Colloid Polym Sci (2008) 286:1443–1454
DOI 10.1007/s00396-008-1915-x

ORIGINAL CONTRIBUTION

Interactions of poly(2-methacryloyloxyethyl phosphorylcholine) with various salts studied by size exclusion chromatography

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Received: 31 March 2008 / Revised: 2 August 2008 / Accepted: 3 August 2008 / Published online: 22 August 2008

Abstract The objective of this research is to develop a relationship between salt type and concentration to poly (2-methacyloyloxyethyl phosphorylcholine) (PMPC) zwitterionic polymer solution behaviors. In particular, polyelectrolyte hydrodynamic volumes were analyzed through size exclusion chromatography in relation to the addition of various salts at various concentrations. The salt properties examined were salt concentration, ionic strength, solution pH, cation type/size, anion type/size, valency, and configuration. It was found that the effect of ion properties is related to mechanisms associated with the geometry of the polyelectrolyte. The negative charge group of the polyelectrolyte situated closer to the backbone (inside) is less important to the change in hydrodynamic volume than the positive charge group situated at the end of the side chain (outside). The extensive amount of data generated in this study provides a strong background for possible accurate formulation of a theory based on the salt effect on PMPC polyelectrolyte solution behavior.

Keywords Polyelectrolyte · Salt effect · Hydrodynamic volume · Zwitterionic polymer · Polybetaine · Size exclusion chromatography · Ionic strength

Introduction

Water-soluble polymers, both ionic and nonionic, are of great interest in industry due to their extensive applications in papermaking, oil recovery, water treatment, and mineral processing areas. The unique behavior of ionic polymers has also resulted in increased research [1, 2]. Zwitterionic polyelectrolytes (carrying both positive and negative charge) in particular can exhibit very unique properties, especially in solution, which allows for many possible developments, such as associative thickener, emulsifier, rheology modifier, solubilizer, colloids, etc. [3]. While these polymers have been used in various industries, it is their emergence in the cosmetic, pharmaceutical, and biomedical industries that is at the forefront of their research today. Their unique properties make them an ideal candidate for applications such as drug delivery, protein separation, enzyme immobilization, and biosensor development [3, 4].

Polyelectrolyte solution behavior is particularly unique due to its combination of long-chain molecule (polymer) properties and charge interaction properties. The ionic groups present on polyelectrolyte chains lead to both intra- and inter-macromolecular interactions whose strength and range vary with charge density and charge type, among other factors. Short-range interactions affect the stiffness or persistent length of the chains, while long-range interactions affect the excluded volume or the volume occupied by the chain. These interactions are much more important for polyelectrolytes than uncharged macromolecules since they can be considerably stronger and longer in range [5]. Inter- and intra-macromolecular interactions are both greatly influenced by the presence and concentration of electrolytes or salt in solution. These small ions act to screen polyelectrolytes from charges present on themselves and the charges of other polyelectrolytes in the solution, through interactions between the bound charges on polyelectrolyte and mobile charges in solution.

Polyelectrolyte solution theory involves the incorporation of electrostatic interactions as the main driving force

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for solution behavior. The complexity intensification of polyelectrolyte solution properties is further enhanced by the presence of counterions in solution. In an aqueous environment, macromolecules are subject to a wide range of forces and interactions, in part due to the polarity of water molecules. These interactions are present to varying degrees and can include some or all of the following: Van der Waals forces, polar and/or hydrogen bonding, electrostatic interactions, as well as Brownian movement [6]. Polyelectrolytes also interact with other polyelectrolytes, which are governed greatly by Coulombic forces [7]. Counterions in an aqueous solution act as a screening agent of the repulsive effects of like-charges along the chains, which can result in a less linear (or stretched/expanded) profile [8]. Although screening does occur with counterions, screening can be greatly enhanced by the addition of dissolved salts or electrolytes into the solution, as is often the case with polyelectrolyte solutions [9]. Increasing the salt concentration increases the screening effect. This involves the use of the Poisson–Boltzmann equation, as an extension of the Debye–Hukel system [5].

The true complexity of salt-added polyelectrolyte solutions is yet to be discovered, but some theoretical models have been developed for solutions both with and without salt [2, 10–16]. A very important experimentally observed phenomenon particular to polyelectrolytes in solution is the so-called polyelectrolyte effect. In salt-free solutions, the polyelectrolyte undergoes chain extension as the like-charges on each monomer unit electrostatically repel each other. On the other hand, when salt is added to the solution, the ions act to screen the like-charges from one another and the polyelectrolyte adopts a semi-collapsed random coil conformation resulting in a decreased hydrodynamic volume. Although polyzwitterions are considered polyelectrolytes, they have been shown to exhibit an “anti-polyelectrolyte effect.” This results in an opposite observation of solution conformation. In salt-free conditions, the polyelectrolyte is in a collapsed random coil (or globular) conformation due to the electrostatic attraction between oppositely charged groups. When salt is added, this attraction is shielded, and the polyelectrolyte undergoes a globular to expanded coil conformation transition, thus increasing the hydrodynamic volume [17]. It should be pointed out that this effect depends greatly on the type of zwitterionic polymer. For example, copolymers of alternating anionic and cationic monomer units are likely to experience the effect to a greater extent than those containing a single monomer unit type with both cationic and anionic groups [18]. The extent of both polyelectrolyte and anti-polyelectrolyte effects depends on the parameters particular to each polyelectrolyte. Some interesting papers include the examination of divalent salt ion influence [19, 20], reviews of polyampholyte solution behavior [4, 7], and solution effects on polyelectrolyte and polyampholyte microgels [21].

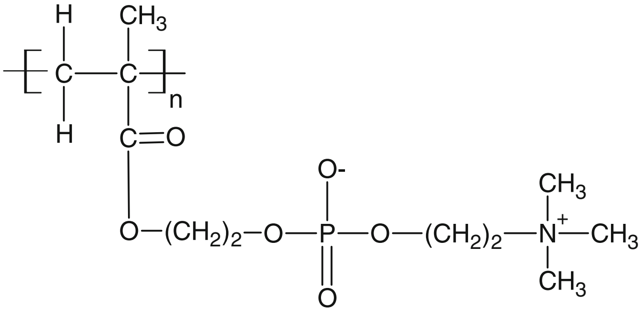
Various techniques such as X-ray scattering, dynamic light scattering [22], nuclear magnetic resonance, viscometry [23], atomic force microscopy [24], and electrical conductivity measurements have been successful to varying degrees at examining polyelectrolyte solution properties. Size exclusion chromatography (SEC, also called gel permeation chromatography) is another technique which has been a very popular method of characterization for various types of polymers but there are many issues surrounding its use in polyelectrolyte studies. One area of particular interest is the minimization of non-size exclusion effects and other sources of error associated with polyelectrolyte characterization studies [2, 25–33].

