental data points are necessary to collect.

Figure 35. The moment-average electrophoretic mobility ($\mu_m$) of the block copolymer peak at different buffer concentrations plotted against the ratio of block lengths ($n_u:n_c$) for all PAM-b-PVPA samples.
Despite this apparent trend in $\Delta \mu_m$ with the block composition ratio, the $S$ value for PAM(10K)-b-PVPA(1.5K) does not match the trend found in the PAM(5K)-based samples. This suggests a different nature of the solvated block copolymer chains compared to the other samples in solution. For all block copolymer samples, $S$ values were found to be in alignment with polyelectrolytes found in Ibrahim, Allison and Cottet (2012), this supports that separation and detection is of discreet polyelectrolyte chains and not particulates or nanoparticles whose composition can be disperse. The experimental $\Delta S$ between block copolymer samples was found to be predominantly affected by the length of the anionic block ($n_c$), therefore the change in mobility with charge composition and corresponding $D(\mu)$ can be compared between samples with high precision where the neutral block species do not change in length.

Table 12. $\bar{\mu}_m$ in $10^{-8}$ m$^2$V$^{-1}$s$^{-1}$ for different buffer concentrations and $S$ values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>25 mM</th>
<th>110 mM</th>
<th>200 mM</th>
<th>300 mM</th>
<th>5 mM (cal.)</th>
<th>$S$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAM(5K)-b-PVPA(0.5K)</td>
<td>1.079</td>
<td>0.844</td>
<td>0.629</td>
<td>0.629</td>
<td>1.374</td>
<td>0.300</td>
</tr>
<tr>
<td>PAM(5K)-b-PVPA(1.5K)</td>
<td>1.543</td>
<td>1.147</td>
<td>0.940</td>
<td>0.927</td>
<td>2.009</td>
<td>0.328</td>
</tr>
<tr>
<td>PAM(5K)-b-PVPA(2.5K)</td>
<td>1.833</td>
<td>1.287</td>
<td>1.020</td>
<td>1.074</td>
<td>2.461</td>
<td>0.362</td>
</tr>
<tr>
<td>PAM(10K)-b-PVPA(1.5K)</td>
<td>1.025</td>
<td>0.778</td>
<td>0.590</td>
<td>0.615</td>
<td>1.360</td>
<td>0.340</td>
</tr>
<tr>
<td>PVP(8K)-b-PVPA(0.5K)</td>
<td>1.548</td>
<td>0.991</td>
<td>0.926</td>
<td>0.833</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>P(VPA-s-AA)</td>
<td>n.d.</td>
<td>3.586</td>
<td>3.725</td>
<td>n.d.</td>
<td>2.867*</td>
<td>0.535</td>
</tr>
</tbody>
</table>

*measured not calculated

n.a. = not applicable – the error far exceeded the acceptable limit

n.d. = not determined/measured

In addition to the block copolymer samples a statistical copolymer P(VPA-stat-AA) has been assessed for comparison of how different compositions can affect the $S$ values and relative mobilities of polyelectrolytes in free solution CE.

4.1.2.1.2 Resolving Disperse Polyelectrolytes

Several chemical species are present in the block copolymer samples that all exhibit different mobilities in CE separation. These species can be broken down into three categories, intended block copolymers, homopolymer impurities and synthetic artefacts. Some of these
species were difficult to resolve by initial CE-CC protocols performed in Section 3.2.2, neutral residual homopolymers in particular comigrated with the tail ends of either the EOF or block copolymer peaks. Thus measurements of total sample purities were not determined only the specific anionic homopolymers were quantified. PVPA homopolymers always had complete separation from the corresponding block copolymer peaks allowing good quantification by CE. However there were some species that had a tendency to overlap with the block copolymer peaks such as the presence of MADIX agent impurities and neutral homopolymers. These convoluted block copolymer regions have the potential to cause significant error in distribution measurements. Accurate polymer dispersities under these conditions are not possible.

Initial polymer separations were performed in NB200 buffer under the conditions listed in Section 2.2.1.1. This buffer concentration was decided upon as it closely matched the optimum conditions found in previous separations of P(EG-b-VPA) samples. In these conditions complete separation of PEG and PVPA homopolymers from the block copolymer peak were achieved, however in P(AM-b-VPA) samples only partial separation of PAM homopolymers was achieved. Different buffer concentrations were applied for resolving the PAM peak from the block copolymer and water peaks. In Section 3.1.1.2.2 CE of the block copolymers in NB25, NB110 and NB200 buffer concentrations demonstrated no significant separation of this peak from the neighbouring peaks, however in NB300 buffer the peak is well resolved from both the water and block copolymer peaks (Figure 17-(D)). Using these conditions an analysis of $W(\mu)$ for the block copolymers can be conducted with high certainty of accurate distributions near the left-half of the analyte peak.

