SIZE EXCLUSION CHROMATOGRAPHY OF PMPC AND ITS INTERACTION WITH SALTS

SIZE EXCLUSION CHROMATOGRAPHY OF POLY (2-METHACRYLOYLOXYETHYL PHOSPHORYLCHOLINE) AND ITS INTERACTIONS WITH VARIOUS SALTS

By

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ABSTRACT

My current thesis is regarding the application of Gel Permeation Chromatography (GPC) equipment and principles to the study of polyelectrolyte configuration in solution. The main focus of this study is the effect of salt on the hydrodynamic volume/solution properties of polyelectrolytes and the ability of GPC to effectively determine the degree of variation. This involves the comparison of different salt types and concentrations in aqueous solution. The specific polyelectrolyte examined is poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC), a zwitterionic polymer (i.e. one having both positive and negative charges on the monomer). PMPC is a fairly new and promising polyelectrolyte for use in protein drug delivery and biomaterial surface applications. Understanding the effects of salt on this polyelectrolyte will aid in the development of technologies involving this polymer as well as other zwitterionic polymers.

Different salts were utilized to formulate an effect of salt ions so that a systematic analysis could be performed. Using a control as reference it was determined that the salt does have an effect on the solution properties of the polyelectrolytes, as expected. The specific properties examined were characterized into two categories; solution properties and ion properties. Solution properties involved the investigation of salt concentration, solution ionic strength and solution pH effects. For ion properties, salts were selected on the basis of ion charge, charge type and ion size, so that the effects of both the cation and anion components could be analyzed. Two parameters, namely, ion size/type and ion valency were examined for both the cation and the anion. In addition the configuration of the anion was also investigated. Specific conclusions found in this study were as follows:

1) The salt concentration has an effect up to a certain "saturation" point.
2) The ionic strength has no visible effect (any effect is related to the concentration component).
3) There is no observable solution pH effect.
4) There is no observable cation effect. This includes no cation type/size or cation valency effect.
5) There is a significant anion effect.
6) A smaller anion has a greater effect than a larger anion.
7) A divalent anion has a greater effect than a monovalent anion.
8) A monatomic anion has a greater effect than a polyatomic anion.

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 which is situated closer to the backbone (inside) is less important to the change in hydrodynamic volume resulting from ionic interactions with the salts since it is shielded by the positive charge group situated at the end of the side chain (outside). The observed phenomena were also explained by other chemical and physical properties such as charge density and ionic potential.

In addition to the original plan of study, other phenomena were observed and later explained, such as the presence of four distinct regions associated with salt concentration and the

variation in the degree of hydrodynamic volume change with different molecular weight samples.

The application of aqueous GPC equipment (including a differential refractive index detector (DRI)) and principles to the study of polyelectrolyte solution effects minimizes the detection equipment required and, provides sufficient resolution and accuracy for examination of solution properties while remaining time and cost effective. The project discoveries have shown that size exclusion chromatography provides an excellent means of obtaining a complete and accurate set of correlations between polyelectrolyte charge and salt effects.

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1 Introduction

1.1 Polyelectrolytes

Water-soluble polymers have drawn a lot of attention in recent years due to their environmentally friendly production conditions (water-based). These polymers can be divided into two main groups, ionic and nonionic character. Nonionic polymers are those that are soluble in water but do not contain or develop ionic groups in solution. Some examples of nonionic polymers include poly (vinyl alcohol) and poly (acryl amide). Ionic polymers on the other hand, exhibit charged groups when in solution and are often called polyelectrolytes (Bruessau, 1992). Polyelectrolytes can be defined as "macromolecules which, in a suitable solvent, usually water, carry a large number of ionized or ionizable groups (Finch, 1983)." Thus, a polyelectrolyte is any polymer that becomes charged upon dissolving in a polar solvent, most often water. The ion concentration can vary between different polymers but is usually on the order of one ion per monomer unit. Polyelectrolytes can be categorized as anionic (negatively charged), cationic (positively charged) or zwitterionic (carrying both positive and negative charge). Polyelectrolytes can also be separated into synthetically produced, such as poly (acrylic acid) and poly (vinylamine), naturally occurring, such as proteins and nucleic acids, or a combination of both, such as modified polysaccharides. Some common synthetic anionic-forming functional groups include sulfate and phosphate, and cationic examples include amine and ammonium.

Polyelectrolytes or ion-containing polymers have often been shaded in mystery, as little fundamental knowledge of them is known. P.G. de Gennes (Physics Noble Laureate) described ion-containing polymers as the "least understood form of soft-condensed matter (Seery & Dobrynin, 2004)." Full journal issues have been devoted to the field of polyelectrolytes, such as the June 1999 issue of Langmuir, the August 2003 issue of Journal of Physical Chemistry B and the October 2004 issue of Journal of Polymer Science B. In the introduction of the Journal of Polymer Science B October issue the editors clearly show the growing interest in this field with a chronological bar graph, as can be seen Figure 1.1. The growth is a clear indication of increasing industrial interest and the introduction of polyelectrolytes into a wider variety of application fields.

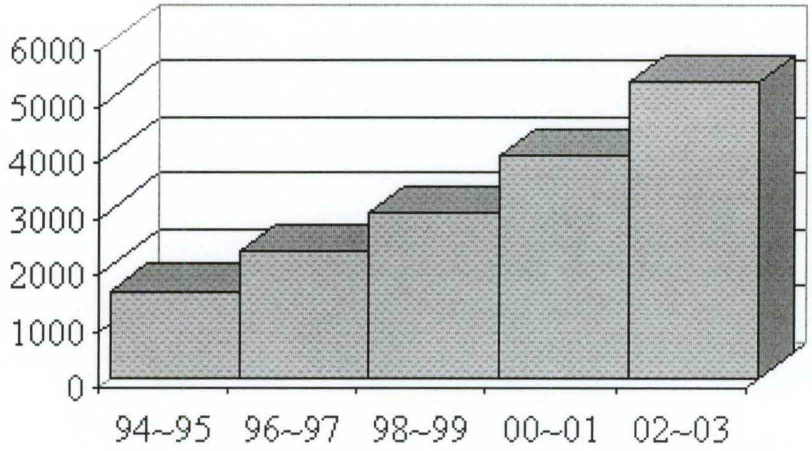


Figure 1.1: Number of publications in leading scientific journals on the topic of ion-containing polymers (Reprinted with permission from John Wiley & Sons, Inc.) (Seery & Dobrynin, 2004)

Zwitterionic polyelectrolytes are polymers that contain both positive and negative functionalities along the backbone and are also referred to as amphiphilic polyelectrolytes, polyzwitterions, polybetaines or polyampholytes (Tripathy, Kumar, & Nalwa, 2002). Some confusion about these polymers is due to the fact that various sources will distinguish differently between each name. Zwitterionic polyelectrolytes can be polymers consisting of a repeating single monomer unit, which contains both an anionic and a cationic group, denoted as polybetaine or copolymers consisting of both cationic and anionic monomers (i.e. separate anionic and cationic monomer units), often denoted as polyampholytes. In other cases, polyampholytes, although they have been considered to have similar properties to polyelectrolytes, are distinctly differentiated from polyelectrolytes by their acid and basic groups. In this case, polyampholytes are charged macromolecules, which carry both acid and basic groups, such as proteins and synthetic copolymers consisting of both acidic and basic monomer building blocks (Dobrynin, Colby, & Rubinstein, 2004). It should be noted that in general, but not necessarily in all cases, all charged polymers are refereed to as polyelectrolytes, regardless of their properties. For simplicity, a list of the different types of polyelectrolytes, their generic structure and some examples of each can be found in **Table 1.1**.

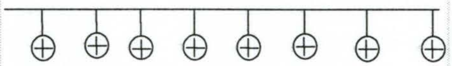
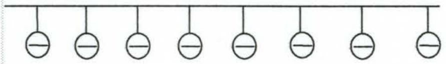
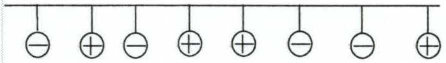
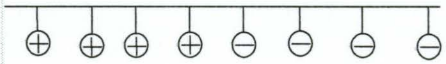
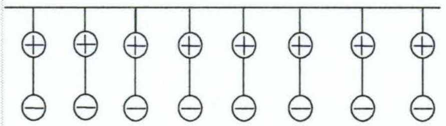
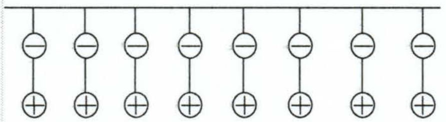
Polyelectrolyte Type	Sub-type	Description	Schematic	Examples
Cationic		Positive charge groups		Poly(vinylpyridine), Poly (ethyleneimine) Poly(dimethylaminoethyl methacrylate) (PDMAEMA)
Anionic		Negative charge groups		Poly(sodium styrene sulfonate) Poly(acrylic acid) Poly(methacrylate acid) (PMAA)
Zwitterionic	Random Polyampholyte	Positive & negative charges on different monomer units Random distribution		Gelatine Poly [acrylamide-co-sodium 3-acrylamido-3-methylbutanoate-co-(3-acryl-amido-propyl)-trimethylammonium chloride]
	Block Polyampholyte	Positive & negative charges on different monomer units Block distribution		PDMAEMA-b-PMAA Poly(sodium styrene sulfonate)-b-poly[4-(dimethylamino) methyl-styrene]
	Polybetaine Positive-Negative	Positive & negative charges on same monomer unit Positive charge closest to back bone		Poly [4-(2-acrylamido-2-methylpropanedimethylammonio) butanoate/hexanoate] Poly[3-(2-(N-methylacrylamido)-ethyl-dimethyl-ammonio) propane sulfonate]
	Polybetaine Negative-Positive	Positive & negative charges on same monomer unit Negative charge closest to back bone		Poly (2-methacryloyloxyethyl phosphorylcholine) Poly(N-isopropylacrylamide phosphorylcholine)

Table 1.1: List of different types of polyelectrolytes.

The lack of knowledge surrounding polyelectrolytes is possibly due to the fact that "polyelectrolyte" is an umbrella term, which encapsulates the various types of charged polymers, and therefore is a field within which very different properties can be observed. It is because of the lack of knowledge that a large amount of research is being conducted to better understand this field.

1.1.1 Applications

Water-soluble polymers are of great interest in industry due to their environmentally friendly, nontoxic production conditions and their associated lower costs. This is of course a driving force for the development and understanding of polyelectrolytes, which in most cases are water-soluble. In addition, scientific curiosity has also been a driving force for polyelectrolyte research due to the "mystery" surrounding such molecules, particularly these molecules in solution. (Natz & Andelman, 2002).

Zwitterionic polymers can exhibit very unique properties, especially in solution, which allows for many possible applications, such as associative thickeners, emulsifiers, rheology modifiers, solubilizers, colloids, etc (Tripathy et al., 2002). While these polymers have been used extensively in various industries, such as papermaking, ceramics and cosmetics, it is their emergence in the bio-medical industry that is at the forefront of their research today. Being dually charged allows for binding with multiple entities, which are characteristically different. This can be very applicable in drug delivery where polyelectrolytes can assist in various delivery schemes (e.g., injection, oral administration or transplantation) as a carrier, a protective barrier, a stabilizer or as the drug itself (Tripathy et al., 2002). Their unique properties can make them an ideal candidate for other biomedical applications such as protein separation, enzyme immobilization and biosensor development (Dobrynin et al., 2004). When conducting research on these polymers, it is very useful to determine their solution characteristics since most applications will require the polymer to be in solution, such as bodily fluids. Zwitterionic polymers are also of interest in the biomedical field because they can often be used to model naturally occurring polyampholytes.

Due to their wide range of properties, polyelectrolytes have already shown to be extremely useful in a large range of specific applications. Polyelectrolytes are often employed as a component of ionic polymer-metal composites (IPMC). These composites have the potential for large dynamic deformation and are thus very applicable as sensors, actuators or even as artificial muscles. Hydrophobically modified polyelectrolytes (HMP) have also been used in the biomedical field. Due to their unique ionizable and hydrophobic groups, HMPs have been examined for use in drug delivery systems, protein stabilization, etc. Another very interesting area of application for polyelectrolytes is in multilayer membranes. Alternating layers of cationic and anionic polyelectrolytes is an excellent method for the formation of dense separating layers often on the scale of 0.5nm to 3nm thickness. Another benefit of this material is its ability to be functionalized or alternately modified to decrease the incidence of fouling, which is arguably the most pressing issue in membrane separations (Tripathy et al., 2002). A further, more comprehensive understanding of polyelectrolytes and their properties is likely to lead to a wide range of new applications and the improvement of current applications.