In addition, a series of papers has been published that aimed to discover ideal system conditions for SEC analysis of polyelectrolytes, such as calibration methods and tools, column packing types and mobile phase optimization, in terms of solution pH, salt, and other additives [1, 33–39]. Some models have also been developed that predict polyelectrolyte behavior in SEC columns under various solution conditions [40–42].

This work utilizes SEC to compare changes in hydrodynamic volume of a zwitterionic polyelectrolyte when in the presence of various salt concentrations and types. Although SEC is often noted as an excellent tool for molecular weight determination, it is also very valuable in hydrodynamic volume studies. Due to the exclusion of standard requirements when determining hydrodynamic volumes, many of the inaccuracies found in molecular weight determination of polyelectrolytes by SEC are not an issue. All of this work requires only relative hydrodynamic volumes or changes in hydrodynamic volumes not precise molecular weights; therefore, in the context of this current study, the only issues that need to be addressed are in relation to secondary or non-size exclusion effects. Due to the nature of our system, some of the secondary effects can be immediately discounted, such as ion exchange and ion inclusion. The remaining secondary effects as well as other sources of error, such as aggregation, can also be disregarded, thanks to the extensive research already conducted by other researchers.

The specific polyelectrolyte we examined was poly(2-methacryloyloxyethyl phosphorylcholine) or PMPC as shown in Scheme 1, which is a relatively new zwitterionic polyelectrolyte, owing both its positive and negative charges to the phosphorylcholine group of the monomer. MPC polymers are water soluble.

Its polymerization has achieved good control using various living polymerization techniques. Although production is still more difficult than other polymers used in similar applications such as poly(ethylene oxide), there



Scheme 1 Poly(2-methacryloyloxyethyl phosphorylcholine)

exists some real advantages to the use of PMPC. The relatively recent interest is mainly due to its phosphorylcholine (PC) end group, which can also be found on the outer membrane of erythrocyte cells. Therefore, polymers containing this end group are considered biomimetic and have been shown to resist initial immune response such as protein absorption and cell adhesion. Extensive work on this particular polymer has basically been limited to studies concerning grafting of PMPC with examination of protein adsorption response [43–45], bio-applicability of PMPC in terms of degradation rates and platelet adhesion [46–49], the effect of pH on interfacial absorption [50], and polymerization kinetics [51]. While no research has been conducted on the full PMPC polymer in terms of solution properties, some solution studies have been conducted on other polymers containing the PC end groups [18, 52, 53].

Although all previous experimentation has not included any salt studies, other than ionic strength effects [54], they have succeeded in showing the wide applicability of the PMPC polymer, specifically in the biomedical fields. Understanding of the solution behavior of polyelectrolytes containing biological-like end groups such as phosphorylcholine is also likely to aid in the understanding of biological functions and reactions [15].

Looking at the whole picture, relatively little is known about polybetaine solutions. Despite the high number of publications concerning polyelectrolytes, no studies have been reported that give a complete and comprehensive examination of the interaction of polybetaines with salt. Polybetaine is an ampholytic polymer in which pendant groups have a betaine-type structure; it is a type of zwitterionic polymer. This lack of knowledge provides an excellent opportunity for discovery, which can be very useful for both fundamental knowledge and industrial application.

In this work, we propose a comprehensive method for the examination and analysis of polybetaine solution behavior under various salt solution conditions. By utilizing size exclusion chromatographic techniques, a clear methodology of polyelectrolyte–salt interactions can be designed. The clearly outlined method can also be applied to the study of other polyelectrolyte types (cationic, anion, and polyampholytes), and the resulting fundamental knowledge is beneficial for understanding all polyelectrolytes in solution.

Experimental section

Equipment

SEC analysis was conducted using a Waters® 2690 Separation Module equipped with a 2410 differential refractive index detector and four Waters® Ultrahydrogel Linear (packed with a hydroxylated polymethacrylate-based gel) columns enclosed within a heating unit. Retrieval and analysis of the SEC data was done with the Waters Millennium ^{32}® Chromatography Manager software program.

Mobile phase preparation and conditions

The solvent consisted of three parts: deionized water from the Barnstead EasyPure® II Ultrapure water system, sodium azide, and a salt of choice. The sodium azide (NaN_3) at a concentration of 0.1% *w*/*v* was used to deter bacteria growth within both the solvent reservoir and the SEC columns. This concentration has been shown to be a sufficient amount for this purpose while minimizing changes of solution properties. A mobile phase containing the sodium azide additive alone was used as the control. All the salts were purchased from Caledon Laboratories, except potassium chloride and the sodium azide, which were purchased from Sigma-Aldrich Canada.

Polyelectrolyte sample preparation

Polyelectrolyte samples were obtained from fellow researcher Wei Feng, who previously synthesized PMPC through atom transfer radical polymerization. Experiments previously conducted by Feng with use of the SEC determined the molecular weights to be approximately 1,500, 3,000, 15,000, 30,000, and 60,000 g/mol. These experimental values were in agreement with the theoretical values. During polymerization, salts such as copper bromide are used. To ensure the removal of the majority of the salt ions, dialysis was run for 5 days on all polyelectrolyte samples.

The polyelectrolyte solution samples were made using a concentration of 1 mg of polyelectrolyte per milliliter of solvent. After a sample of approximately 1.5 g was dissolved and mixed for 5 min, it was then filtered and transferred to the SEC specific vials. Samples were then run immediately to ensure that the time in solution was the same for all samples with the same molecular weight value. The system parameters and conditions were column temperature, 35°C; detector temperature, 40°C; flow rate, 1 mL/min; sample concentration, 1 mg/mL; sample run time, 55 min; base elutant, deionized water; additive, 0.1% *w*/*v* NaN_3.

For each different solvent condition, five samples were prepared, one for each molecular weight ranging from 1,500 to 60,000 g/mol. In order to ensure reproducibility, every sample set included a repeat of the 1,500 g/mol molecular weight sample. This was then compared with the original run to determine the degree of equipment reproducibility.