An increase in the buffer concentration results in changes to the mobilities of all species in a sample. Buffer concentration NB ≥ 300 mM in CE of P(AM-b-VPA) samples, produces a high resolution peak of the PAM residual homopolymers but result in a decreased resolution of the PVPA homopolymer peak. Charged residual homopolymers exhibited broad mobility peaks in comparison to the neutral homopolymers. Due to separation in CE dominated by charge-to-friction, different chain lengths of the charged homopolymers will migrate at different velocities up to a critical conditions chain length (see Section 3.1.1.1.2). Thus the separation of PVPA homopolymer reveals more compositions spread over wider peak range with lower $w(\mu)$ intensity than the corresponding neutral species. In NB300 buffer this low signal intensity compared to the either the PAM or block copolymer peaks reduces the detection of PVPA homopolymer content. In order to quantify the residual homopolymer
content and determine accurate distribution of compositions for the block copolymer samples, several separations must be performed to satisfy the needs of each property.

Block copolymer peaks for each sample were observed to be broad in comparison to each of the homopolymer peaks. The width of these peak distributions like the homopolymer peaks is dependent on the concentration of the buffer solution. In Figure 36 the position and width of the \( W(\mu) \) for each block copolymer shifts toward higher \( \mu \) with an increase in buffer concentration. This causes the runtime of the CE separation to increase in order to retrieve a complete migration of the analyte. As with charged PVPA homopolymers the distribution of mobilities is becoming more spread along the horizontal with a lower buffer concentration. Theoretically a complete resolution of all chain species is possible if an infinitely long capillary is used to separate a finite solution of analyte. What this means for the current distributions is that block copolymers of like composition are migrating further apart. This change in \( W(\mu) \) will affect calculations of the average-mobility moments outlined in Section 4.1.3.2 which determine the dispersity values of composition.
Figure 36. Stacked CE-CC separations of P(AM-b-VPA) block copolymers with varying \( n_C : n_U \) ratios, in different NB buffer concentrations. All separations performed in high sensitivity fused-silica capillary \((l_t = 99.5 \text{ cm}, l_d = 8.5 \text{ cm})\).

Another feature this graph brings up is the presence of the MADIX impurity previously observed in separations of homopolymer and other RAFT/MADIX synthesized copolymers around \( \mu = 1 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1} \) (Figure 27). Similarly this ‘peak’ appears in all block copolymer samples above and like the other peaks exhibits a shift in mobility with buffer concentration. It has been noted that determining distributions of compositions for the block copolymer is complicated by the presence of this species in the middle of the analyte peak. By changing the concentration of the buffer this impurity peak can be shifted away from the block copolymer peak, at NB300 the peak appears around \( 1.2 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1} \) and continuing to shift closer to the EOF through NB200, NB110 and at NB25 (not pictured above, see Appendix 4) migrates at a \( \mu \) around \( 0.6 \times 10^{-8} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1} \). The MADIX impurity peak was near completely separated from the block copolymer peak at NB buffer concentration < 25 mM. Though the peak was no longer overlapping significantly with the analyte peak, homopolymers for each species were not visible at these concentrations. The \( W(\mu) \) of the
block copolymer peaks also showed a trend of increasing with lower buffer concentrations, resulting in higher resolution of the disperse copolymer chains but at a reduced detection of weight-fractions. Alternatively, higher buffer concentrations also resulted in a decreased MADIX impurity peak toward higher mobility but could not be fully removed from the block copolymer peak. At NB300 the P(AM-b-VPA) copolymer peak became narrower in all samples. For PAM(5K)-b-PVPA(0.5K) all visibly different composition population peaks such as found in NB110 at $\mu_{\text{max}} = 1.2, 0.65$ and $0.38 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ were reduced to a single $\mu_{\text{max}} = 0.75 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$, a similar effect was observed in other PVPA block lengths.