1.2 Solution Properties

1.2.1 Polyelectrolytes in Solution

Polyelectrolyte solution behavior is particularly unique due to its combination of long-chain molecule (polymer) properties and the charge interaction properties. The ionic groups present on the polyelectrolyte chains lead to both intra- and inter-macromolecular interactions whose strength and range vary with charge density and charge type, among other factors. Intra-macromolecular interactions, as the name denotes, simply describe the interactions of charges with other charges on the same polyelectrolyte chain. Polyelectrolyte inter-macromolecular interactions occur between charges on different polyelectrolyte chains and can be characterized as either long or short range. Short-range interactions affect the stiffness or persistent length of the polyelectrolyte, while long-range interactions affect the excluded volume or the volume occupied by the polyelectrolyte. These interactions are much more important for polyelectrolytes than uncharged macromolecules since they can be considerably stronger and longer in range. Most often these interactions are significant enough that they cannot be ignored, as is often the case with dilute uncharged macromolecules. (Hara, 1993).

One of the main factors that affect the inter-macromolecular interactions is the polyelectrolyte concentration in solution. This aspect has such an influence on the solution properties of polyelectrolytes that most experiments conducted to examine other aspects of polyelectrolyte solutions had to be carried out at the "limit of finite dilution". Even experiments that are carried out under fixed polyelectrolyte concentrations often result in ill-defined conclusions due to the occurrence of complex concentration effects. In most cases the concentration effects have either been ignored or merely qualitatively accounted for. At present, the most successful inclusion of concentration effects into solution studies has involved mathematical or theoretical models (as will be discussed in a later section). Even though models have shown some progress, the overall conclusion reached with these models is that they lack the necessary complexity observed in real polyelectrolyte solutions. It is for this reason that most researchers have turned to infinitely dilute sample practices but this has also been of particular difficulty due to the lack of characterization techniques that are applicable at such low polyelectrolyte concentrations (Hara, 1993). Differential refractive index and nuclear magnetic resonance are two methods that have shown to exhibit sufficient sensitivity in some polyelectrolyte systems (Wu, 2004).

The differentiation between dilute, semi-dilute and concentrated polyelectrolyte solutions is of particular importance when discussing polyelectrolyte configurations. The conformational state of a polyelectrolyte will vary between two extremes, namely the collapsed random coil and the rod-like chain (see Figure 1.2). The relationship between concentration and polyelectrolyte configuration is attributed to viscosity and osmotic pressure (Bennemann, Brouers, & Quitmann, 1982), as well as like-charge repulsion at higher concentrations. Therefore, a polyelectrolyte will take on a rod-like configuration at dilute concentrations and random coil configuration at higher concentration. Obviously, the concentrations at which shape change occurs can vary greatly depending on the polyelectrolyte characteristics. Some aspects that greatly effect the "critical" concentration for conformational change include charge density, valency, molecular weight and chain length.

Figure 1.2: Polyelectrolyte configurations; random coil to rod-like.

This can be further understood by the linear charge density of a polyelectrolyte:

$$|\beta| = Zq / L,$$

where q is the elementary charge and Z is the number of the elementary charges fixed on the chain, whose contour length is denoted by L. From this, one can see that in dilute solutions (where inter-macromolecular interactions are minimal) an increase $|\beta|$ will result in an increase in hydrodynamic volume (decreasing the contour length), eventually reaching rod-like form. This does however require that the solution reach near infinite dilution and that there be an absence of electrolytes in solution (addition of which will be discussed later) (Finch, 1983).

A clear understanding of neutral polymer solution behavior has been in place for many years. This has included a set of theories for either flexible or semi-flexible chains. In flexible chain length statistics, also called the freely jointed chain model, the end-to-end radius, which is related to the hydrodynamic volume and polymer conformation, is simply related to the number of monomers in a chain (N) and the effective monomer size (a), where the mean squared end-to-end radius is denoted by:

$$\left\langle R_N^2 \right\rangle = Na^2$$

The semi-flexible chain statistics elaborates on this theory to include chain stiffness or elasticity that can be related to the persistence length (ℓ_0) or the average radius of curvature (allowing for thermal fluctuations). Combining this with the contour length of a chain (L), the end-to-end radius is thus denoted by:

$$R^2 = 2\ell_0 L + 2\ell_0^2 \left(e^{-L/\ell_0} - 1\right), \qquad \text{where } N = \frac{L}{2\ell_0}.$$

This equation has shown to give fairly accurate calculations of end-to-end radii when compared to experimental data (Natz & Andelman, 2002).

While these models have shown accuracy in neutral polymers, they cannot, for obvious reasons, be related to polyelectrolyte samples and thus other theories have been developed to deal with the differences between charged and neutral polymers. Polyelectrolyte solution theory involves the incorporation of electrostatic interactions as the main driving force for solution behavior. This is the main cause for the complexity intensification of polyelectrolyte solution properties, which is further enhanced by the presence of counterions in solution. Polyelectrolyte models deal with the presence of electrolytes, which are either counterions and/or salt ions in solution (depending on the salient conditions) and thus a discussion of such will continue in the next section.

1.2.2 Polyelectrolytes and Electrolytes 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 and Brownian movement (Van Oss, 1994). When polyelectrolytes are in solution they encounter different interactions with both electrolytes in the solution and the water molecules. Polyelectrolytes will also interact with other polyelectrolytes, which are governed greatly by Coulombic forces (Lowe & McCormick, 2002). The system that is examined in this work is entirely aqueous and therefore the discussion of electrolytes and polyelectrolytes in solution will be limited mainly to such systems. Polyelectrolyte solutions contain counterions in three different forms; free ions moving outside the volume occupied by the polyelectrolyte, ions that are bound but free to move within the polyelectrolyte-occupied volume, and ions that are bound to specific charged groups of the polyelectrolyte (Oosawa, 1971). When there is an absence of added salt, the total charge of the counterions in solution is equal to the total charge carried by the polyelectrolytes. Counterions, in an aqueous solution, act as a screening agent of the repulsive effects from like-charges along the chains, which can result in a less linear profile, as outlined in a previous section (Šegudovic, Sertic, Kovac-Filipovic, & Jarm, 1995). 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. In this case, the solution is made up of water (a polar solvent), polyelectrolytes, counterions and dissolved polar/ionic solids. The polarity of the salt determines whether or not it is soluble in water. Its specific solubility on the other hand is determined by the free energy of ion hydration and the free energy of the salt and water (Murrell & Jenkins, 1994). Therefore the balance of the three free energies determines the solubility of the salt. With the presence of four elements in the system the complexity surrounding the understanding of solution properties increases greatly. All of the aforementioned inter- and intra-macromolecular interactions are greatly influenced by the presence and concentration of electrolytes in the solution. These small ions act to screen the polyelectrolytes from charges present on itself and the charges of other polyelectrolytes in the solution. The screening is due to interactions between the bound charges on the polyelectrolyte and the mobile charges in solution.

Increasing the salt concentration will increase the screening effect. Theoretically if the added salt concentration Cs is greater than or equal to the charged polyelectrolyte concentration ZCp (i.e. if Cs ≥ ZCp), the charged polyelectrolytes will behave relatively similar to an uncharged macromolecule, with only small differences. Attempts have been made to account for the small differences by use of a theoretical scaling factor. This involves the use of the Poisson-Boltzmann equation, as an extension of the Debye-Hückel system. The Debye-Hückel theory deals with ordinary or simple electrolyte solution, where ions are viewed as spherical moieties and the Poisson-Boltzmann theory works with polyelectrolytes and their likeness to cylindrical entities. In most cases, the later is a vast approximation and will only apply when the Cs ≥ ZCp. Due to this, the approximation has not proven to be sufficient enough except as a rough estimate (Hara, 1993). It should also be noted that increasing the salt concentration could affect the quality of a solution. In most cases, the solution salt concentration will reach a point upon which the solution becomes a non-solvent resulting in the precipitation of the polyelectrolytes.

The true complexity of salt-added polyelectrolyte solutions is yet to be discovered but some theoretical models have been developed for both cases; without and with salt presence in solution:

$$\upsilon(r) = \frac{\ell_B}{r} \qquad (1) \qquad\qquad \upsilon_{DH}(r) = \frac{\ell_B}{r} e^{-\kappa r} \qquad (2)$$

$$\text{where } \ell_B = \frac{e^2}{\varepsilon k_B \mathrm{T}}, \quad \kappa^2 = 8\pi z^2 \ell_B c_{SALT}$$

and $k_B\mathrm{T}$ = thermal energy, e elementary charge, ε = medium dielectric constant. The first equation accounts for the Coulombic interactions in the absence of salt and the second equation shows the Debye-Hückel potential $\upsilon_{DH}(r)$ which when multiplied by the charges of the salts ions, z_1 and z_2, gives the effective (screened) electrostatic interaction, $z_1 z_2 \upsilon_{DH}(r)$. As can be seen by comparison of these two equations, the presence of salt can considerably increase the complexity of a polyelectrolyte solution. Although the second equation is used on occasion to give preliminary interaction properties for polyelectrolyte-salt solutions, it lacks significant accuracy primarily due to under- or over-stating assumptions. Thus, one would be led to believe that models predicting polyelectrolyte solution behavior, in the presence of salt need to be far more complex then the models, which have been previously developed. This may also lead one to believe that the only accurate means of predicting salt effects is through experimental methods (Natz & Andelman, 2002).

One 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 configuration resulting in a decrease in 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 configuration. In salt-free conditions, the polyelectrolyte will be in a collapsed random coil (or globular) conformation due to the electrostatic attraction between oppositely charged groups. When salt is added, the attraction is shielded and the polyelectrolyte undergoes a globular-to-extended-coil conformation transition, thus increasing the hydrodynamic volume (Fevola, Bridges, Kellum, Hester, & McCormick, 2004). 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 it to a greater extent than polymers containing a single monomer unit type with both cationic and anionic groups. Therefore in summary, while polyelectrolytes in solution are likely to shrink in the presence of electrolytes, polyzwitterions in solution can experience the opposite, namely chain expansion, as the concentration of electrolytes is increased (Miyazawa & Winnuk, 2003). The extent of both polyelectrolyte and anti-polyelectrolyte effects will obviously depend on the parameters particular to each polyelectrolyte or polyzwitterion and is therefore not always observed in every case.

To conclude, the effect of salt on polyelectrolytes has been studied to varying degrees. Various experimental methods and mathematical theories have been applied to polyelectrolyte solutions to determine the general effect of salt. Further discussion of both empirical and theoretical systems will be continued in the Previous Experimentation section of this work.

1.3 Poly(2-methacryloyloxyethyl phosphorylcholine)

Poly (2-methacryloyloxyethyl phosphorylcholine) or PMPC is a relatively new zwitterionic polyelectrolyte, owing both positive and negative charges to the phosphorylcholine group of the monomer. (Figure 1.3)

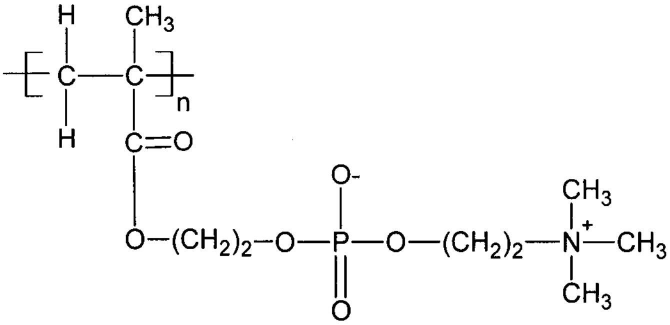


Figure 1.3: Poly (2-methacryloyloxyethyl phosphorylcholine)

Ishihara *et al* first produced the MPC monomer in 1998 (Ishihara et al., 1998). From this development extensive work has been conducted involving the use of the MPC molecule in biocompatibility studies and the biomedical applicability of PMPC (W. Feng, Zhu, Ishihara, & Brash, 2005; W. Feng, Brash, & Zhu, 2004; Ueda, Watanabe, Ishihara, & Nakabayashi, 1991). Its polymerization has achieved good control using various living polymerization techniques, including the relatively new and advantageous atom transfer radical polymerization (ATRP) method (Wang, Miyamoto, Hirano, Seno, & Sato, 2004). Although production is still more difficult then other polymers used in similar applications such as poly (ethylene oxide) (PEO), there exists some real advantages to the use of PMPC. This polyelectrolyte is of great importance and is becoming a highly researched polymer due to its various applications in the biomedical industry. The relatively recent interest is mainly due to its 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 adsorption and cell adhesion. It was suggested in 1998 that phospholipid polymers showed decreased amount of adsorbed proteins on the surface and it was concluded that this is likely due to the high water content (Rojas, Claesson, Muller, & Neuman, 1998), but the exact anti-thrombogenic mechanism is not clearly understood. Ishihara, in reference to PMPC, again proposed the binding of water molecules as the cause for the non-thrombogenic behavior. In detail, it is believed that the high content of bound water molecules leads to the creation of a hydration layer which in turn leads to the deterrence of protein and cell surface adhesion (Ishihara et al., 1998). It can be concluded that regardless of mechanism, a biomaterial containing a polymer with the PC group is likely very desirable and widely applicable.