Reproducibility was evident from initial results. By comparing the difference between the first and second run for each solvent condition, it was found that the average difference was 0.0226 min and that the greatest difference was 0.081 min. To further examine reproducibility, variance was determined from the 1,500 g/mol sample in the 0.3M $CaCl_2$ solution with $n=4$. The variance was calculated to be 0.03584 min and can be seen in Fig. 1 where the variance was applied to all five molecular weight samples for the $CaCl_2$ data of 0.0005- and 0.3-M concentration sample sets.

Results and discussion

For this study, the salt properties were divided into two main categories: solution properties and ion properties. The former involve the characteristics of the solution as a whole entity and how changes in these characteristics affect the polyelectrolyte solution behavior. These properties include salt concentration or molarity, ionic strength, and solution pH. The ion properties category is divided into two subsections, namely cation effect and anion effect. Within each subsection, the size/type and the valency of the ion are examined. The anion section involves the additional investigation of ion configuration, which examines any differences in effect between monatomic and polyatomic anions.

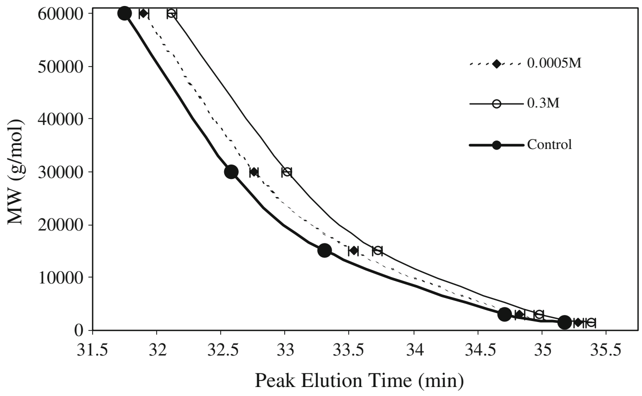


Fig. 1 Graph to show reproducibility of data. *Error bars* show variance that was calculated to be 0.03584 with $n=4$. Curves are of $CaCl_2$ solution at 0.0005- and 0.3-M concentration, and the control samples

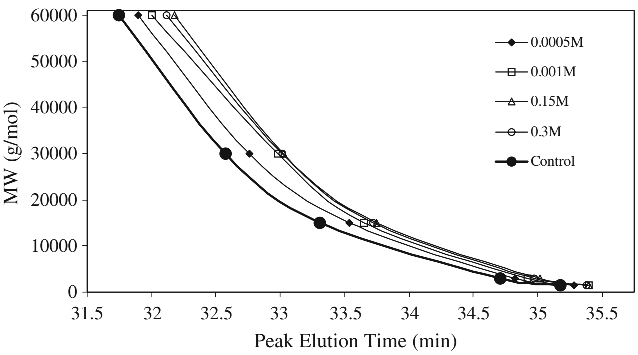


Fig. 2 Effect of $CaCl_2$ salt concentration on elution time of PMPC

Solution properties

Effect of salt concentration

In order to examine the effect of salt concentration on the hydrodynamic volume of PMPC, a variety of concentrations of calcium chloride, varying between 0.0005 and 0.3M were used (see Fig. 2). Each curve shows the peak elution time for all five molecular weight samples. A decrease in hydrodynamic volume is denoted by a shift to a higher elution time (shift to the right).

It can be said that all of the salt concentrations have an effect on the hydrodynamic volume, as evident in reference to the control sample, which contains only 0.1 wt.% of sodium azide (NaN_3). A comparison between the 0.0005- and the 0.001-M curves shows that an increase in salt concentration decreases the hydrodynamic volume of the polyelectrolyte. A further comparison of the three highest salt concentration seems to suggest that higher concentrations of salt have little to no greater effect on the hydrodynamic volume than the 0.001-M concentrations. A more in-depth study of salt concentration was conducted in order to get a clearer understanding of salt concentration at all levels.

Figure 3 shows the effect of seven different calcium chloride salt concentrations on the 1,500- and 3,000-g/mol molecular weight PMPC samples. For clarity, only two molecular weight series are shown, but it should be noted that similar results were found for all molecular weight samples ranging from 1,500 to 60,000 g/mol. Error bars representing the maximum variance of 0.081 min have been used to show that the trends are not a result of experimental errors.

The two curves each contain four regions: (1) the hydrodynamic volume decreases with increasing salt concentration, (2) the hydrodynamic volume remains constant with increasing salt concentration, (3) the hydrodynamic volume

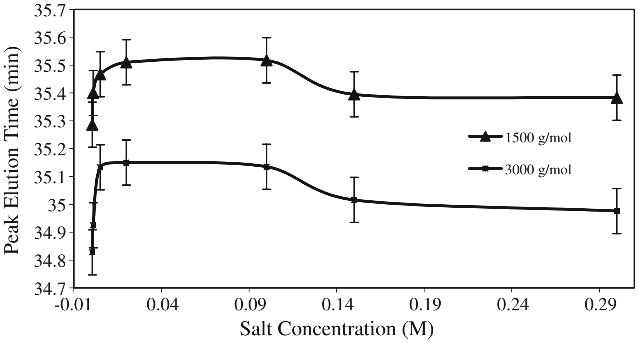


Fig. 3 $CaCl_2$ concentration effect past the "saturation point," as seen by two different molecular weights

increases with increasing salt concentration, and (4) again, the hydrodynamic volume does not change with increasing salt concentration. The first two regions were already observed in an earlier section but the last two sections show a new phenomenon.

In order to explain the mechanism associated with the four distinct behavioral regions, a more complete picture of the salt effect must first be conducted. Therefore, this will be discussed once all experimental parameters are analyzed.

Effect of solution ionic strength

Another important property when dealing with solution properties of polyelectrolytes is the apparent ionic strength of the solution. Ionic strength of a solution can be determined by the following equation:

$$I = \frac{1}{2}\sum_i c_i Z_i^2$$

where c_i is the ion concentration and Z_i is the charge on the ion [9]. In this work the ionic strength is calculated using only the salt component and not the sodium azide component for simplicity reasons. This is assumed to not be of concern since all solutions carry the additional ionic strength from the sodium azide and that since the sodium azide is in such low concentration.

For this investigation, two salts were chosen, namely sodium sulfate (Na_2SO_4) and sodium nitrate ($NaNO_3$). It should be noted that the ionic strength for the 0.1-M Na_2SO_4 and the 0.3-M $NaNO_3$ solutions are 0.3 mol/L (see Fig. 4). Comparison of these curves shows that different values of hydrodynamic volume are attained. If ionic strength were an important parameter, one would expect that solution of the same ionic strength would result in similar hydrodynamic volume values. Since this is clearly not case, even when considering the predetermined variance, some other parameter must be responsible for the hydrodynamic volume changes. Once the parameter (or parameters) is determined, it is likely to dominate over ionic strength. For this reason, it is believed that, although it cannot be concluded that ionic strength plays no role in the hydrodynamic volume changes of PMPC, it is fairly clear that it is not the only variable, and it is likely not a very important variable in the effect of salt on the hydrodynamic volume of PMPC.