Comparatively the P(EG-b-VPA) block copolymer samples were less susceptible to this issue as the MADIX impurity appeared well resolved from both population peaks of the analyte (Figure 37). However the overall UV detection of peaks on average was lower than P(AM-b-VPA) and P(VP-b-VPA) samples presenting a problem for quantification of the homopolymer species and weight-fractions of the block copolymers. Since the MADIX end-groups of the chains are UV visible around 290 nm wavelength, detection of species by end-group was attempted to improve absorbance as well as provide the number-average of chain compositions ($C_n$) outlined in Section 4.1.3.2.
Figure 37. CE-CC separation of PEG(2.5K)-b-PVPA(1K) block copolymers in NB200 buffer in fused-silica capillary ($l_c = 100.0$ cm, $l_d = 8.5$ cm). UV detection taken from DAD set to 195 and 290 nm wavelengths.

In all cases the conditions needed to satisfy investigation of one property were different from those of another particularly when measuring the distribution of mobilities for the block copolymers and obtaining accurate purity measurements.

4.1.3 Converting Distribution of Electrophoretic Mobilities into Composition Distributions

As explained in the previous section the raw electrophoretic migration data from a CE separation can be converted into electrophoretic mobility moments, allowing accurate measurement of the distribution of block copolymers by their charge composition. $D(\mu)$ does not provide a complete analysis of the composition of each analyte chain, the influence of the charged and uncharged components on the $W(\mu)$ is not well understood thus relating the dispersities of these distributions to a composition is challenging. In CE-CC the charge of a polyelectrolyte can be estimated by its $\mu$, given Eq. (1), which correlates with the charge density ($\xi$) of the whole block copolymer via Eq. (18). Here charge density accounts for the ratio of block lengths with different net charge in relation to electrophoretic mobility. The
mobilities of the charged block ($\mu_C$) and the neutral block ($\mu_U$) affect the electrophoretic mobility of any given double hydrophilic block copolymer ($\mu_{BC}$). This relationship is a ratio represented in the equation as ‘$\alpha$’. By obtaining $\alpha$ in CE and converting $\mu$ to $\xi$ the composition of a DHBC in terms of the ratio of charged to neutral blocks can be estimated.

### 4.1.3.1 Converting $w(\mu)$ into Weight Distribution of Compositions

Detection methods in CE other than direct UV absorbance have been investigated on copolymers to provide the charge composition and distribution of charge densities ($D(\xi)$). It has been demonstrated with the use of contactless conductivity detectors for non-UV absorbing species[201] as well as in conjunction with UV DAD detectors for characterisation of micelle surfactants[202]. Other techniques such as indirect UV detection are capable of determining the effective charge and thus charge density of neutral-anionic diblock copolymers of PEO[203]. This example, like other CE techniques, obtains a distribution of peaks (observed as a drop in UV absorbance from the background) in time ($t_m$), that relate to the composition of the block copolymer. However in Anik et al. (2009) they found that the drop in UV absorbance can be related to the weight fraction of the effective charge densities ($w(\xi_{\text{eff}})$) if a probe with the same charge as the solute is used. The transformation of the detector ordinate from $w(\mu)$ (weight fraction as a function of electrophoretic mobility) to $w(\xi)$ (weight fraction as a function of charge density composition) is similar to the conversion used in Section (4.1.1.3) for transforming UV absorbance from the electropherogram.

Using this conversion on the data collected by CE-CC can generate distribution of the charge densities for the DHBCs of interest. Without the need for secondary detectors or background probes a charge density composition can be determined from the sample electropherograms. As with Eq. (10) for obtaining $w(\mu)$ from the initial UV signal, here $w(\xi)$ is calculated using the mathematical relationship between $\mu$ and $\xi$ by dividing the ordinate in the electrophoretic mobility distribution by the derivative of Eq. (18) as follows:

$$w(\xi) = \frac{w(\mu)}{\left(\frac{d\xi}{d\mu}\right)}$$  \hspace{1cm} (24)

Having both the equations for chemical charge density ($\xi$) Eq. (19) and electrophoretic mobility for a neutral-anionic block copolymer ($\mu_{BC}$) Eq. (16), the derivative of the charge composition ($\frac{d\xi}{d\mu}$) can be rearranged as follows:
Substitution of Eq. (25) into Eq. (24) allows the weight fraction of charge composition to be assessed in terms with known values from the following relationship:

$$w(\xi) = w(\mu) \frac{[\mu(\alpha-1)+\mu_0]^2}{\alpha \mu_0}$$  \hspace{1cm} (26)

These values can be determined experimentally from the mobilities of the charged homopolymers ($\mu_0$) and block copolymers ($\mu$) respectively. Below is displayed the distribution of charge densities for block copolymers of PAM-$b$-PVPA with differing charged block lengths.