Understanding of the solution behavior of polyelectrolytes containing biological-like end groups (such as phosphorylcholine) is also likely to help in the understanding of biological functions and reactions. It should furthermore be noted that due to the complex ionic behavior and the biomimickry of PMPC polyelectrolytes, work conducted on PMPC and similar polymers is likely to be very beneficial in the biomaterials/tissue engineering fields as well as life science in general (Vlachy, 1999).

1.4 Gel Permeation Chromatography

The field of chromatography has been around for many years and has grown to include a wide range of methods. This thesis work greatly employs the use of gel permeation chromatography (GPC) for the study of polyelectrolyte solution behavior. This type of chromatography is often termed gel filtration chromatography (GFC), size exclusion chromatography (SEC) or steric exclusion chromatography (SEC). The basic setup of a GPC machine is shown in Figure 1.4.

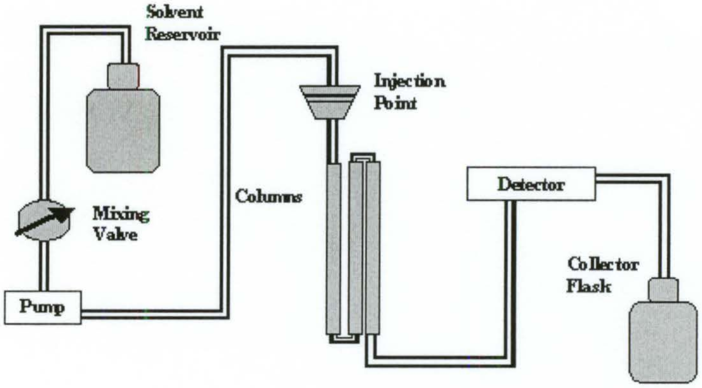


Figure 1.4: Gel permeation chromatography set-up.

It involves a set of components, namely a reservoir (or a set of reservoirs), a pump, a sample injection instrument, columns (can be one single column but in most cases there is more than one), a detector and a waste or fractionation collector.

1.4.1 Brief History

The size exclusion method has been around since the mid- to late-1950s when maize starch packed columns were used to separate samples of proteins in aqueous mobile phases (Wu, 1999). In 1959, the company Pharmacia developed a cross-linked dextran gel (Sephadex), specifically for use in GPC applications. Sephadex was found to successfully separate proteins based on their molecular weight when in aqueous environments. By the mid-1960s, the instrumentation was in place and development went into the manufacturing of various column packings. By 1964, an organic equivalent (Styragel®) was developed by the Waters Associates company, which is still considered the leading company in the GPC field (now called Millipore-Waters). The Styragel® column packing (rigid, cross-linked styrene/divinyl benzene resins) allowed for the first extensive use of organic-phase separation solvents and thus the separation of many more types and classes of polymers (Hunt & Holding, 1989; Wu, 1999). It was also at this time that a procedure for measuring molecular weights and molecular weight distributions was formulated and the term “gel permeation chromatography” was conceived (Mori & Barth, 1999). From that point to the present, the equipment and methods have become increasingly sophisticated. One of the main advances made since

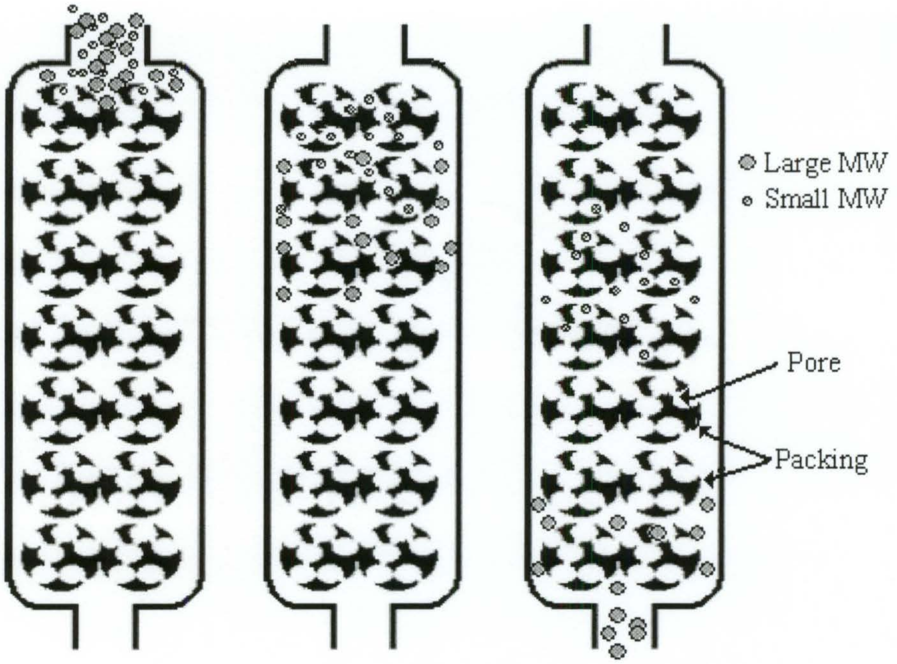
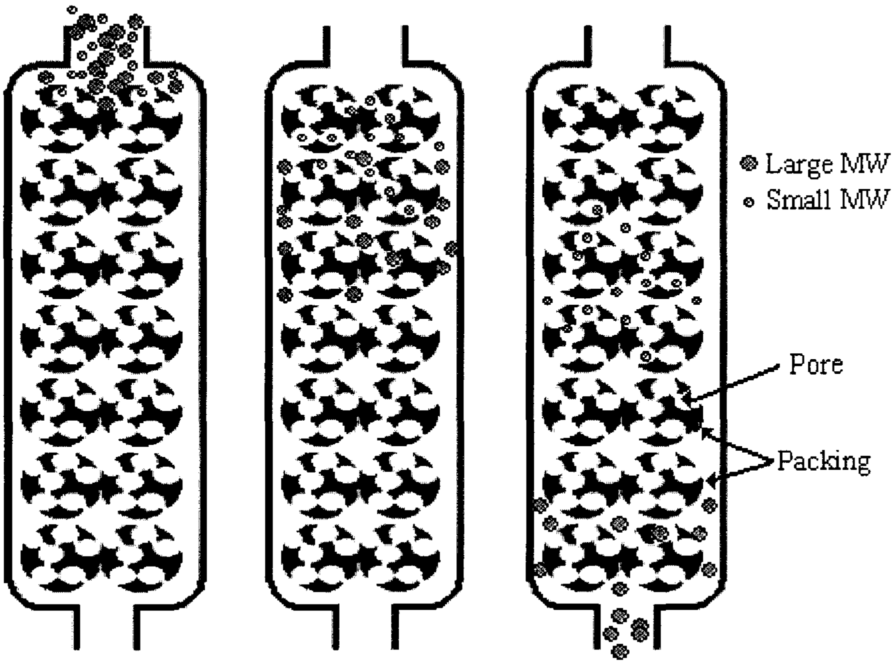


Figure 1.5: GPC separation of two different molecular weights (large and small).

The mobile phase (of various types) is continuously pumped through the system. Once the detector achieves a stable baseline, an injection of the sample is initiated. The sample is a solution of mobile phase and polymer, which can vary in concentration but usually ranges between 0.5 and 5 mg/ml. Once the sample injection stream reaches the columns, the separation begins. Smaller sized polymers will take longer to flow through the columns as they are able to penetrate the pores of the column packing more than the larger sized polymers. This process can be a comparison of distance traveled since the smaller particles will be traveling in and out of the pores (or more pores) thus resulting in a longer pathway. Once the polymers have been extruded from the columns, they pass through a detector where they are measured either in terms of concentration (differential



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With the use of standards and software, one can determine peak molecular weight, number average molecular weight, weight average molecular weight, polydispersity index and peak broadness, among other variables. Figure 1.6 shows an example of sufficient separation of different molecular weight peaks in aqueous GPC.

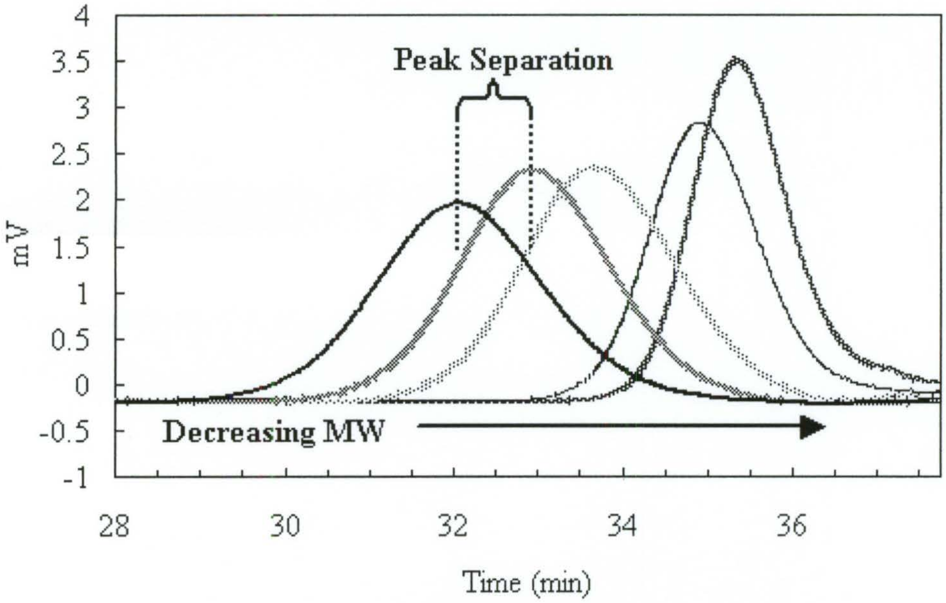


Figure 1.6: GPC peaks for different molecular weights. The separation between alternate peaks is due to many factors including number of columns, polydispersity, solvent and molecular weights.

1.4.3 Detectors

In recent years, with the emergence of advanced column technology, GPC use as an analysis tool has increased and thus the presence of an online detector has risen. Online detectors, as opposed to offline detectors, are part of the flow cycle. These detectors are often situated directly after the columns. Offline detectors are usually employed after the sample has run through the system and been collected. Further processing of the sample in often required in order to optimize certain properties for these detectors. For example, concentration adjustments may need to be made since the sample is now spread over a flowing mobile phase. The online detector's obvious benefits are attributed to the fact that a second step/stage is not required thus decreasing the probability of error. Error can however occur regardless of whether online or offline detectors are used. Such error can be attributed to contamination, polydispersity or peak spreading (increases with increasing flow path lengths), detection interference (temperature, electrical), pump fluctuations, air bubbles present in the system, improper system equilibration (i.e. mobile phase flushing) and mobile condition changes (temperature, concentration, etc.).

The detectors available for GPC analysis fall into three main categories, concentration-based, structure-selective and molecule-weight-sensitive detection. Although there can be many different variations, in general there are only about six different detectors used in conjunction with GPC equipment and each has its advantages and disadvantages. The differential refractive index (DRI) detector is probably the most commonly used, especially in industry. The DRI is a concentration-based detector which measures the difference in refractive index between a compartment of merely mobile phase and a compartment of the flowing mobile phase through which the injected sample will pass. Due to this, it is very important to ensure that two things occur. Firstly, the detector must be thoroughly flushed with the current mobile phase as to deject any traces of previous mobile phases. Secondly, the polymer or sample must have a significantly different refractive index compared to the mobile phase so that a difference can be adequately detected. Through the refractive index signal, the DRI detector will show the changes in polymer concentration (as indicated by peak height) and thus is able to determine polydispersity. One of the main reasons for its popularity is its low cost relative to other types of detectors (Mori & Barth, 1999). Another benefit is that the polymer sample is not altered in any way by the DRI detector, which can be the case with other detectors.