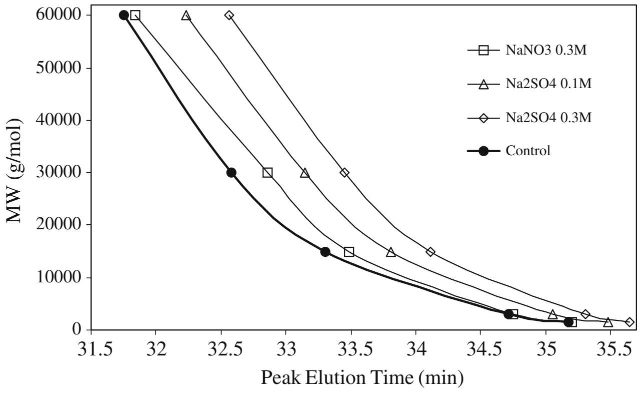


Fig. 4 *Graph* shows effect of ionic strength. Ionic strength of $NaNO_3$ at 0.3 M = ionic strength of Na_2SO_4 at 0.1 M

Effect of solution pH

All of the solutions produced throughout this work had a pH value between 7 and 8.5. Although this pH range is fairly small, it is important to determine if pH has any significant effect on the hydrodynamic volume of PMPC. In order to examine the pH effect, two new salts were utilized, namely disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4). The pH was found to be 8.9 and 5.2 for Na_2HPO_4 and NaH_2PO_4, respectively. In solution, both salts form anions of phosphate (PO_4^{3-}), hydrogen phosphate (HPO_4^{2-}), and dihydrogen phosphate ($H_2PO_4^{-}$), and both contain the sodium cation. Examination was done with three salt solutions, namely 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4, and 0.15 M Na_2HPO_4. The first two salt solutions provide a comparison at the same salt concentration. The 0.15-M Na_2HPO_4 was used to determine whether or not "saturation" had occurred. It is important to address saturation, since without reaching the saturation point, concentration effects may alter values. As can be seen in Fig. 5, all three curves fall on the same values. Due to the similarity of the solution characteristics, it can be concluded with high probability that the pH difference between the different solutions has no effect on the solution behavior or hydrodynamic volume of the PMPC polymer.

Since no significant difference was observed with the large pH range (approximately four points) used in this study, it is accurate to state that a pH range of approximately 1.5 points (as with all the other salts) does not directly alter the hydrodynamic volume of PMPC.

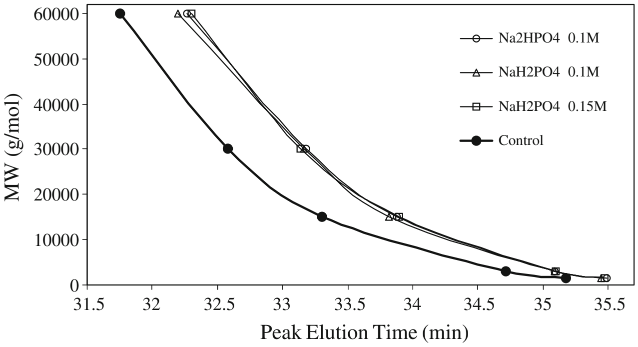


Fig. 5 Graph to examine effect of solution pH. PH values: Na_2HPO_4~5 and NaH_2PO_4~9

Ion properties

Effect of cation size/type and valency

In order to determine the effect of cation size and/or type, appropriate salts need to be selected, which consist of the same anion and differently sized cations. We chose sodium chloride (NaCl) and potassium chloride (KCl) salts. The molecular mass of Na^+ and K^+ are 22.99 and 39.10 g/mol, respectively, which gives a significant mass range. It should be noted that the radius of K^+ is significantly larger due to the presence of additional electron orbitals, 3p and 4s,and therefore, it can be said that the K^+ cation is considerably larger in both volume and mass than the Na^+ cation.

In Fig. 6, the curves of both KCl and NaCl at 0.1 M concentration are compared. Since the anion type, anion concentration, salt concentration, and cation valency are the same for each curve, any difference can be directly attributed to the cation size/type. The great similarity of the two curves leads to the conclusion that the K^+ and the Na^+ ions affect the hydrodynamic volume to the same degree. Therefore, no cation size/type effect occurs with respect to hydrodynamic volume change.

To examine the cation valency effect, the salts chosen were NaCl, with monovalent Na^+ and $CaCl_2$, with divalent Ca^{2+}. The comparison of these two salts is slightly more complex due to the fact that a 1-M $CaCl_2$ solution contains twice as many Cl^- ions and three times the value of ionic strength as a 1-M NaCl solution. Since it was previously determined that the ionic strength effect is linked to the salt concentration, it is not necessary to examine this aspect, but for a complete methodology, a comparison of the same ionic strength was also conducted.

Figure 7 shows that comparison of the monovalent and divalent cations. In order to fully address all possible parameter variations, three concentrations of $CaCl_2$ were compared to a 0.3-M NaCl solution. $CaCl_2$ at a concentration of 0.3M directly compares to the 0.3M NaCl solution under constant salt concentration. The 0.15-M $CaCl_2$ solution compares to the 0.3-M NaCl solution under constant anion concentration, and the 0.1-M $CaCl_2$ solution compares to the 0.3-M NaCl solution under constant ionic strength. On an immediate observation, it can be seen that the four curves all fall on the same values (well within the variance, 0.03584). From this, it can be concluded that the cation valency has no effect on the hydrodynamic volume of PMPC.

Effect of anion size/type and valency

In order to examine the anion size/type effect on the hydrodynamic volume of PMPC, two salts were selected that contain anion molecules of significantly different size. In this case, KCl, with the Cl^- anion and KI with the larger I^- anion were used, where the molecular mass of the Cl^- anion and the I^- anion are 35 and 128 g/mol, respectively. Although it is not necessary to maintain the same cation within both salts, due to the conclusion above that the cation has no effect, it is beneficial to specifically compare the variation due to anion size/type. This is also achievable since both anions are monatomic and monovalent in nature.