![Graph showing the distribution of charge densities for block copolymers of PAM-$b$-PVPA with differing charged block lengths.](image)

**Figure 38.** Weighted distribution of chemical charge densities for CE-CC separations of PAM-$b$-PVPA block copolymers in NB200 buffer. $\alpha$ values for conversion were determined using $\mu_0 = 3.7 \times 10^{-8}$ m$^2$·V$^{-1}$·s$^{-1}$ and $\mu = \mu_{BC}$ at maximum. All distributions were normalised to $\xi_{\text{max}}$ of 1.

As expected increases in the PVPA block length coincide with a change in the charge composition of the block copolymer. The shift in $\xi$ is significant between samples PAM(5K)-
b-PVPA(0.5K) (purple) and PAM(5K)-b-PVPA(1.5K) (green) and PAM(5K)-b-PVPA(2.5K) (orange), where each peak start appears nearly identical the ξ_{max} is different between samples. Most apparent is the large difference in peak areas when comparing copolymers of PVPA blocks of \( M_n = 0.5 \text{ Kg} \cdot \text{mol}^{-1} \) with 1.5 and 2.5 Kg·mol⁻¹ respectively. The smaller number of charged subunit species (PVPA) in the first sample contribute less to the electrophoretic character of the copolymer especially considering the significantly larger neutral block (PAM), as such this sample exhibits the smallest weight fraction of charge compositions.

It should be noted that a different \( \alpha \) for each sample was calculated in Figure 38. The degree to which the rescaling factor affects the distribution is not known for all samples, it has also been demonstrated that the relative change in \( W(\xi) \) for any \( \alpha \) of arbitrary value are not equal between samples[39]. This implies that composition distributions cannot be compared between samples unless some standard were used to measure the sample against in each CE separation.

**4.1.3.2 Distributions of Charge Densities for Neutral-Anionic DHBCs**

In CE separations the electrophoretic mobilities are mathematically related to the charge composition of a polyelectrolyte, the dispersity of distributions \( D(W(\mu)) \) are proportional to the distribution of charged species in the polymer chains. However any subunit species whether charged \( n_c \) or uncharged \( n_u \) in the chain will contribute to the total hydrodynamic friction of the polymer. For neutral-anionic DHBCs in this work, the additional friction from the uncharged block will affect the apparent electrophoretic mobility proportional to the length of both charged and uncharged blocks Eq. (16). The dispersity values calculated from these distributions, as demonstrated in Eq. (11) and (12), approximate the heterogeneity of charge densities of these block copolymers. Charge density on the other hand directly relates the distribution of compositions \( D(c) \) and \( D(W(\mu)) \) as a ratio of the number of charged-to-uncharged units in a polyelectrolyte. In salt solution the neutral-anionic block copolymer will complex with the BGE forming a polyelectrolyte with a specific charge density depending on the concentration of background ions (buffer solution) and the block composition. This determines the effective charge density and is unique to each chain in the sample. Thus a specific composition and electrophoretic mobility can be determined from this relationship: Using Eq. (19) and (26) where \( \alpha \) is known the weight distribution of charge densities \( W(\xi) \) or simply charge compositions \( C(\xi) \) can be calculated from the distribution of electrophoretic mobilities of a polyelectrolyte.
Typically \( D(C) \) inform about the broadness of distributions and can be determined by the ratio of weight-average compositions \( (C_w) \) to number-average compositions \( (C_n) \). Detection of subunits in a polyelectrolyte either by direct or indirect UV absorbance provides the weight fraction of polymers in a peak allowing \( C_w \) to be calculated. End-groups of single absorbing species detection is necessary to provide the \( C_n \) which is challenging in CE of polymers without this group. The DHBCs studied in this work have a MADIX end-group that is detected at a higher UV wavelength than the constituent monomer subunits, though the sensitivity of this species once samples undergo separation is too low to provide precise measurements.

For polyelectrolytes as with the determination of \( D \) of \( W(\mu) \) (see Section 1.3.3.3), \( D(C) \) can be calculated in a similar manner by measuring the ratio of moments in a distribution of compositions shown below[169]:

\[
D(C) = \frac{C_w}{C_m}
\]  

Where the fractions of monomer subunit species in the copolymer chains, denoted by \( C \), are expressed as chemical charge densities \( (\xi) \) the above can be calculated from \( W(\xi) \). For list of moments and corresponding composition averages \( C_m \), \( C_w \), and \( C_n \) see Appendix 5.