The evaporative light scattering detector is the other so-called concentration-based detector even though it actually measures mass. As the flowing mobile phase flows through the detector, the eluent is evaporated leaving behind the less volatile sample, which is then measured by light scattering. The result is a set of incremental mass measurements that can be more or less considered a continuous stream. The main disadvantage of this detector is that it requires full evaporation of the eluent along with no evaporation of the sample, which may be difficult to achieve. Some less popular detectors in the concentration-based category include flame ionization detectors and density detectors (Mori & Barth, 1999).

Another fairly popular type of detector is the laser light scattering (LLS) detector. Detectors of this type are able to measure molecular weight of polymers directly and are thus in some cases more desirable. Light scatterers work on the principle that when radiation is passed through a sample the elements in the sample will scatter the light in all directions. At a specific light intensity, the scattering can be related to the molecular weight, size, number and shape of the polymer in the solution. Through correlation of the various measured parameters, the molecular weight can be calculated. Over the years many different types of laser light scattering detectors have been developed, such as multi-angle laser LS (MALLS), low-angle laser LS (LALLS), dual-angle laser LS (DALLS), etc. All types are particularly beneficial for different systems and all have their individual advantages and drawbacks. In general, all LLS require that a DRI detector also be available. This is necessary since both the refractive index of the solvent (η_o) and the specific refractive index increment ($d\eta/dc$) are required for the molecular weight calculations. The need for the dual detection setup is often cited as the main drawback to LLS detector methods (Mori & Barth, 1999).

The capillary viscometer is the other type of molecular-weight sensitive detector. Viscosity measurements are based on the fact that a polymer in solution increases the viscosity with increased molecular weight, at low concentrations. The relationship between molecular weight (M), viscosity and hydrodynamic volume (V_h) can be easily shown by the following equation:

$$V_h = [\eta]M,$$

where $[\eta]$ is the intrinsic viscosity. This method works well with SEC since the injector samples are in sufficiently low concentration. In a capillary viscometer, the pressure drop or pressure difference is measured, which is directly correlated to the viscosity of the solution. One main drawback is the resultant flow-rate and temperature fluctuations from the pressure drops, which can alter the accuracy of the SEC measurements, particularly molecular weight distributions (Mori & Barth, 1999).

The structure-selective class includes the UV and infra-red (IR) detectors as well as various other offline detectors, such as fluorescence, nuclear magnetic resonance and mass spectrometers. These types of detectors measure properties specific to the sample or polymer but are only sensitive to specific components of the sample (Mori & Barth, 1999). One main drawback to these types of detectors is that they often are associated with degradation or alteration of the sample during analysis.

Sometimes a laboratory will combine multiple detectors either to obtain required variables (as with LLS and DRI), to measure more than one variable (such as concentration and molecular weight) or to improve accuracy (two measurements per sample). Although the multi-detector setup will successfully measure more than one variable, it has yet to be proven that increased accuracy is obtained. This is due to such obstacles/problems as peak broadening resultant from inter-detector volumes. Opinions will be contradictory regarding which detector or detectors provide the best results depending on the source.

1.4.4 Calibration Methods

In order to quantify the data obtained from the detectors outlined above, a form of calibration with standards needs to be employed. The two main types of calibration are direct or universal calibration. The standards used in the direct calibration method can be of either narrow or broad molecular weight distribution (MWD). Narrow MWD standards are the more commonly used since the calibration of the instrument is simpler. The method involves the injection of a set of standards into the GPC stream and the construction of a calibration curve. The calibration curve is a graph of the logarithm of molecular weights against the elution time. A sample calibration curve can be seen in Figure 1.7.

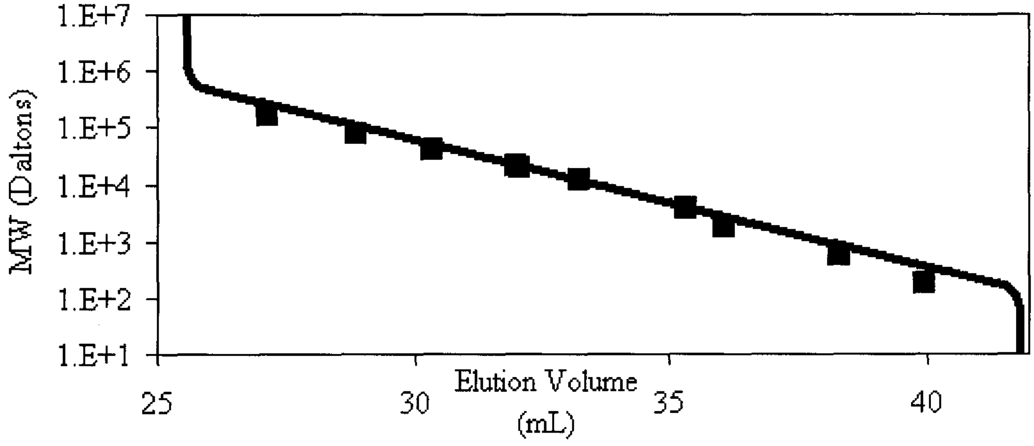


Figure 1.7: Sample calibration curve. Semi-logarithmic plot of elution time versus molecular weight.

Once the calibration curve is constructed, a sample of the same polymer, with an unknown molecular weight can then be analyzed for determination of molecular weight. For polymers that are different than the narrow MWD standards, the molecular weight determined must be recorded as an "equivalent molecular weight" in relation to the standard polymer type. The most common narrow standards available are poly(ethylene oxide/glycol) (PEO/PEG) and polystyrene (PS), therefore samples are often cited with PS or PEO equivalent molecular weights. These standards can produce both the number-average molecular weight ($\overline{M}_n$) and the weight-average molecular weight ($\overline{M}_w$), by the following equations:

$$\overline{M}_n = \frac{\sum H_i}{\sum \frac{H_i}{M_i}} \qquad \overline{M}_w = \frac{\sum H_i M_i}{\sum H_i},$$

where H$_i$ is the height of the peak or the concentration of the polymer in a polymer fraction.

For broad MWD standards, the method is more difficult but is sometimes necessary when narrow standards are not available. The difference is attributed to the fact that while narrow molecular weight standards have a known polydispersity or MWD, broad standards usually do not (Kostanski, Keller, & Hamielec, 2004).

Direct calibration can be used with nuclear magnetic resonance and mass spectrometry detection techniques. Employing these methods is often done when appropriate molecular weight standards are not available. Drawbacks to these methods include high expense, sample alteration and inaccuracy (Kostanski et al., 2004).

Up to this point the calibration methods have been one of two cases, either the polymer molecular weight is determined from calibration curves made from the same polymer type, solvent and temperature or that the molecular weight determined is considered to be “relative” to the polymer standard. One alternative to this is to employ the universal calibration method. This method uses the Mark-Houwink equation:

$$[\eta] = KM^{\alpha}$$

where is $[\eta]$ the intrinsic viscosity, M is the molecular weight, and K and α are Mark-Houwink constants. For polymer samples (subscript 2) of known K and α, the true molecular weight can be calculated if the same is known for the polymer standards (subscript 1) by the following equation (Kostanski et al., 2004):

$$\log M_2 = [1/(1+\alpha_2)]\log(K_1/K_2) + [(1+\alpha_1)/(1+\alpha_2)]\log M_1$$

It should be noted that universal calibration does have many issues, which can cause error in molecular weight determination. The viscosity determination and the retention volume are among the most significant sources of error and it was found in 1990 that they could account for at least a 15% error. Due to this, the determination of molecular weight by GPC/SEC with universal calibration was and is still considered, to a certain degree, debatable. (Malfait, Sloot, & Van Cauwelaert, 1990).

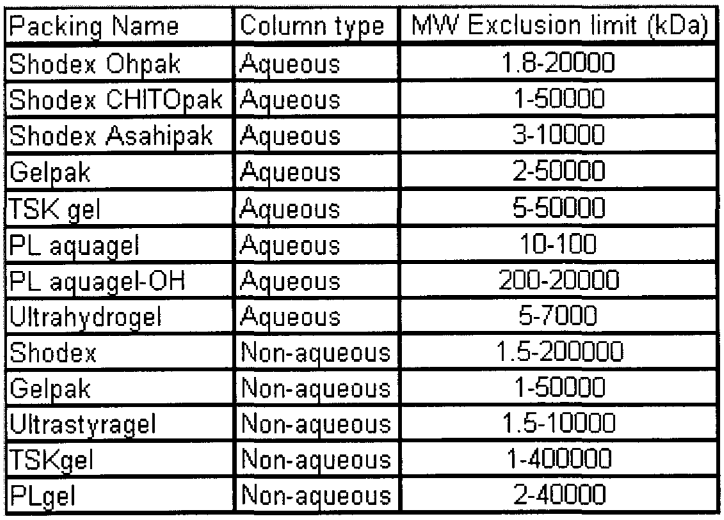
1.4.5 Gel Permeation Chromatography Types and Applications

In the GPC field, two main types of systems can be found, namely, organic and aqueous. These names refer to the type of mobile phase present in the system. In organic systems, solvents are often tetrahydrofuran (THF), toluene or dimethyl formamide

(DMF), while the aqueous system involves water-based mobile phases with various additives, buffers and salts.

Current GPC systems use various types of configurations and column types. Some systems employ a multi-solvent reservoir system. This enables an elaborate and efficient means of examining different molecules in different solvents simply by varying the mobile phase source conditions. The many types of columns and column packings are available so that the optimum system can be configured for the type of molecule being analyzed. For example silica-based SEC columns are quite often employed for biopolymer analysis and purification but would be a bad choice for high molecular weight polymers (>5 million Da.) due to their limited pore size. A better suited column for high molecular weight polymers are those containing polymer-based packings, such as polystyrene-divinylbenzene, since they can be synthesized with pore sizes greater than 4000Å (Wu, 2004). A list of some of the most commonly used column packings can be seen in Table 1.2.

Gel permeation chromatography has two main streams, namely, analytical and preparative. In most cases, analytical is purely for the determination of molecular weight and MWD of known polymers and the quantitative and qualitative determination of components in a sample. Preparative on the other hand is used for purification of samples and the collection of fractions (be it MW fraction or component type fractions). For obvious reasons, preparative is usually less accurate but greater in scale then analytical and can often result in decreased resolution due to larger sample masses (Mori & Barth, 1999).



Packing Name	Column type	MW Exclusion limit (kDa)
Shodex Ohpak	Aqueous	1.8-20000
Shodex CHITOpak	Aqueous	1-50000
Shodex Asahipak	Aqueous	3-10000
Gelpak	Aqueous	2-50000
TSK gel	Aqueous	5-50000
PL aquagel	Aqueous	10-100
PL aquagel-OH	Aqueous	200-20000
Ultrahydrogel	Aqueous	5-7000
Shodex	Non-aqueous	1.5-200000
Gelpak	Non-aqueous	1-50000
Ultrastyragel	Non-aqueous	1.5-10000
TSKgel	Non-aqueous	1-400000
PLgel	Non-aqueous	2-40000

Table 1.2: List of some commercially available SEC columns (Mori & Barth, 1999).

1.4.6. Problems Associated With Polyelectrolyte Analysis by GPC

Polyelectrolyte characterization by GPC has had much difficulty due to what is called non-size-exclusion effects. Non-size exclusion effects include electrostatic effects, ionic interactions between the column and the sample, adsorption of sample onto the column packing and solvophobic association (Bruessau, 1992). Intra- and inter-molecular electrostatic effects (as outlined above) are a result of repulsive forces between like-charges on the same polymer and between polymers of like-charge. The degree of electrostatic repulsion will vary depending on many aspects, of which salt concentration is the most important. Polyelectrolyte concentration is also very important in this aspect but only in higher concentrations than is often used in GPC samples. Due to the great effect of counterions and added salt counterions on the polyelectrolyte's hydrodynamic volume the molecular mass cannot be easily determined or accurately quantified. Another highly researched non-size-exclusion effect involves the various interactions between the sample and the column. Although this can occur in nonionic polymers, it is by far more evident in polyelectrolyte sampling. Interaction between the solute (i.e. the polyelectrolyte) and the column packing can occur in three forms; ion exclusion, ion inclusion and ion exchange. In ion exclusion (a repulsive situation) the polyelectrolyte would likely have the same charge as the surface of the column packing resulting in the exclusion of the polyelectrolyte from pores in the packing that it would otherwise sterically and volumetrically be able to enter. This ultimately results in a premature elution of the polyelectrolyte sample at the exclusion limit and an overstatement of molecular weight or hydrodynamic volume. Ion inclusion is slightly more complicated than the exclusion case in that it involves the diffusion of the polyelectrolyte counterions into the pores of the column packing. In order to establish electroneutrality between the pores and the remaining volume, the polyelectrolytes will be forced into the pores. The outcome of such is the delayed elution time (i.e. lower molecular weight recorded compared to actual) and the presence of an electrolyte peak at high elution times.