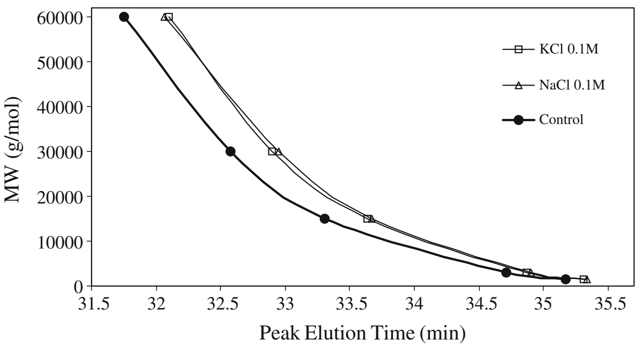


Fig. 6 Graph examining the difference in effect between Na^+ and K^+ ions. $Na^+ < K^+$

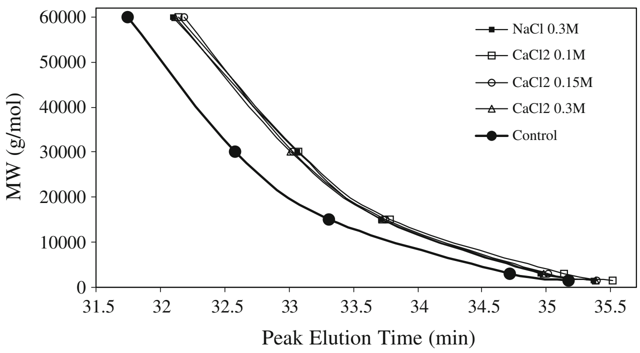


Fig. 7 Graph examining the difference associated with cation valency. Monovalent Na^+ is compared with divalent Ca^{2+}

By comparing the KCl and the KI curves in reference to the control curve, found in Fig. 8, one can see that clear conclusion can be made about the size/type effect. The KI curve seems to have no effect on the hydrodynamic volume of PMPC since all values fall on the control curve. The KCl curve on the other hand seems to have a substantial effect on the hydrodynamic volume of PMPC as compared to the control curve. With a shift to the right (i.e., higher elution time) of all molecular weight values, it can be concluded that the KCl greatly decreases the hydrodynamic volume of PMPC when in solution, or in other words, a smaller salt anion affects the hydrodynamic volume of PMPC more than the larger salt ion.

By giving particular attention to the KI curve, one can see a very interesting phenomenon. This salt appears to have no more influence on the polyelectrolyte hydrodynamic volume than the control case, which contains simply NaN_3 0.1% *w*/*v*. The conclusion is that the larger anion has less of an effect, and in the case of the very large I^- ion, it has no effect at all. One may think that a possible reason for the lack of effect of the larger ion is due to the steric hindrance. Although this seems plausible, it is believed that other factors play a greater role in the large anion's inability to alter the hydrodynamic volume of PMPC.

A similar examination of the effect associated with the anion valency was conducted with sodium nitrate ($NaNO_3$) and sodium sulfate (Na_2SO_4) salts. The NO_3^- and SO_4^{2-} anions provide a good comparison between monovalent and divalent salt ions, while maintaining the same cation. These anions are of comparable size, where the molecular weight of NO_3^- and SO_4^{2-} are 62 and 96 g/mol, respectively. In addition, both anions are polyatomic, and thus, both have polarity.

By comparing concentrations of 0.3 M for $NaNO_3$ and 0.15 M for Na_2SO_4, both the concentration of the cation and the number of negative charges in solution from the anion are equivalent. Figure 9 shows these two concentrations along with the control curve.

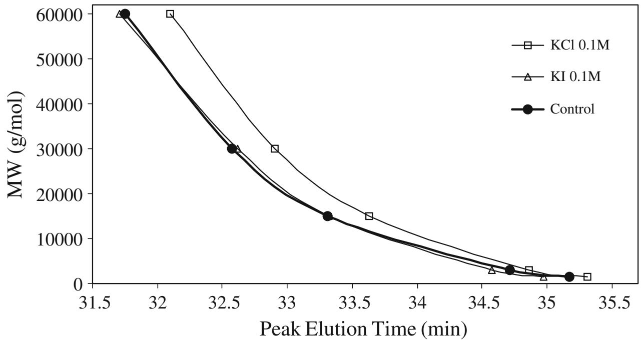


Fig. 8 Graph examining the difference in effect between Cl^- and I^- ions. $Cl^- < I^-$

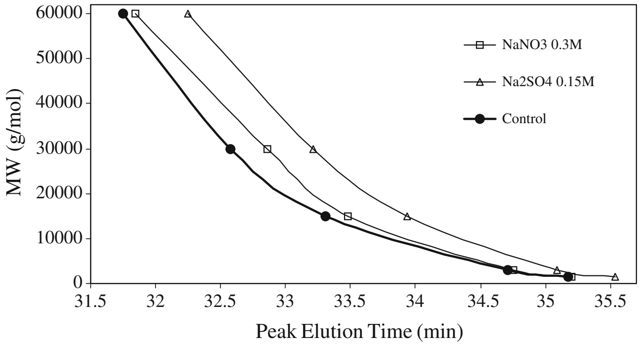


Fig. 9 Graph examining the difference associated with anion valency. Monovalent NO_3^- is compared with divalent SO_4^{2-}

It is observed that the SO_4^{2-} anions have a considerably greater ability to decrease the hydrodynamic volume of PMPC than the NO_3^- anions. This is approximately on the order of 0.5 min, which can be considered quite a significant shift, especially when taking into account that the time lapse between the 1,500- and the 3,000-g/mol sample is only about 0.5 min. From these observations, two conclusions can be made: (1) Both the NO_3^- and SO_4^{2-} anions decrease the hydrodynamic volume of PMPC, and (2) the divalent SO_4^{2-} decreases the hydrodynamic volume to a significantly greater extent than the monovalent NO_3^-.

Effect of anion configuration

For the anion configuration study, NaCl and $NaNO_3$ salts were used to examine the difference between a monatomic Cl^- ion and a polyatomic (four atoms) NO_3^- ion. In Fig. 10, we compare both salts at 0.3 M, which allows for equivalency of other solution properties such as solution concentration, solution ionic strength, cation concentration, and anion valency.