4.1.4 Comparison of Composition Distributions in DHBCs

It has been discussed in Section 4.1.3 that the distribution of electrophoretic mobilities in a separation of polyelectrolytes is related to the charge density of the analyte chains. In block copolymers the charge density is indicative of the ratio of neutral to charged species and their effect on the mobility of the polyelectrolyte chains in CE. Thus charge density can be treated as the composition of a neutral-anionic DHBC, since this can be calculated from the electrophoretic mobility a dispersity value of these compositions can be determined. However in order to acquire the charge densities of a block copolymer the rescaling factor \( \alpha \) must be obtained, this can be determined from the relative influence of each block on the apparent electrophoretic mobility of the copolymer. In this section the rescaling factor and distributions of compositions \( (D(C)) \) are compared for the PAM and PVP-based block copolymers.
4.1.4.1 Rescaling Factor $\alpha$

To calculate $\alpha$ as the relative impact of each block on the electrophoretic mobility of the copolymer Eq. (17) can be used to represent this factor in a linear plot of the change in relative electrophoretic mobilities ($\Delta \mu_{\text{rel}}$) against the block length ratios ($n_U : n_C$). $\alpha$ in this context can be obtained from the slope of the function shown in Figure 39.

![Figure 39](image)

Figure 39. Linear plots of the change in $u_{\text{rel}}$ represented by $\mu_0/\mu - 1$ against $n_U/n_C$. Mobilities were taken from NB200 buffer CE-CC separations. $\lambda$ is determined from the slope of these functions.

Despite having obtained the $\alpha$ from these plots, the factor can only be applied to each of the CE separations that $\mu$ was taken from. This is because different buffer and separation conditions affect the $D(\mu)$ of the polymers non-monotonically in Sections 4.1.2.1.1 and 4.1.1.3.1. This has been observed also in a plot of $\alpha$ against buffer concentration determined in Meinel (2016). As such a complete list of $\alpha$ for all buffer concentrations used has been determined in Table 13.

Table 13. Rescaling factors for all block copolymers assessed in this work. The neutral block lengths have not been provided in the samples as most shared the same DP, PAM(5K) and PAM(10K) DHBCs showed the same $\alpha$ and so were combined.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>PAM-$b$-PVPA(0.5K)</th>
<th>PAM-$b$-PVPA(1.5K)</th>
<th>PAM-$b$-PVPA(2.5K)</th>
<th>PVP-$b$-PVPA(0.5K)</th>
<th>PEG-$b$-PVPA(0.5K)</th>
<th>PEG-$b$-PVPA(1K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB25</td>
<td>0.111</td>
<td>0.185</td>
<td>0.280</td>
<td>0.088</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>NB110</td>
<td>0.147</td>
<td>0.252</td>
<td>0.420</td>
<td>0.135</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
A trend was observed where increasing $\alpha$ occurred with an increase in the block length of PVPA in the PAM-based DHBCs. However this trend was not reflected in the PEG-based samples though these were only determined in one set of buffer concentrations. The implication of these trends is that the charged block has a dominant effect on the distribution of $\mu_{rel}$ more so than the neutral block. Further examination is necessary to determine the relationship for PEG-based samples.

### 4.1.4.2 $D(C)$ of Block Copolymers

The distribution of compositions ($D(C)$) of the block copolymers in this work is determined as the dispersity of $W(\xi)$ which can be determined analogous to $D(W(\mu))$ by comparing the weighted fractions of $\mu$ as a function of $\xi$ (see Section 4.1.3.2). Using the $\alpha$ value determined in Section 4.1.4.1 a $D(C)$ for PAM and PEG-based block copolymers were obtained at each of the buffer concentrations (Table 14). Dispersity values were not calculated for PVP-based samples and obtaining a ‘true’ composition distribution of these materials requires a measure of the number of block copolymer chains at each $\mu$-moment along the distribution. This has not yet been in achieved, however the weighted charge compositions determined here provide a closer look at the distribution of these species.

**Table 14.** $D(C(\xi),1,0)$ for PAM and PEG-based block copolymers.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>PAM(5K)-b-PVPA(0.5K)</th>
<th>PAM(10K)-b-PVPA(1.5K)</th>
<th>PAM(5K)-b-PVPA(1.5K)</th>
<th>PAM(5K)-b-PVPA(2.5K)</th>
<th>PEG(2.5K)-b-PVPA(0.5K)</th>
<th>PEG(2.5K)-b-PVPA(1K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB25</td>
<td>1.7077</td>
<td>1.54856</td>
<td>1.66044</td>
<td>1.46766</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>NB110</td>
<td>1.38929</td>
<td>1.33004</td>
<td>1.44247</td>
<td>1.34718</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>NB200</td>
<td>1.35476</td>
<td>1.30602</td>
<td>1.42049</td>
<td>1.3956</td>
<td>1.00105</td>
<td>1.03304</td>
</tr>
<tr>
<td>NB300</td>
<td>1.53663</td>
<td>1.00591</td>
<td>1.542</td>
<td>1.36211</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