Ion exchange occurs mostly with silica gel and some polymer–based packings, where the packing contains anionic silanol or carboxyl groups that can undergo ion exchange with cationic polyelectrolytes. Ion exchange is therefore an enthalpic interaction resulting in a negative ΔH. (Bruessau, 1992). This type of secondary effect will result in the elution of the polyelectrolyte either at a volume greater than the total void volume or no elution of the sample at all. Adsorption of sample onto the column packing can be either reversible or irreversible and can occur through three methods, ion exchange (as just mentioned), hydrophobic interaction and hydrogen bonding. Hydrophobic interactions and hydrogen bonding involve non-ionic groups and hydrogen moieties, respectively of both column and sample. Any adsorption event can lead to the polyelectrolyte sample remaining in the column for a longer period of time (or indefinitely), which in turn would lead one to believe that the polyelectrolyte has a lower molecular weight than in actuality. Solvophobic association simply involves the sample not dissolving or not remaining dissolved in single form (Bruessau, 1992). In this case, the detector is often reading dimers (two polymers together), trimers (three polymers together), etc., thus resulting in overestimated molecular weights.

In addition to those mentioned above, some other, less common effects can occur. Polyelectrolyte's charged groups could have a significant effect on the viscosity of the solution, as can the concentration of polyelectrolyte in solution. This is often associated with prolonged resident times in the columns and the generation of a recorded molecular weight below the actual molecular weight (Mori & Barth, 1999). Other less common or less significant effects include viscous fingering and concentration effects.

Various methods have been outlined to combat the secondary effects that can occur. Some of these methods include reducing the pH to a value specific to the polyelectrolyte which will suppresses dissociation, increasing or decreasing the concentration of electrolytes (ionic strength), or adding organic solvents, surfactants and/or ionic modifiers to mobile phase (Bruessau, 1992; Mori & Barth, 1999). In addition, the development of new columns packing has resulted in better outcomes for a wide range of systems.

Another problem often associated with GPC analysis of polymers is the lack of distinction between branched and linear or un-branched polymers. This problem also extends to other polymer geometries and architectures but is most cited in reference to branched polymers. Due to the different solution conformations of different polymer architectures, it is likely that polymers that are branched are mistaken for un-branched polymers of either lower or higher molecular weight and vice versa. Figure 1.8 clearly demonstrates this phenomenon. It shows that polymers of different molecular weight can have the same hydrodynamic volume. This is also often the case with polyelectrolytes of different charge density, as this will greatly affect the hydrodynamic volume of the polyelectrolyte due to repulsive and attractive forces between charges.

Figure 1.8: Sketch of linear (un-branched) and branched polymers. Both have different molecular weights but same hydrodynamic volumes.

When analyzing any sample with the GPC or SEC method for the purpose of obtaining molecular weight or other parameters, it is most important that good reproducibility is achieved. Once this goal is met, the rest of the issues can be identified or addressed. Therefore, as long as reproducibility is evident and the researcher is aware of the non-size exclusion effects that could occur, GPC can be a very valuable tool even for the often-troublesome polyelectrolyte samples.

1.5 Background

1.5.1 Previous Experimentation: Polyelectrolytes Solution Studies with GPC

As stated previously, GPC is a very popular method of characterization for various types of polymers but there have been many issues surrounding its use in polyelectrolyte studies. This section will outline various strategies that have been developed to either deplete or account for sources of error associated with polyelectrolyte characterization studies, such as non-size-exclusion effects.

For polar polymers, molecular weight determination has been difficult due to their peculiar behavior in solution. A frequently employed solvent of N,N-dimethylformamide (DMF) has helped to overcome some of the obstacles surrounding such methods (Šegudovic et al., 1995). Although this solvent is not aqueous, some research has been conducted examining the effect of added salt. One very popular paper, written by Hann in 1977, involved an examination of the effect of lithium bromide (LiBr) on the hydrodynamic volumes of polyester-based polyurethanes. Due to the high polarity of such polymers, they tend to exhibit polyelectrolyte behavior in solution and thus can be correlated to polyelectrolyte solution studies. This study concluded that the addition of the electrolytic salt stabilized effects, such as polymer association, column interaction and chain extension allowing for more accurate chromatographic analysis (Hann, 1977). Another more recent study again examined the effect of LiBr salt on SEC with DMF solvent. In this study, they examined copolymers of maleic acid and styrene. They found that the addition of LiBr resulted in the chromatographs shifting to a longer elution time, thus denoting smaller molecular weight or hydrodynamic volume. Although this phenomenon was observed for some of the copolymers, it was not evident for those with higher values of sulfonation, which is responsible for the strong polyelectrolyte behavior. With a higher polyelectrolyte effect, higher concentrations of LiBr where required to screen out the charge. In any case, the calibration curve from polystyrene standards was found to only give relative values and thus is insufficient for accurate MW determination (Šegudovic et al., 1995). All three studies are likely valuable in pointing to effects of salt in aqueous systems of polyelectrolytes. Due to the nature of this current work, the remaining literature review will be limited to aqueous systems.

A particularly relevant paper was published in 2005 which reported on solution properties of poly(diallyldimethylammonium chloride) (PDADMAC) using GPC coupled with refractive index (RI) and multi-angle laser light scattering (MALLS) detectors. It was first concluded that the addition of sodium chloride (NaCl) to the aqueous mobile phase provided a better calibration curve (i.e. more linear) as compared to the addition of sodium bromide (NaBr) or lithium chloride (LiCl). NaCl also showed a sample displacement to higher elution times, denoting a smaller hydrodynamic volume. Using the MALLS detector, they were also able to compare the elution time to the root mean-squared radius of gyration. By graphing the two parameters against each other, they were able to show the possibility of larger molecules being retarded in the columns and eluting at later times. They were unable to explain the reason for this phenomenon but simply stated that it must be related to molecular architecture and that the molecules eluted at longer times were unimportant since the calibration curves were only to be applied within the linear segment (Marcelo, Tarazona, & Saiz, 2005).

Another paper used the same setup to characterize the polyelectrolyte, poly(dicarboxylatophenoxy phosphazene) with the addition of a UV detector. The purpose of the study was also to investigate how experimental parameters, such as ionic strength, column resolution and injection volumes could be manipulated to minimize non-size-exclusion effects. In terms of ionic strength it was found that molecular weight values were unchanged when the concentration of NaCl was reduced from 0.42M to 0.14M. Due to the lack of incremental values, no solid conclusions were made from this finding. They did however conclude that injection volume had little-to-no effect on molecular weight determination accuracy and that column resolution was greatly affected by its history. The final conclusion they made was that the concentration detectors (RI and UV) were more accurate in determining molecular weights as compared to the molecular weight sensitive MALLS detector (Andrianov & Le Golvan, 1996).

A group at the University of Valencia in Spain has published a series of articles, in various journals over a span of 9 years, from 1987 to 1996. This series is entitled, simply "Solution Properties of Polyelectrolytes" and encompasses many different characterization methods and polyelectrolyte types. Each individual paper aims to take steps into the further understanding of polyelectrolytes by looking at specific systems and expanding from such. Their first three papers used GPC methods to examine the effect of polyelectrolyte concentration and ionic strength on molecular weight and elution volume results. Each resulted in a variation of a semi-empirical model that they suggested could predict concentration effects. The model's validity was minimally tested with the same system but was not extended to other systems (Abad, Braco, Soria, Garcia, & Campos, 1987a; Abad, Braco, Soria, Garcia, & Campos, 1987b; Soria, Garcia, Campos, Braco, & Abad, 1988). In a later paper, this group was able to show that if shielding of polyelectrolytes from pores is complete then all polyelectrolytes are eluted at the same time, regardless of molecular weight due to complete pore exclusion (Campos, Garcia, Porcar, & Soria, 1994). This conclusion is of great importance to all polyelectrolyte studies, since complete exclusion from pores is easily and immediately identified.

In addition to the result altering factors associated with GPC, such as adsorption and repulsion, one also needs to consider the occurrence of aggregation or association of polymers in solutions, especially when dealing with amphiphilic associative polymers, namely polyelectrolytes or hydrophobically-modified polymers. Researchers in Russia conducted experiments to examine the effect of such on the GPC-MALLS molecular weight determination. They specifically examined hydrophobically-modified poly (acrylamide)-based polymers in water-acetonitrile mixed aqueous solutions. While they did observe aggregates for the highly charged and highly hydrophobic polymers in the solution, the aggregates were found to be quite unstable and were thus disrupted during GPC analysis. In other words, the impacts and shearing forces present in the GPC-MALLS stream were large enough to break up the aggregates so that the polymers were individually detected. This suggests that other polyelectrolytes, which have a tendency to aggregate, have a high likelihood of dissociation during GPC analysis (Blagodatskik et al., 2002).

1.5.1.1 Calibration, Column Packings and Mobile Phase Optimization

A series of papers has been published in addition to those mentioned above that aimed to discover ideal system conditions for GPC analysis of polyelectrolytes, such as calibration methods and tools, column packing types and mobile phase optimization. Although many polyelectrolytes were examined, there is significantly more data available for anionic polyelectrolytes as opposed to cationic and zwitterionic polyelectrolytes but the general principles are often in place so that theories can be extend to other types of polyelectrolytes. This subsection will serve as a brief outline of some of these studies.

The Valencia group conducted a study on the effectiveness of new Ultrahydrogel™ aqueous SEC packings in three types of mobile phases, namely salt-free water, sodium nitrate solution and buffer-containing water at different pH values. In addition they were able to optimize chromatographic parameters to obtain sufficient and accurate separation. These parameters included sample concentration, injection volume, eluent pH and ionic strength of eluent. By altering the conditions, the hope was to deplete secondary or non-steric interactions with the columns, such as electrostatic adsorption, electrostatic exclusion and hydrophobic partitioning. They were able to determine that even with a mobile phase of salt-free water, a pronounced ion-exclusion effect was not seen and a reasonable (but not optimal) separation was achieved. A mobile phase of 0.2 M acetate buffer (pH 4.0) was found to provide good resolution in both cationic (e.g. poly(2-vinylpyridine) (P2Vpy)) and anionic (sodium polystyrenesulphonate (NaPSS)) polyelectrolyte samples. (Perez-Paya, Braco, Campos, Soria, & Abad, 1989). It should be noted that while the optimization studies are valuable for pointing to optimal conditions, the results need to be considered merely an example due to the large effect of polyelectrolyte type, molecular weight and concentration on separation characteristics. In other words, optimization conditions will be specific for each polyelectrolyte. Research was then expanded to examine the effect of operation variables on calibration curves for silica-based columns, such as polyelectrolyte concentration, ionic strength and pH (Soria et al., 1990). The conclusion made was that a set of conditions was found that completely suppressed ion-exclusion effects when characterizing NaPSS with silica-based packing. The combination of these two papers might lead to the conclusion that Ultrahydrogel™ packings are much more suited to polyelectrolyte characterization than the silica-based packings. This issue was addressed in a 1993 paper by the same group, which compared the elution behaviors on two organic-based column packings. They were able to conclude that both of the organic-based column packings are more convenient than the traditional silica-based packings for aqueous GPC. In addition, they made the obvious conclusion that optimization of operating condition is specific to each solute/support system (Garcia, Porcar, Campos, Soria, & Figueruelo, 1993b). This could also be expanded to say that other parameters, such a polyelectrolyte type and concentration, salt concentration and temperature, alter the optimization conditions and thus must also be considered.

Yet another paper in this series examined a variety of salts and their ability to deplete non-exclusion effects in GPC analysis. They varied both salt concentration and the nature of the salt (i.e. salt type). A conclusion was made that a certain level of mobile phase ionic strength was required to "screen out" repulsive forces between the negatively-charged polyelectrolytes and negatively-charged packing surfaces. It was also stated that the experimentally determined ionic strength was necessary to combat the polyelectrolyte effect, resulting in the polyelectrolytes attaining a random coil configuration (Volet & Lesec, 1994). Although they were able to state that both the screening of repulsive forces between the column and the polyelectrolytes and the screening of the charges on polyelectrolyte from each other were, in combination, responsible for the prolonged elution times, the researchers did fail to address the very important issue, of available pore volume. It would be very interesting to discover how the screening event affected the available pore volume, in a quantitative measure.