It is clearly observed that the Cl^- ion decreases the hydrodynamic volume of PMPC to a greater extent than the NO_3^- ion. At this point, it can be concluded that due to the

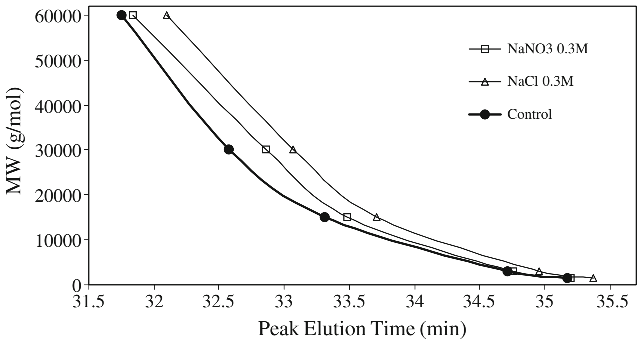


Fig. 10 Graph examining the effect of anion configuration through comparison of the monatomic Cl^- ion and the polyatomic NO_3^- ion

equivalency of the other solution and ion properties, a monatomic anion has a greater effect on the hydrodynamic volume of PMPC than a polyatomic anion. It should, however, be noted that while the examination of the Cl^- and NO_3^- ions does rule out most of the other solution and ion properties, it does not discount effects associated with anion size/type. Due to the difference in size (molecular masses of Cl^- and NO_3^- are 35 and 62 g/mol, respectively), the conclusion of anion configuration cannot be fully verified at this time.

Effect of other properties related to the anion

In addition to the structural mechanism, other factors point to the reasoning behind the effect of the various anion characteristics: type/size, valency, and configuration. One reason may be related to energy minimization of the system. The driving force for the interaction between the SO_4^{2-} anion and two cationic charge groups may be associated with the neutralization of the −2 charge. The divalent nature of the SO_4^{2-} ion requires that it "binds" with two positive charges (in other words, two cationic groups) to achieve neutrality. Due to the fact that this neutralization results in a lower energy state, the anion's ability to "pull" two cationic groups together is high, much more so than a monovalent anion. If this rationale is true, it can be concluded that, while the surface charge density is very important when comparing anions of the same valency, it is not as important when comparing ions of different valency (i.e., −1 compared to −2). It should also be noted that anion parameters such as ionization energy, electronegativity, and electron affinity all follow similar trends to the dissociation constant.

The most obvious explanation of the combined property phenomenon involves the effect of ionic potential on the hydrodynamic volume, i.e., the ability of the anion to decrease the hydrodynamic volume of PMPC through ionic interactions with the polyelectrolyte cationic group. Through examination of the data presented here, it is believed that two processes occur. Distinguishing between these processes is important when remarking on the effect of solution properties versus that of ion properties. The first process involves screening of repulsive forces, as explained earlier. This mechanism is linked to the solution properties discussed with respect to ionic strength and concentration. The second process is significantly more complex. It involves specific ionic interactions between the polyelectrolyte charge groups and the anions in solution. It is this process that is concerned with the ionic potential of the anion.

One way to describe ionic potential is through surface charge density. When addressing the fact that the monatomic anion, Cl^- has a greater ability than the polyatomic NO_3^- ion to decrease the hydrodynamic volume of PMPC, it may be insightful to examine how their surface charge densities differ. If we utilize the Stoke's radii (r_s) of Cl^-, 1.21 Å, and NO_3^-, 1.29 Å, [55] and assume that the anion is of spherical shape, the surface area can be calculated by $A=4\pi r_s^2$. From this, the surface charge density can be calculated by a ratio of anion charge to anion surface area. The resulting values for the Cl^- and NO_3^- anions are 5.435 and 4.782 charge/nm^2, respectively, or in other words, each negative charge covers 0.184 and 0.209 nm^2 of surface area for Cl^- and NO_3^-, respectively. These values clearly show that the surface charge density of Cl^- is higher than that of NO_3^-. Recalling that the Cl^- anion had a greater effect on the hydrodynamic volume of PMPC, it makes sense that Cl^- would have a higher surface charge density. A higher surface charge density means a higher ionic potential and a greater ability to interact with oppositely charged groups.

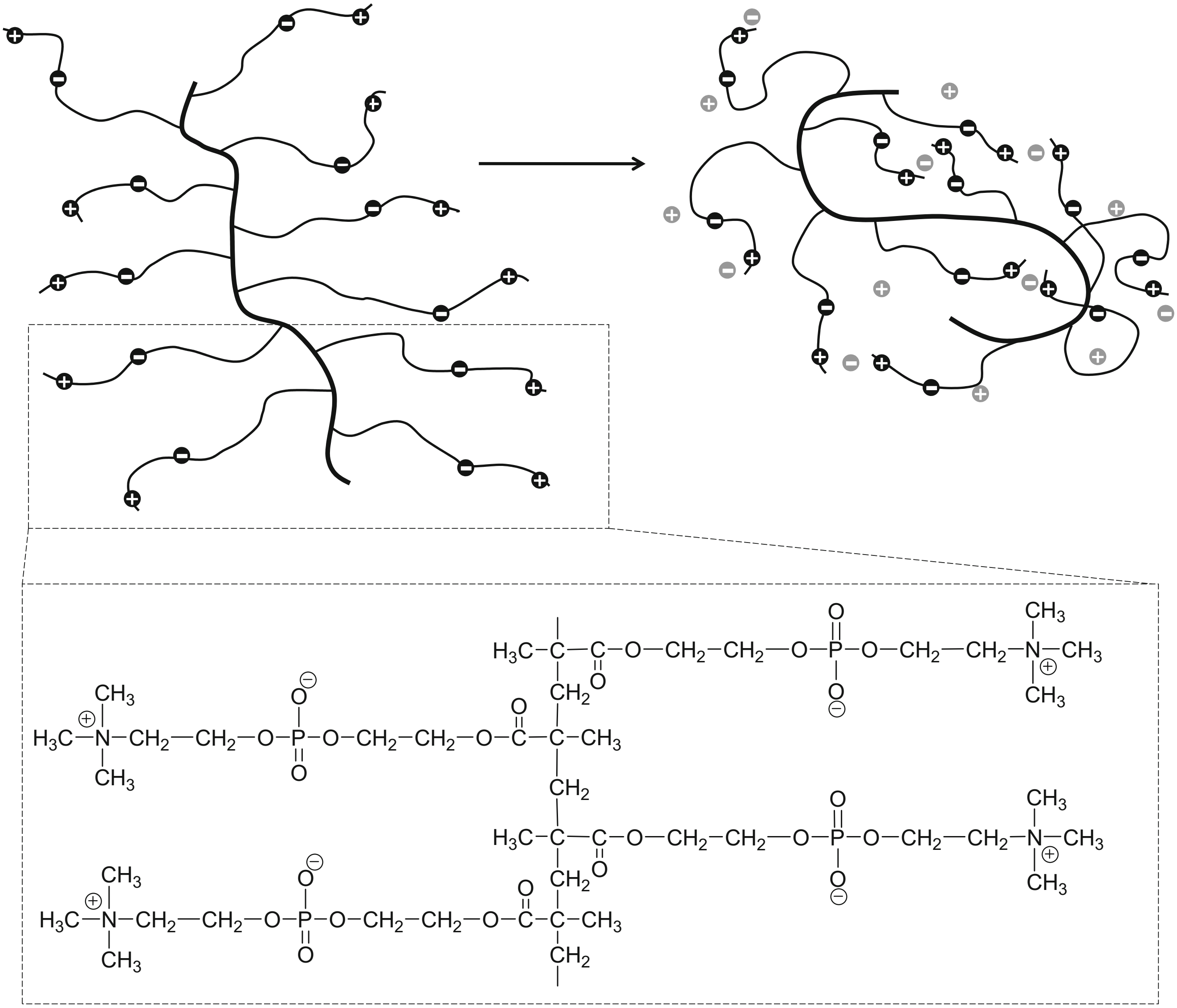
If we extend this investigation to include the SO_4^{2-} anion, we find that the results do not provide agreement with the data. Calculating the surface charge density for the polyatomic SO_4^{2-} anion, which has a Stokes radius of 2.31 Å [55], we find that each negative charge covers 0.335 nm^2 (2.983 charges/nm^2). From the data, it is expected that the surface charge density of SO_4^{2-} would be greater than that of Cl^- and NO_3^- due to the fact that it has a greater effect on the hydrodynamic volume of PMPC. Unexpectedly, we find that the opposite is true, and therefore, we find that the surface charge density trend does not apply to all of our data. Further reflection produces two possible reasons for discrepancy between the experimental data and theoretical values. One reason is the aforementioned energy minimization of the system (for the SO_4^{2-} anion), and the second is related to the inaccuracy of the surface charge density calculation, since the anions were assumed to be spherical in nature. In order to examine the validity of the former examination of a possibly more accurate volume charge density, comparison was done.