### 4.2 CHAPTER CONCLUSIONS

Block copolymers are rarely characterised in terms of the distributions of compositions in literature, though heterogeneity in the ratio of block compositions is known to affect the properties of these materials. In this chapter, a method for the composition assessment of DHBCs with novel ‘smart’ properties has been developed using CE-CC. Comparisons of the distributions of electrophoretic mobilities for block copolymer samples has demonstrated the effectiveness of this technique in the separation of detection of different block compositions.
species. The dispersity values obtained in this chapter provide a relative distribution of the charge densities in terms of theoretical block length ratios. For assessment of polymer separations by CE in general, the correction of the horizontal as migration time ($t_m$) to an electrophoretic mobility ($\mu$) is discussed and shown to reduce the relative error between repeat separations.

In summary weight fractions of each species in the block copolymers has been assessed in a qualitative manner and provides identification of different compositions in the samples. This technique may provide supplementary characterisation for the distribution of compositions and structures not yet obtained in the literature.
Chapter 5 – CONCLUSIONS

5.1 CONCLUSIONS

Purity and composition are important aspects to consider in the construction of polymer-based products, particularly in block copolymers that mix multiple monomeric and polymeric constituents. In a commercial setting, it is standard operating procedure to screen these materials for quality assurance, this includes the potential of impurities in samples that can lead to product failure or simply the properties lacking the capabilities desired by the manufacturer. Both outcomes are influenced by the synthetic process utilised in copolymer production. Furthermore with growing emphasis on the application and design of new ‘smart’ macromolecules in academia it is imperative that these materials are well characterised in order to translate them into products.

Neutral-anionic double hydrophilic block copolymers (DHBCs) containing vinylphosphonic acid (VPA) units are of interest for their high charge capacity in controlled molecular structures. In addition these block copolymers are constructed with a range of neutral adhesive and biomimetic blocks, these species include acrylamide (AM), N-vinylpyrrolidone (NVP) and ethylene glycol (EG) units. VPA-based polymers are difficult to synthesize with narrow distributions of compositions. RAFT/MADIX is an important controlled radical polymerisation technique that has shown great potential for the synthesis of VPA-based block copolymers used in this work. These samples have been assessed in terms of purity and compositions using capillary electrophoresis in the critical conditions (CE-CC). Characterisation of block copolymers by common polymer separation methods such as SEC rarely observe the presence of homopolymer impurities. In the separations of the DHBCs of interest by CE-CC this work shows that homopolymers from both charged and uncharged species can be separated from the block copolymer. Quantification of the homopolymer content was performed using standard concentration curves. The relative weight-fractions of homopolymer impurities increased with the $\bar{M}_n$ of the PVPA block length in the copolymers. Therefore the methodology demonstrated in this work provides a clearer picture of the purity of RAFT/MADIX synthesised DHBCs.

Block copolymer heterogeneities were also assessed through the distributions of migrating species determined by CE-CC. Electropherograms of these block copolymers were converted into weighted-distributions of electrophoretic mobilities ($W(\mu)$). This converted
data provides much higher accuracies of the relative distribution of these species correcting for instrument error and changes in the EOF between separations. The relationship between the charge of the smart DHBCs and their composition was examined based on their electrophoretic mobility ($\mu$) as they separated in the capillary. The dispersity of these $W(\mu)$ for the block copolymer peaks was calculated as a ratio of the average $\mu$-moments similarly to $D$ or dispersity of molar masses. These $D(\mu)$ provided information about the heterogeneity of distributions in terms of the ratio of charged-to-uncharged blocks and their effect on the $\mu$ of the block copolymers. For all block copolymers examined higher DP of PVPA resulted in lower $D(\mu)$.

These results provide a framework for quick and inexpensive characterisation of DHBCs interesting for future nano-technologies as well as a literature for the improvement of the RAFT/MADIX and any controlled polymerisation synthetic technique.