Other research has been conducted to determine the optimal mobile phase composition for polyelectrolyte molecular weight studies. One such work looked at the utilization of organic solvents as an additive to aqueous mobile phases (Kuhn et al., 1993). By adding different concentrations of organic solvent adsorption onto the columns was depleted while the quality of the solvent could be improved. This is of great interest since in most cases salts are added to the aqueous mobile phase for deterring adsorption but often result in decreased solvency thus leading to precipitation of the polyelectrolytes on the columns. When the universal calibration data was checked against viscosity measurements they found that the data conformed nicely. Through this work, the researchers were able to demonstrate that regardless of column material, SEC can be successfully preformed for both cationic and anionic polymers given the proper eluent or mobile phase. One drawback to the use of organic solvents, even in low concentrations, is the sensitivity of the aqueous column packings to such elements and thus this method must be limited to columns that are not affected by organic solvents.

One particularly interesting paper from the Valencia group involves an empirical correlation between pure SEC and SEC involving secondary effects under different eluent ionic strengths and pHs (Perez-Paya, Braco, Abad, Soria, & Campos, 1991). In order to account for the secondary effects involved in polyelectrolyte SEC, a correlation was made between the experimental elution volumes of uncharged polymers (V_e) and experimental elution volumes of polyions (V_e'):

$$V_e = V_o + K_{SEC} V_p \qquad\qquad V_e' = V_o + K_{SEC}' V_p,$$

where V_o is the void volume, and K_{SEC} and K_{SEC}' are the partition coefficients for pure SEC and for SEC accounting for secondary effects, respectively. If V_e and V_e' are considered to be of the same molecular weight, their partition coefficients can be related by the following equation:

$$K_{SEC}' = K_{SEC} K_p$$

The K_p term is the partition coefficient for secondary effects and can also be expressed in terms of pore volume (V_p):

$$V_p^* = K_p V_p,$$

where V_p^* is the virtual pore volume or the "effectively seen" pore volume. This value accounts for secondary effects in which $K_p<1$ denotes the occurrence of secondary effects. V_p^* can be correlated to residence time in the columns. Thus when $V_p^* > V_p$ there are attractive interactions between the columns and the polyions and when $V_p^* < V_p$ repulsive interactions are taking place. Values were determined and then tested by proteins and polypeptides of known hydrodynamic volumes and molecular weights. They found that although this correlation could be applied, the complexity of the systems requires a much more sophisticated approach, especially with complex biopolymers. It should be noted that the K_p values are specific to polymer properties (type, molecular weight and charge), elution conditions (temperature, pH, ionic strength, etc.) and column packing composition and thus empirical data must be gathered for each system. (Perez-Paya, Braco, Abad, Soria, & Campos, 1991).

As mentioned in the first section, polyelectrolyte solution behavior is extremely complex and thus very little is known about the field. In order to combat the lack of understanding of polyelectrolyte solutions, extensive research has and continues to be conducted around the world. GPC has proven to be a valuable tool for polyelectrolyte solution studies and with continued research into the improvement of GPC accuracy, this method is likely to become even more valuable.

1.5.2 Previous Experimentation: Other Polyelectrolyte Solution Studies

Up to this point, all of the solution studies that we have examined have in some way involved GPC analysis. Although GPC is a very valuable tool, other methods such as X-ray scattering, dynamic light scattering, nuclear magnetic resonance, viscometry and electrical conductivity measurements have been successful to varying degrees at examining polyelectrolyte solution properties. The following is a short sample of some interesting and relevant work in the field of polyelectrolyte solution behavior.

A paper published in 2002 reported on a study which examined the effect of added salt on the intermolecular correlation between electrostatic repulsion in flexible polyelectrolyte solutions and ionic strengths of the solution, using small-angle neutron and X-ray scattering. They were able to separate the scattering function into inter- and intra-molecular components, by improving on previous methods. From preliminary tests of these methods, they concluded that both inter- and intra-molecular parts are responsible for the scattering function and that the persistent length of the polyelectrolyte in solution could be adequately determined under various ionic strengths with this method. The persistent length parameter allowed for the determination of the degree of screening effects caused by the added salt (Nishida, Kaji, Kanaya, & Shibano, 2002). Although the researchers have developed a promising method for evaluation of polyelectrolyte solution behavior studies they did not report any sets of results or conclusions obtained from its application. Therefore, the accuracy of this method as compared to other methods, such a GPC has yet to be proven.

The Valencia group later expanded their examination of NaPPS by GPC (as described in Section 1.5.2) with a study that looked at salt-free solutions containing two polyelectrolytes of like-charge; one being NaPSS and the other being sodium poly(L-glutamate) (NaPLG). They were able to determine viscometric behavior of both polyelectrolytes as a function of concentration, molecular weight and pH through the use of an Ubbelohde viscometer. This work resulted in the formulations of two equations that were able to predict viscosity-related solution behavior of polyelectrolytes under salt-free conditions (Parets, J. Garcia, Soria, & Campos, 1990).

As mentioned above, increasing the salt concentration can affect the quality of a solution eventually resulting in the solution becoming a non-solvent causing the polyelectrolyte to precipitate out. One experiment examined the influence of divalent salt ions on alternating polyelectrolytes, poly(maleic acid-alt-styrene) (NaMA/St) in terms of solution stability. Since the binding of salt to polyions in solution can lead to polyion contraction, dehydration, decreased solubility and eventually precipitation, they examined a range of values of polymer concentration, salt concentration and temperature. They found that the instability of solution occurred at different concentrations for different salts and they established the following order: $Ba^{2+} > Ca^{2+} > Mg^{2+}$, where Ba^{2+} had the greatness tendency to promote instability of the polymer. They commented that the reason for such a trend was due to the cation's differences in size, hydrophilicity and binding form (Jarm, Sertic, & Šegudovic, 1995). This study was later extended to examine similar properties of the sulfonated form of NaMA/St. It was found that the sulfonated form was more stable and thus more soluble than the original (Jarm, Kovac-Filipovic, & Šegudovic, 1995).

In addition to these experiments there has also been considerable interest in the polyampholyte solution behavior. Recalling from the first section, polyampholytes are often (but not always) characterized as polyelectrolytes consisting of both acid and base groups that can undergo ionization in solution leading to the formation of cationic and anionic groups, thus becoming zwitterionic polymers. Two review papers have been published in recent years that outlined the current understanding surrounding amphoteric polymers in solution (Dobrynin et al., 2004; Lowe & McCormick, 2002). The first paper, written in 2002, examined the synthesis and aqueous solution properties of two types of zwitterionic polymers, namely polyampholytes and polybetaines. These authors distinguished between the two types as follows: polyampholytes contain alternately charged (anionic and cationic) groups on different monomer units and polybetaines contain anionic and cationic groups on the same monomer unit. They summarized a collection of work concerning polyampholyte solutions, which led to the following characteristics: they can exhibit both the polyelectrolyte and anti-polyelectrolyte effects, those near overall neutral charge exhibit the anti-polyelectrolyte effect, those that are either strongly negative or strongly positive exhibit the polyelectrolyte effect and polyampholytes have an isoelectric point, at which block copolymers (i.e. not statistical) tend to precipitate out of solution, and the viscosity and coil size are minimized. Polybetaines were likewise summarized with the following attributes: they exhibit the anti-polyelectrolyte effect with the addition of salt, they are often not soluble in pure water due to the formation of ionic cross-linked networks, they are biocompatible due to their high hygroscopic nature and copolymer betaines can exhibit polyelectrolyte behavior at lower pHs (Lowe & McCormick, 2002). The second paper, which focused solely on polyampholytes, was published in 2004. This review also examined polyampholyte solution properties but most of the findings were a repeat of those outlined in the previous review. In addition, a review of polyampholyte interactions with polyelectrolytes and other surfaces were reviewed as well as popular polyampholyte theoretical models (Dobrynin et al., 2004).

1.5.2.1 Polyelectrolyte Solution Models

In addition to the abundance of experimental polyelectrolyte solution studies conducted, there has also been a significant amount of theoretical or mathematical models developed to predict polyelectrolyte solution behavior, in recent years. Some models have already been presented in this work; many others have been developed concerning polyelectrolyte solution behavior. The preliminary models described earlier are theory based and have become widely accepted. This section serves to outline some of the more recent theories or models in order to give readers an idea of the volume of work currently being conducted in such fields.

A paper published in 1998 theoretically examined semi-dilute polyelectrolyte solutions with added salt using various charge distribution cases; smeared, annealed, permuted, quenched and polyampholytes. Each case accounts for different possible charge distributions, for example the permuted model contains charges that are fixed on the chain but able to move along the chain. Methods such as mean-field equation derivations, free energy calculations and random phase approximation were used to compare the range of charge distributions. They concluded that each distribution alternately affected the free energy and that with all cases there existed limitations with the mean-field and the random phase approximations. They were able to name higher correlations and high degree fluctuations as parameters that were necessary to neglect in order to achieve results (Borukhov & Orland, 1998).

Another paper examining various polyelectrolyte models was published in 1999 by Vlachy as a review of recently developed models associated with ionic effects on polyelectrolyte solutions beyond the Poisson-Boltzmann theory. The first model examined is called the cell model, which assumes that the macroions repel each other and thus are situated at large distances from one another. The second model is the one-component model, which effectively treats the solution like a one component fluid of macroions. This results in the assumption that macroion interaction occurs only with other macroions since it is essentially the only component. The last model, the isotropic model is actually a collection of various theories that accounts for all or most of the inter-particle correlations. In this model, the macroion or polyelectrolyte, which is depicted a hard or soft sphere immersed in a solvent, is simulated on a level closer to the microscopic level than the previous two models. The first two models were described as fairly easy to solve but limited in interaction accountability. The last, more complex model, though likely more encompassing, is very difficult and time-consuming to solve. Furthermore, it still relies on simplifying assumptions thus resulting in the incomplete interaction representation. The last conclusion is that the approximation of the polyelectrolyte to a spherical geometry is likely to be a gross approximation that will greatly affect the results (Vlachy, 1999).

Another model that approximates the polyelectrolyte shape equates it to a cylindrical rod and studies the nonspecific binding of salt to these polyelectrolytes. In this case the polyelectrolyte unit is viewed to be infinite, inflexible and impenetrable while the counterions and co-ions are considered to be uniform diameter, charged spheres. This model is based on a density function theory, which examines preferential interaction of both divalent and monovalent ion concentrations (from salt). One benefit of this model is its ability to add a solvent component (water) as a contributor to the polyelectrolyte-ion interactions. They concluded that by accounting for effects of divalent ion presence the model can better predict correlations in the interactions, where the widely accepted linear Poisson-Boltzmann theory cannot. (Patra & Yethiraj, 2000). Although more variables are incorporated within this model it is by no means complete. Again the assumptions made during the formulation of the theory, such as polyelectrolyte as cylinders and salt ions as uniform spheres, must be considered when assessing the validity of such a model. Once more, this model cannot be directly correlated to the actual polyelectrolyte geometries but may be useful in pointing to a general salt effect.

Researchers at the Swiss Federal Institute of Technology experimentally related chemical structure and macromolecular parameters, such as monomeric molar mass to the partial molar and specific volumes. The aim of this correlation was to create an in-depth comparison between experimental work done on relatively new, high-precision equipment to the so-called additivity schemes developed by Durchschlag and Zipper, and Gianna and Lepori. An extensive examination of a wide range of different polyelectrolytes was done through density measurements. They found that each additivity scheme showed somewhat good correlation to the experimental results but each had their indidvidual strongpoints. The Gianna and Lepori model was more accurate for polycations while the Durchschlag and Zipper model was better at predicting anionic biopolymer volume parameters. The work presented was stated as a useful tool for improving current theoretical models and was the first extensive empirical-theoretical comparison of polyelectrolyte solution models, using advanced experimental techniques (Wandrey, Bartkowiak, & Hunkeler, 1999).

One paper from the International Symposium on Polyelectrolytes held in 2002 demonstrated a new model that attempted to simulate salt-free polyelectrolyte solutions with mixed-valence counterions. This model used a rod-like polyelectrolyte solution system with the Monte Carlo method and compared it to numerical solutions from the previous mentioned Poisson-Boltzmann equation. The purpose of this comparison was to examine the influence of counterions on ion distribution. Parameters such as ion size in relation to the counterion condensation theory and ion-ion correlations were considered. It was concluded that the Monte Carlo simulation showed a considerably larger accumulation of the multivalent ion compared to the Poisson-Boltzmann system. This greater accumulation is likely to lead to the improved screening of the polyelectrolyte thus affecting its hydrodynamic volume and solution configuration. The conclusions were not tested by experimental means and no distinction was made as to the "more correct/accurate" method but the work did serve to show that extremely diverse results could be generated when comparing two different models (Nishio & Minakata, 2003).