Obtained values from the literature set the anion volumes of I^-, NO_3^-, Cl^-, and SO_4^{2-} at 0.072, 0.064, 0.047, and 0.091 nm^3 (0.0455 nm^3 for volume per negative charge), respectively [56]. By comparing the values of volume per negative charge for the four anions used in this work, we find that the charge density increases as follows: $I^- < NO_3^- < Cl^- < SO_4^{2-}$, which is identical to the observed order of increasing effect on the hydrodynamic volume of PMPC. Since charge density is related to the size of the anion, the valency of the anion and the configuration (related to volume and surface area) of the anion, it can be said that combination of all the ion properties determines the degree of effect on the hydrodynamic volume. From this exciting discovery, it can be concluded that many solution and ion characteristics are responsible for changes to the hydrodynamic volume of PMPC.

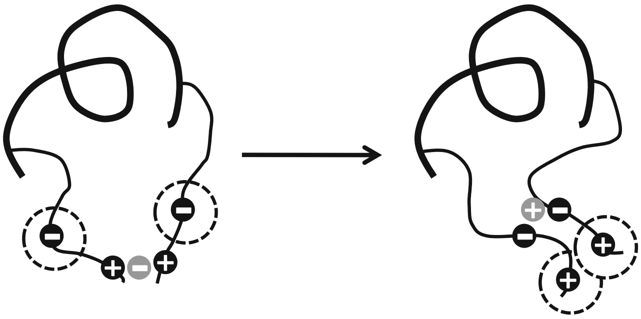
Polyelectrolyte–salt interaction mechanism

Although there may be cation presence within the polyelectrolyte volume, our results have shown that the cation is not responsible for the change in hydrodynamic volume. Furthermore, it has been shown that the anion component of the salt has a substantial effect on the hydrodynamic volume of PMPC. The reasoning behind the lack of cation effect and the existence of the anion effect can be visualized through Scheme 2. The scheme shows how the polyelectrolyte likely behaves in the presence of salt ions. The left-hand diagram shows how a polyelectrolyte adopts a linear configuration in solution due to repulsive forces between charge groups on different side chains. The right-hand diagram shows how, with the addition of salt, the polyelectrolyte is able to obtain a more random coil configuration, effectively decreasing the hydrodynamic volume of PMPC. It should be pointed out here that the PMPC samples used in this work have low molecular weights in a range of oligomers. Such short chains could not coil in a conventional sense and are actually expanded chains. In this text, we used the two terms interchangeably for convenience. The screening of repulsive forces and the divergence of side chains through ionic interactions with the salt anions in solution allow for the polyelectrolyte to attain this random coil configuration. It can be seen that pairs/trios of side chains, both adjacent and far are brought together by ionic interactions with the salt anions in solution, in which two or more polyelectrolyte charge groups essentially “share” a salt anion.

The lack of influence of the cation is related to the structure of PMPC in terms of side chain flexibility, steric



Scheme 2 Schematic showing how salt ions in solution can decrease the PMPC hydrodynamic volume