5.2 FUTURE WORK AND DIRECTIONS

The $D(\mu)$ for the block copolymers is a relative measure of the width of the $W(\mu)$, for narrow peaks this distribution is low and thus a smaller value is obtained for the dispersity. However this value does not directly relate the distribution of compositions ($D(C)$) of the block copolymers. This value can be calculated as was done in Section 4.1.4.2 for block copolymers with known $\alpha$ values. $D(C)$ of the PVP-based block copolymer was not assessed due to a lack of time and samples with different block length ratios. Change to the buffer concentration resulted in changes to peak width of the block copolymers, this also affected both the $D(\mu)$ and $D(C)$ in different ways, neither of which produced a linear trend. As such the relationship between these two dispersity values is not understood nor their relationship with the composition for block copolymer species fully known. As such more samples of block copolymer with varying block lengths in both the charge and neutral block species are necessary to elucidate this relationship.

As was briefly mentioned at the end of Section 4.1.2.1.2 the true distribution of compositions for a block copolymer may be obtained were two separate criteria known, those being the number-average weight fraction of chains with known composition and the total number of compositions. This has the potential to be determined for the PEG-based block copolymers as the MADIX transfer agent end-groups are UV visible in the 290 nm wavelengths while the subunits are not. Assuming each chain has only one of these end-groups the total fraction or number of chains can be determined at this wavelength, while the
usual detection used in this method at 190 nm will detect each subunit that complexes with the BGE. Thus a separation based on charge density composition can be directly related to the number-average DP of the block copolymers. However this is dependent on the detection of the end-group which has not produced quantitative signals in some cases. Further injections of block copolymers of decreasing DP may allow a quantitative measurement of the end-group if the effect of chain length is known.

In terms of purity, homopolymer species from the neutral block species were easily observed in all neutral-anionic DHBC separations with the exception of PEG homopolymers. Conversely the PVPA homopolymers were very difficult to detect and quantify in all but the P(EG-b-VPA) samples, this was the case even in the optimal NB buffer conditions. Given the difference in $n_U : n_C$ block length ratios one might expect the much larger neutral species to be detected more easily, but even when DHBC samples are spiked with PVPA homopolymers the change in relative peak height ($\Delta w(\mu)_{rel} = 10.5 \%$) and area ($\Delta A_{rel} = 22.8 \%$) with PVPA concentration remains much too low in comparison to the change in relative neutral homopolymer peak height ($\Delta w(\mu)_{rel} > 310 \%$) and area ($\Delta A_{rel} > 600 \%$). This suggests that quantification may be more complicated than initially expected. As well as the homopolymers, some species that were not quantified but exhibited much stronger peaks were the residual VPA impurity and the MADIX impurity. Both of these species must be assessed to understand and improve the synthesis of the DHBCs.

On the method development of CE-CC as a means to quantify the purity and compositions of smart DHBCs, conversion of the raw electropherogram data into the $W(\mu)$ plots is a relatively new technique for assessing the separations of analytes. The conversion to the weight distribution of mobilities is proven to be more repeatable than the electropherogram plots especially when determining the peak maximum as shown in Section 3.1.1.1.1, however the template for the conversion affects the scaling of the horizontal as well as the vertical axes in a non-linear manner. For peaks with high mobilities this can affect the position of the baseline in such a way that obtaining the correct start and end points can be challenging by visual observation. Even worse is the potential of introducing error into the dispersity measurements. Therefore to conclude that accurate peak measurements are being made from data in these cases a baseline correction must be applied, however selecting where and what baselines are used based on an arbitrary visual observation may not be sufficient nor does it reveal how that affects the distribution of data points in the plot or dispersity measurements. It was planned to perform round-robin tests involving researchers with
varying degrees of experience with CE to baseline correct many different datasets from various synthetic polymer samples using a common protocol. This could not be attempted during the timeframe of the Master of Research program but is important for future research in this field.

Determining the distribution of structures in these DHBCs is important for grading the synthesis by RAFT/MADIX. As stated previously more block copolymers with different architectures and chemical distributions are necessary to validate the analytical methodology being developed in this work. Future work combining the synthetic procedure with online CE has the potential to elucidate the mechanism of polymerisation and can be used to improve the product.
APPENDIX 1: Bias from transformation of Electropherogram into $W(\mu)$

Figure 40. Artificial peaks generated with uniform height (maximum UV = 10 Au) and FWFM (full width at full maximum = 0.5 min) have been plotted into (A) an Electropherogram and this distribution has been transformed in (B) $W(\mu)$ using the same conditions as the CE electropherogram data for P(AM-b-VPA) samples in NB200 buffer.

The transformation of the raw electrophoretic migration data into the distribution of electrophoretic mobilities is presented in Figure 1. The change in the peak height is not uniform across the distribution, and the proximity of neighbouring peaks increases with mobility. Declining baselines will result in the following trends in each distribution:
Figure 41. Artificial peaks generated with uniform absolute height (maximum UV = 10 Au) at a decreasing baseline, FWFM (full width at full maximum = 0.5 min) have been plotted into (A) an Electropherogram and this distribution has been transformed in (B) \( W(\mu) \) using the same conditions as the CE electropherogram data for P(AM-b-VPA) samples in NB200 buffer.