In the tenth addition of the "Solution Properties of Polyelectrolyte Solutions" series, mathematical theory was used to formulate a so-called "master equation" which predicted the effect of ionic strength on the size and shape of polyelectrolytes (Garcia, Porcar, Campos, Soria, & Figueruelo, 1994). This group developed this "master equation" from previous work involving a polynomial coefficient, which relates elution volume to ionic strength (Garcia, Porcar, Campos, Soria, & Figueruelo, 1993a). Specifically the equation is to be applied to calibration curves to improve the molecular weight determination process through SEC. This equation was developed from fundamental equations and proposed a semi-empirical correlation between the elution volume (or time) and ionic strength. Although this is able to account for repulsive interactions between the polyelectrolytes and the column packing, it does not predict effects associated with adsorption, hydrophobic interaction or hydrogen bonding within the columns (Garcia et al., 1994). This was latter expanded to include the a parameter for an "effective barrier" between the polyelectrolytes and the packing, which is a balance of a hypothetical repulsion layer and the theoretical expansion of pore size due to attractive forces (Garcia, Porcar, Figueruelo, Soria, & Campos, 1996).

Although this section is by no means an extensive review of polyelectrolyte solution models, it does serve to show the general level of complexity and wide range of variables involved in currently available models as well as the degree of accuracy that has been achieved.

1.5.3 Previous Experimentation: PMPC, PC Copolymers and Other Polybetaines

In comparison to other valuable polymers and polyelectrolytes, PMPC has seen relatively little activity in terms of solution characteristics. This may be linked to the fact that PMPC is expensive and difficult to produce and thus all research involving PMPC is minimal compared with less expensive and less complicated polymers. Nonetheless

PMPC has already shown its potential for application in many fields and thus any research conducted to expand the knowledge base of this polymer is beneficial.

Extensive work on this particular polymer has basically been limited to three groups, namely Ishihara and Nakabayashi's group at the University of Tokyo, Armes and Lewis' group in the University of Sheffield UK, Zhu and Brash's group at McMaster University. Zhu's group has a number of papers involving the study of PMPC grafting by atom transfer radical polymerization from silicon wafers and the adsorption response of proteins on these surfaces (W. Feng et al., 2005; W. Feng et al., 2004; W. Feng, Brash, & Zhu, 2006). The Ishihara-Nakabayashi group has conducted various studies involving the bio-applicability of the PMPC polymer, such as degradation rates, platelet adhesion and studies on block copolymers contain a PMPC block (Ishihara, Nishiuchi, Watanabe, & Iwasaki, 2004; Uchiyama, Kiritoshi, Watanabe, & Ishihara, 2003; Ueda et al., 1991). These groups have not done any experimentation involving solution properties of PMPC in the presence of salt. However, Armes et al. conducted a study in 2005 on the solution properties of a di-block phosphorylcholine copolymer. Specifically, they examined the effect of pH on interfacial adsorption of the copolymer with a small sub-study that looked into salt effects. Although this resulted in a conclusion that that salt sensitivity was minimal in the effect of copolymer interfacial adsorption onto to silicon oxide, the study did not address whether or not salt affected solution properties such as hydrodynamic volumes (Zhao et al., 2005). The only other paper that reported the effect of salt on PMPC was by Wang et al. and involved its effect on polymerization kinetics. They found that a metal cation of larger radius has a greater accelerating effect on polymerization (Wang et al., 2004).

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 phosphorylcholine (PC) end groups. Two such studies examined the solution properties of hydrophobically modified PC-based polymers (polybetaines) (Miyazawa & Winnuk, 2002; Miyazawa & Winnuk, 2003). One looked at the polyelectrolyte aqueous solution effects in the presence of surfactants while the other compared solution properties in water and in mixed solvents. It was discovered that the ionic strength and pH of the solution greatly affected the assembly of the phosphorylcholine groups in aqueous solutions. The association of the polymers, in salt-free solutions (of both water and chloroform), was quite evident due to strong interactions between ion pairs (Miyazawa & Winnuk, 2002). They also concluded that divalent salt ions had a considerably greater effect on the expansion of the polybetaines in solution than monovalent salt ions (Miyazawa & Winnuk, 2003). In addition, other studies have been conducted on PC group containing polymers involving surface dynamics (Ruiz, Hilborn, Leonard, & Mathieu, 1998).

Although all previous experimentation has not included any salt studies they have succeeded in showing the wide applicability of the PMPC polymer, specifically in the biomedical fields. This has thus created a basis for the value associated with solution studies of the PMPC polymer.

1.6 Applications

By examining the wide range of research that has been conducted on polyelectrolyte solutions, one can see the importance of such a filed. On the other hand, one can also see that much more research is essential to its understanding. Greater knowledge of salt effects on polyelectrolyte solutions is likely to be very beneficial in many different areas. These areas include all stages of development and manufacturing in industry as well as biological phenomena recognition. In the developmental stage, it is very important to identify effects of salt on materials in order to develop new materials that exhibit desired responses in their application environments. In terms of manufacturing, a comprehension of how the presence of salt can alter polymer characteristics, such as chain length and molecular weight distribution will allow for better control over various properties. This aspect can also extend to the formation of non-linear polymer architectures, such as star and brush structures, and surface attachment, in the form of grafting to and from surfaces. Surface coating capabilities could also be greatly affected by the presence of salt in terms of polymer coating thickness and density. Lastly, greater knowledge of polymers and polyelectrolytes that have similarities to biological elements can help to further our comprehension of biological behavior.

1.7 Research Objectives

The objective of this research is to develop a relationship between salt type and concentration to the poly (2-methacyloyloxyethyl phosphorylcholine) (PMPC) zwitterionic polymer solution behavior. In particular, polyelectrolyte hydrodynamic volumes are analyzed through gel permeation chromatography in relation to the addition of various salts at various concentrations. By conducting a detailed analysis, conclusions can be reached in regards to the effect of specific ions in solution. The salt characteristics examined will include concentration, ionic strength, solution pH, charge type (i.e. anionic or cationic), ion size, ion valency and ion configuration. The extensive amount of data produced during this study will form a strong background for the accurate formulation of a theory based on the salt effect on PMPC polyelectrolyte solution behavior. The viability of a theory developed from a large array of research experimental parameters will allow for its extension to other zwitterionic polymers as well as both cationic and anionic polymers. Comprehensive experimental data will also aid in the interpretation of empirical models currently available and those yet to be developed. By better understanding polyelectrolyte solution properties, advancements in areas such as polyelectrolyte synthesis, industrial manufacture, environmental response and the study of biological component functions will be enhanced.

1.8 Contents of Thesis

This thesis will include four chapters, specifically, the introduction, the experimental methods section, the discussion and results chapter, and the conclusions and recommendations chapter. Chapter 1 presents the reader with introductory information regarding gel permeation chromatography (GPC) methods, polyelectrolytes types, current knowledge of polyelectrolyte solution behavior and problems associated with GPC analysis of polyelectrolytes. This section will also serve to outline some of the research that has been previously conducted which is relevant to the current work. Chapter 2 outlines the chemicals and equipment used in this work as well as the process of experimental design. Chapter 3 presents experimental work conducted over the course of the 2-year Master's program involving PMPC solution properties as determined by GPC. This section also gives theoretical explanation for the data obtained and correlation of the data to other chemical properties. The last chapter summarizes the conclusions made and extends some general theories based on the collection and analysis of extensive amounts of data. This chapter will also outline future recommendations in the field of polyelectrolyte solution properties.

2 Experimental Design

2.1 Polyelectrolyte Solutions

As discussed earlier, little is known about polyelectrolyte solutions, mainly due to the presence of charge. Interactions between a charged group on the polyelectrolyte with other components comprising the solution, namely salts, counterions, other parts of the same polyelectrolyte, other polyelectrolytes and even the water molecules themselves result in a highly convoluted and complex system. Despite the increasing number of publications concerning polyelectrolytes, no studies have been reported that give a complete comprehensive examination of the interaction of polybetaines with salt. 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 in the presence of various salts and salt concentrations. 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, polyampholytes) and the resulting fundamental knowledge will be beneficial for understanding all polyelectrolytes in solution.

2.2 Gel Permeation Chromatography Analysis

Although GPC is often noted as an excellent tool for molecular weight determination it is also very valuable in hydrodynamic volume studies. Molecular weight measurements can only be considered relative to the standards and not actual values or in other words as a comparison of hydrodynamic volume. Quite often there is an increased lack of accuracy when comparing to standards in the aqueous GPC analysis of charged polymers. Counterions in the solution, either from the dissociated polyelectrolytes or from solution-added salts, will greatly effect the polymers and most likely in varying degrees depending on such things as charge type on the polymer, charge density, charge location (i.e. backbone vs. polymer arm), etc. Due to the exclusion of standard requirements when determining hydrodynamic volumes many of the inaccuracies found in molecular weight determination of polyelectrolytes by GPC are not an issue. All of our current work requires only relative hydrodynamic volumes or changes in hydrodynamic volumes not precise molecular weights; therefore, in the context of this study the only issues that need to be addressed are in relation to non-size exclusion effects.

Secondary or non-size exclusion effects are particularly an issue when dealing with strong polyelectrolytes, therefore, in order to successfully interpret the GPC results, these effects must either be accounted for or suppressed. Many options have been examined to prevent or discourage this, as mentioned in the first chapter, but it must first be determined if any secondary effects are actually occurring.

Of the previously listed secondary effects some can be immediately discounted, such as ion exchange and ion inclusion. Ion exchange is not likely to happen since this usually only occurs with silica gel or polymer–based packings that contain silanol or carboxyl groups, which is not the case with our packings. In terms of ion inclusion, where the polyelectrolyte is held in the pores by charge neutrality (due to counterion diffusion), the delay in elution time is likely to be approximately the same for all molecular weight samples and therefore it is not an issue since we are only looking at relative hydrodynamic volumes.

Two effects that may be of concern with our work are adsorption of the sample onto column packing and ion exclusion. If attractive forces (adsorption) are strong between the polyelectrolytes and the column packing, the detection of polyelectrolyte is likely to never occur as the polyelectrolytes can permanently adsorb onto the packing. However, if samples are eluting at increasing retention times for increasing molecular weights it can usually be concluded that there are no adsorption effects or that the pure SEC effect far outweighs the adsorption effects, in other words rendering them insignificant (Kuhn et al., 1993). If repulsive forces are taking place the polyelectrolyte is excluded from the pores in the packing (i.e. exclusion effects) and it would be expected that different molecular weights would elute at relatively the same time. Since neither of these events was observed, strong interactions between polyelectrolytes and packing could be ruled out.

On the other hand, weaker interactions are not so easily ruled out. With these occurrences the polyelectrolytes either elute earlier or later then their actual molecular weight/hydrodynamic volume alone would dictate, resulting in incorrect molecular weight determination. Again, since this work does not deal with molecular weight determination this area of interaction is not of concern as long as it is assumed that the effects are minimal and that any effects that may occur are relatively equal in magnitude for different molecular weight samples. When comparing results under different salt concentrations and types, the comparisons were made so as to make any possible non-size exclusion effects irrelevant. Since preliminary experiments have shown that there exists partitioning or time scale differences between all five molecular weights samples (1.5, 3, 15, 30 and 60 kDa) it can be concluded that adsorption or repulsion with the columns is not occurring as per conclusions of previous research (Campos et al., 1994).

Another issue that needs to be addressed to ensure accurate results are obtained is polyelectrolyte aggregation. As seen with other work (Blagodatskik et al., 2002), polyelectrolytes that have a tendency to aggregate have a high likelihood of dissociation during GPC analysis due to shear forces and other like phenomena. PMPC is an example of such a polymer and due to its similarity to previous polyelectrolytes studied is likely to "break-up" as well. Therefore, it can be confidently reported that the polyelectrolytes that are detected are not dimers, trimers, etc. but single polymers.

2.2.1 Detector Selection

When comparing published works involving GPC analysis it is found that a variety of detectors are employed, sometimes in combination. Our work solely employs the DRI detector, which gives the difference in refractive index between the sample in the eluent and the eluent alone. This effectively gives the polyelectrolyte concentration (as shown by the varying peak heights) over the sample run time. This detector was chosen for many reasons. Since only the relative hydrodynamic volumes, and not molecular weight measurements, are required more complex detectors are not necessary. Light scattering equipment could also be used to determine hydrodynamic volume, possibly more easily, but the employed method of SEC with DRI was chosen in part due to its novelty as a technique for in-depth salt solution studies of polyelectrolytes. In addition, the DRI is currently the most common detector found in laboratories and allows for duplicate studies with other polyelectrolytes (i.e. method utilization). Lastly, the DRI detector does not alter the sample in anyway and has show sufficiently high accuracy. Therefore, for these reasons we are confident that the DRI detector is the detector best suited for this project.