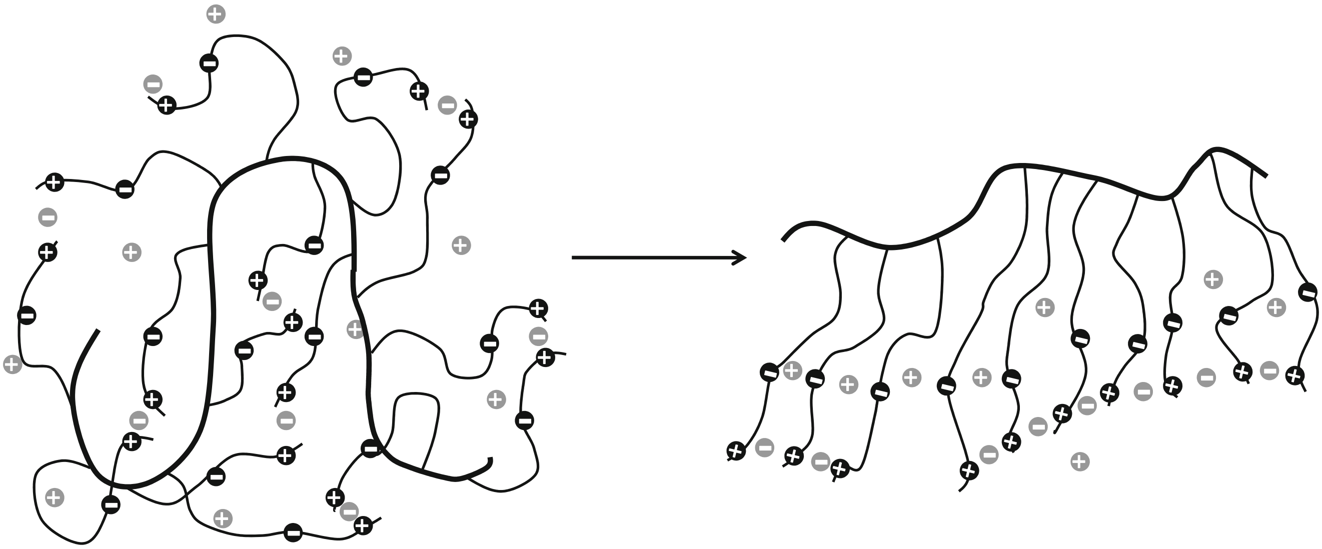


Scheme 3 Schematic showing why the salt anion has an effect and the salt cation does not. Ionic interaction of the polyelectrolyte with the cation can result in repulsive forces and steric hindrance

hindrance, and repulsive forces. Due to the closer proximity of the polyelectrolyte negative charge (anionic group) to the backbone as shown in Scheme 3, it is believed that interactions of solution cations with negative charges from two side chains are not strong enough to overcome these obstacles. The short distance between the negative charge and the backbone does not provide the sufficient flexibility required to bring about significant changes to polyelectrolyte conformation. This is visualized in the right-hand diagram of the scheme. Convergence of two anionic charge groups of the polyelectrolyte to one single salt cation requires that the side chain bend and flex, likely to implausible degrees. In addition, it is believed that the portion of the side chain adjacent to the negative charge [i.e., the $CH_2CH_2N^+(CH_3)_3$ component] causes steric hindrance if the bridge between two side chains occurs at the negative point. Lastly, it is likely that, if the anionic charge groups of the polyelectrolyte are brought together, the cationic charge groups would exert repulsive forces if a salt anion is not in screening position. This can be visualized by the dotted rings in the right-hand diagram in the scheme, which denote the area of charge presence. Here the two dotted rings are likely to overlap, resulting in strong repulsive forces that increase the energy of the system. In order to achieve stability, either the salt anions need to be situated in between these two charges or the side chains must diverge. In contrast, the interactions between an anion with two cations shown in the left-hand diagram of the scheme do not have these obstacles.

Now that it is has been determined that the anion is the superior influence on the hydrodynamic volume of PMPC, we can return to the effect of salt concentration in order to explain the mechanism associated with the four distinct behavioral regions. By isolating the first two regions, it is observed that increasing salt concentration decreases the hydrodynamic volume up to a certain "saturation point," after which further increase in the salt concentration has no additional effect on the PMPC solution properties. The saturation point is believed to occur at a concentration at which all the positive charges present on the polyelectrolytes have been shielded/screened from other polyelectrolyte charge groups, either from the same polyelectrolyte or from another polyelectrolyte in solution. Without screening, repulsive forces between polyelectrolyte charge groups cause chain expansion, which results in a more linear configuration. By screening the charges, repulsive forces are diminished and the polyelectrolyte is able to attain a more random coil structure.

Examination of the third region, where there is a shift back to a lower elution time (i.e., larger hydrodynamic



Scheme 4 Schematic showing how increase in salt concentration can increase the hydrodynamic volume of PMPC through increase in the number of ionic interactions

volume) at high salt concentrations, suggests that the ionic interactions present at lower concentrations have in some way been altered. Upon further evaluation, the current phenomenon seems to be quite logical and can be explained by considering the geometry of the more tightly coiled polyelectrolyte. Since the shift is only slight in comparison to the difference between 0.001 and 0.02 M, it is believed that distant chain end pair interactions are not affected but that changes to the adjacent/near chain end pair interactions are solely responsible. This may be explained by the schematic above (Scheme 4). Here, the increased presence of anions in solution may “pull” the cation end in multiple directions, resulting in a more expanded, linear configuration. The expanded polyelectrolyte is excluded from more column packing pores than the coiled polyelectrolyte, resulting in an earlier elution time and a larger hydrodynamic volume.

Although the transition from one region to another seems to occur at a certain concentration for $CaCl_2$, it is believed that these values are very much dependant on the salt type. Therefore, this study should be considered as a qualification of phenomenon and not a quantification of concentration data when applying to other systems. The presence of a saturation point as described here is likely to be beneficial in explaining other studies involving the effect of salt on polyelectrolyte characteristics [27]. Therefore, our finding provides a basis for other research and proves that extensive concentration ranges are particularly important when examining concentration effects.

Conclusions

In this study, we have gained some valuable insight into the effect of salt on the hydrodynamic volume of polyelectrolytes. Through the analysis of the extensive data obtained, the following conclusions were made: (1) Salt concentration has a significant effect involving four distinct behavioral regions (as explained by ionic interactions taking place in the solution), (2) ionic strength has no visible effect (any effect is related to the concentration component), (3) pH has no observable effect, (4) cation type/size and valency has no effect, (5) a smaller anion has a greater effect, (6) a divalent anion has a greater effect than a monovalent anion, and (7) a monoatomic anion has a greater effect than a polyatomic anion. Concurrent evaluation of the various properties resulted in the explanation of both intra- and inter-molecular polyelectrolyte forces and addressed the alternate mechanisms transpiring. Beyond the study of salt properties, we have succeeded in explaining the variation in the degree of hydrodynamic volume change with different molecular weight samples.

In addition to constructing a comprehensive profile of salt effects on PMPC, we have successfully developed a detailed methodology that can be utilized for various other polyelectrolyte studies. The information attained from such salt studies can be extremely beneficial in many areas of application, both on a large scale in the cosmetics, ceramics, papermaking, and biomedical industries, and for more specific applications such as release kinetics in drug delivery and control of grafting densities in polymerization grafting techniques. The discoveries in this study have shown that SEC provides an excellent means of obtaining a complete and accurate set of correlations between polyelectrolyte charge and salt effects. By following our set of experiments, clear deductive conclusions can be made with confidence.

Acknowledgment We thank the Natural Science and Engineering Research Council of Canada (NSERC) and the Canada Foundation of Innovation (CFI) for supporting this research. J.M. was supported by an NSERC Postgraduate Scholarship for her Masters study at McMaster University. J.M. also thanks Dr. Wei Feng, a team member, for preparation and donation of the PMPC samples.

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