Small peaks around \( \mu > 3 \times 10^{-8} \text{m}^2\text{V}^{-1}\text{s}^{-1} \) are difficult to differentiate from background and measurements of their areas and baseline must be kept in consideration concerning the shape of these baseline trends in the \( W(\mu) \) (Figure 2). Future work establishing a standard method of analysing peaks under these conditions is needed.

APPENDIX 2:

Table 15. List of all buffer solutions used in this work and the concentration codes.

<table>
<thead>
<tr>
<th>Buffer Code</th>
<th>Concentration in mM</th>
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<tbody>
<tr>
<td>NB25</td>
<td>25</td>
</tr>
<tr>
<td>NB110</td>
<td>110</td>
</tr>
<tr>
<td>NB200</td>
<td>200</td>
</tr>
<tr>
<td>NB300</td>
<td>300</td>
</tr>
</tbody>
</table>

APPENDIX 3: Baseline Correction
Baselines were determined in one of two ways: either a baseline based off the electropherogram of the Blank (DMSO) was used as in the case below.

![Electropherogram of PAM-b-PVPA from CE performed in NB300 buffer. Blank injection with only 5 µL of DMSO was injected prior to sample injection to mark to position of the EOF and to determine the baseline.](image)

**Figure 42.** Electropherogram of PAM-b-PVPA from CE performed in NB300 buffer. Blank injection with only 5 µL of DMSO was injected prior to sample injection to mark to position of the EOF and to determine the baseline.

In these cases where all peaks of interest are well defined both at the start and end points of the curve, straight baselines can be used sometimes without the use of the DMSO injection. However not all baselines are uniform or easy to distinguish.

The second method used was the generation of artificial baselines based on the transformation of migration time into electrophoretic mobility. If the position of the start and end points of the migration curve are known then a straight baseline can be generated that follows these points (**Figure 4**). Once that is generated both sample data and baseline can transformed into the $W(\mu)$, in which case the curvature of the data is accounted for by a baseline that follows the same transformation trend (**Figure 5**). This last method is currently under investigation and further improvements are being made to the model as well as understanding how this method affects the treatment of the raw data and dispersity measurements.
Figure 43. Baseline correction in the electropherogram of PVP homopolymer: generating a straight baseline based on the start and end points of the distribution. Artificial baseline (dashed line).
Figure 44. Transformation of figure into $W(u)$: both baseline and sample is transformed.

APPENDIX 4: MADIX agent impurity

The impurity from the MADIX agent has been observed to change mobility in CE of different NB buffer solutions. Separations in NB25 buffer resolved the MADIX impurity from the block copolymer completely. Further investigations with different buffer solutions are needed.
Figure 45. Separations of PVP-b-PVPA in different NB buffer concentrations. Madix agent impurity is highlighted in all $W(u)$.

APPENDIX 5: Average Composition Values

The average compositions in analogy with $\bar{M}_m$, $\bar{M}_w$, and $\bar{M}_n$ in the calculation of $D$, the calculation of $D(C)$ can be determined thusly:

\[
D(C) = \frac{C_w}{C_m}
\]

\[
C_m = \frac{\sum w_k}{\sum w_k c_k^{-1}}
\]

\[
C_w = \frac{\sum w_k c_k}{\sum w_k}
\]

\[
C_n = \frac{\sum N_k c_k}{\sum N_k}
\]

Where $w_k$ and $N_k$ represent the total weight and number of polymer chains of number k and $C_k$ represents the particular composition of polymer chains of number k. Since C is a composition it can be expressed as the fraction of charges or charge density of a copolymer with charge-uncharged blocks.


26. Moad, G.N., Peter;Tsarevsky, Nicolay V;Vana, Philipp;Sumerlin, Brent S;Storey, Robson;Abd-El-Aziz, Alaa S;Craig, Stephen;Yagci, Yusuf;Dong, Jianhua, *Polymer Chemistry Series : Fundamentals of Controlled/Living Radical Polymerization (1)*. 2013, Cambridge, GB: Royal Society of Chemistry.


83. Favier, A., et al., Liquid chromatography at the critical adsorption point (LCCAP) of high molecular weight polystyrene: pushing back the limits of reduced sample recovery, in e-Polymers. 2009. p. 95.