2.3 Experimental Section

Although the literature has shown that large amounts of research have been conducted on polyelectrolyte solutions, the examination of PMPC cannot for obvious reasons be correlated with previous experiments done on cationic or anionic polyelectrolytes, due to its zwitterionic behavior. This has also proven to be the case of other polyelectrolytes that may contain both positive and negative charges.

In terms of salt interactions, ionic strength has been examined for a few systems but little to no research has been focused on the examination of valency, ion size, charge type, etc. with respect to hydrodynamic volume. One reference, discussed in the first chapter used GPC to examine the effect of LiBr salt concentrations on charged copolymers in dimethylformamide (Šegudovic et al., 1995). This set of experiments had a good basis but lacked the complexity necessary to obtain a complete picture of polyelectrolyte-salt interactions in solution. We have expanded on this method to examine the effect of different salt types and concentrations for PMPC in an aqueous system. We believe this will result in a detailed methodology of PMPC ionic interactions with salt in solution, which can in turn be used for other polyelectrolyte investigations.

2.3.1 Equipment

GPC analysis was conducted using a Waters 2690 Separation Module equipped with a 2410 differential refractive index (DRI) detector and four Waters Ultrahydrogel™ Linear columns enclosed within a heating unit. The columns were packed with a hydroxylated polymethacrylate-based gel, whose effective molecular weight range is $1x10^3$ to $5x10^6$ g/mol. The pore size of the column packing is a blend type ranging from

120 to 2000Å. The specifications of this column packing make it an ideal choice for analysis of our polyelectrolyte, as well as many other polyelectrolytes, regardless of charge and thus this work can be easily duplicated for other polyelectrolyte types. Retrieval and analysis of the GPC data was done with the Waters Millennium[32] Chromatography Manager software program.

2.3.2 Preparation of Experiments

2.3.2.1 Mobile Phase Preparation and Conditions

The solvent consisted of three parts, namely deionized water from the Barnstead EasyPure® II ultrapure water system, sodium azide and a salt of choice. The sodium azide (NaN_3) was used as a safety mechanism against bacteria growth within both the solvent reservoir and the GPC columns. It was used in a $0.1\%^{w}/_{v}$ concentration since it has been shown to be a sufficient amount for this purpose while minimizing changes to solution properties. A mobile phase containing the sodium azide additive alone was used as the control. In order to extensively examine the effect of salt on polyelectrolyte solution behavior many salts were employed in varying concentrations. With the exception of sodium azide and potassium chloride, which were purchased from Sigma-Aldrich Canada Limited, all the salts were purchased from Caledon Laboratories Limited. The salts that were used in this study are listed in Table 2.1. Each salt and concentration was selected so that definite conclusions could be made about the effect of concentration, ionic strength, solution pH, ion charge type (positive/negative), ion size, ion valency and ion configuration of the salt on the hydrodynamic volume of the polyelectrolyte.

Salt	0.0005M	0.001M	0.005M	0.05M	0.02M	0.1M	0.15M	0.3M
Na_2SO_4				X		X	X	X
NaCl		X				X		X
KCl						X		X
$CaCl_2$	X	X	X		X	X	X	X
KI						X		X
$NaNO_3$						X		X
Na_2HPO_4		X				X		

Table 2.1: Salts conditions that were utilized in experiments.

Temperatures of 35 °C and 40 °C were chosen for the columns and the DRI detector, respectively. These temperatures fully ensure that temperature fluctuations in the laboratory do not affect the temperature of the sample, to any detectable amount. These temperatures also make certain that the sample remains in solution and in addition are not high enough to affect the polymer in terms of degradation. A Plexiglas box was mounted around both the detector and the column-heating unit (containing the columns) to protect them from surrounding airflow. Elimination of normal room airflow is of particular importance when dealing with the ultra-sensitive DRI detector. A common flow rate of 1ml/min was chosen, taking into account both column sensitivity and time efficiency. Higher flow rates can lead to the degradation of the column packing by shear flow stress but on the other hand it is desirable to minimize the sample run time.

Therefore, a flow rate of 1ml/min provides an appropriate balance between temporal efficiency and column packing sustainability. Maintaining a flow rate of 0.1 ml/min during non-run times also served to prolong the life of the column.

With the current setup and system conditions a very stable base was achievable after a 24-36 hour equilibration run (as outlined in section 2.3.2.3). The stability of the baseline dictates the required concentration of a sample since concentration is directly related to peak height. Therefore, with a flat or stable baseline a smaller peak (i.e. a lower concentration sample) is sufficiently clearer than in a non-stable situation. Due to the stability achieved and maintained in our lab a low concentration sample is easily detected and analyzed.

2.3.2.2 Sample Preparation

Polyelectrolyte samples were obtained from fellow researcher Wei Feng, who previously synthesized PMPC through atom transfer radical polymerization methods. Experiments previously conducted by Feng with use of the GPC determined the molecular weights to be approximately 1500, 3000, 15000, 30000 and 60000 g/mol (see Table 2.2) These experimental values were in agreement with the theoretical values determined earlier and will thus be taken to be factual.

Sample	Molecular Weight (g/mol)	Monomer Units
1	1500	5
2	3000	10
3	15000	50
4	30000	100
5	60000	200

Table 2.2: List of polyelectrolyte samples.

The polyelectrolyte samples were made using a concentration of 1 mg of polyelectrolyte per ml of solvent. This concentration was found to show a sufficient peak while having little effect on the viscosity of the solution. Each sample had a volume of approximately 1.5ml so as to ensure that the extraction of a 40μl injection volume did not include any air bubbles. Minimization of the polyelectrolyte concentration was also done to ensure that the polymer was not wasted. After a sample of approximately 1.5g was obtained, the appropriate of solvent was added and the sample was then mixed for 5 minutes. Once the polymer was completely dissolved the solution was filtered and transferred to the GPC specific vials and loaded into the machine. Samples were then run immediately to ensure that the time in solution was the same for all samples with the same molecular weight value.

For each different solvent condition five samples were prepared, one for each molecular weight ranging from 1500 to 60000 g/mol. These molecular weights provided a sufficient range for this study. In order to ensure reproducibility every sample set included a repeat of the 1500 g/mol molecular weight sample. This was then compared with the original run, to determine the degree of equipment reproducibility, which is presented later in this chapter.

2.3.2.3 System Equilibration

In order to ensure both optimum baseline characteristics and minimization of machine error, the system was equilibrated after each transition to new mobile phase conditions. Altering the mobile phase involves either a change in salt type, salt concentration or both. For each eluent, the system was wet primed and the injector, pump and columns were flushed for 10, 30 and 180 minutes, respectively. In addition to this, a change in salt type required that the system be flushed with pure deionized water, prior to the introduction of a new salt in order to ensure no reaction between the two salt types. After the system was flushed the system was then equilibrated for approximately 24 hours. With each mobile phase a baseline was achieved in which the drift or variation did not exceed 0.1 mV per 100 minutes. This was found to be more than sufficient for the desired polyelectrolyte sample condition (e.g. concentration). A full list of system parameters and conditions can be found in Table 2.3.

Parameter	Setting
Column Temperature	35 degC
Detector Temperature	40degC
Flow Rate	1mL/min
Sample Concentration	1mg/mL
Sample Runtime	55 minutes
Base Elutant	Deionized Water
Additive	0.1%w/v NaN_3

Table 2.3: System parameters and conditions.

2.4 Reproducibility

Reproducibility was evident from initial results and calculations were done to determine the degree of reproducibility of the GPC equipment. In order to ensure reproducibility and accuracy of the results a second run of the 1500g/mol sample was conducted, for every sample set (1500-60000g/mol). By comparing the difference between the first and second run for each solvent condition it was found that the average difference was 0.0226 minutes and that the greatest difference was 0.081 minutes. To further examine reproducibility, variance was determined from the 1500g/mol sample in the 0.3M $CaCl_2$ solution with n=4. The variance was calculated to be 0.03584 minutes and it can be seen in Figure 2.1 where the variance was applied to all five molecular weight samples for the $CaCl_2$ data of 0.0005M and 0.3M concentration sample sets.

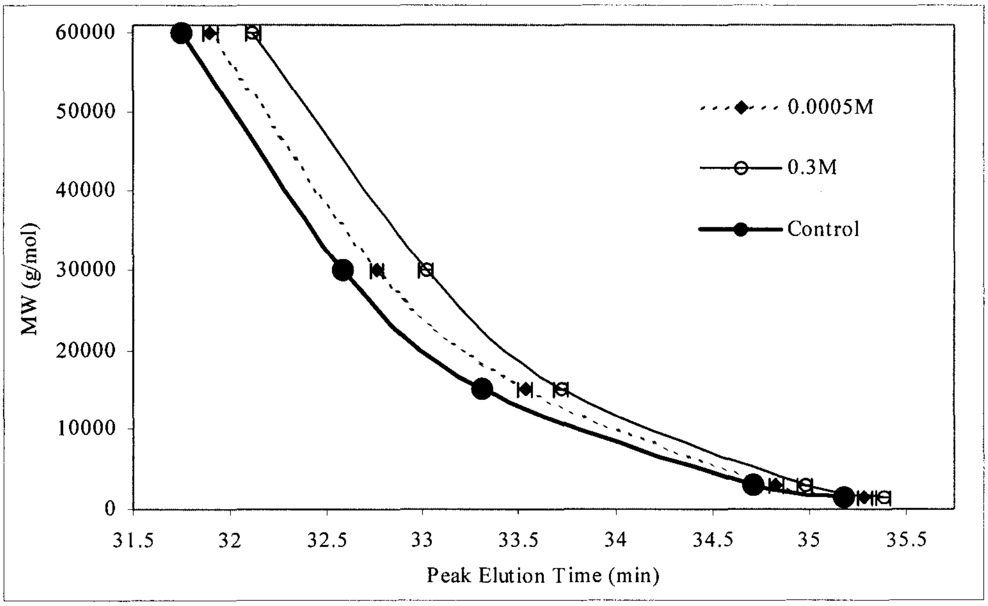


Figure 2.1: Graph to show reproducibility of data. Error bars show variance which was calculated to be 0.03584 with n=4. Curves are of $CaCl_2$ solutions and the control solution with NaN_3 0.1%$^w/_v$.

Due to the very small value it is believed that interpolation of the 1500g/mol sample variance to the other molecular weight samples is acceptable, as comparison between different solutions curves is well outside this range. Both the difference and variance calculation clearly set the range that must be taken into account when analyzing the data.

3 Results and Discussion

3.1 Outline of Properties Examined

The salt properties examined in this thesis work can be grouped 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 will affect the polyelectrolyte solution behavior. These properties include salt concentration or molarity, ionic strength of the solution 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 will be examined. The anion section, for reasons of which will become evident later, will involve the additional examination of ion configuration, which examines any differences in effect between monatomic and polyatomic anions. The ion properties section will conclude with an examination of how ion properties combine to bring about specific effects and how charge density, pKa value and other properties might be related to the trends that have been observed. An explanation of the systematic mechanisms associated with changes in hydrodynamic volume will shred some light onto why certain properties are more important than others. Leading from this a more in-depth study on the solution concentration will be presented to show how different ionic interaction mechanisms can result in four distinct regions of hydrodynamic behavior. The discussion will conclude by examining whether or not the effects observed by one molecular weight are also experienced by other molecular weights of the PMPC polymer. This last section will draw conclusions as to possible reasons for divergent effects between the different molecular weights. A table of the examined properties and the salts used to determine the resultant effects can be seen in Table 3.1.

Category	**Property**	**Salts**
Solution	Salt Concentration	$CaCl_2$
	Ionic Strength	$NaNO_3$ vs.Na_2SO_4
	Solution pH	Na_2HPO_4 vs. NaH_2PO_4
Ion	Cation Size/Type	KCl vs. NaCl
	Cation Valency	$CaCl_2$ vs. KCl/NaCl
	Anion Size	KCl vs. KI
	Anion Valency	$NaNO_3$ vs.Na_2SO_4
	Anion Configuration	NaCl vs. $NaNO_3$

Table 3.1: List of properties examined and the salts used to conclude for each property.

Figure 3.1 shows the molecular structure of the anions and cations in solution from all the salts used. It should be noted that the ion structure can greatly affect its own properties and how they interact with other entities. These structures will become important later on in the chapter, as a fuller understanding of the solution dynamics is unveiled.

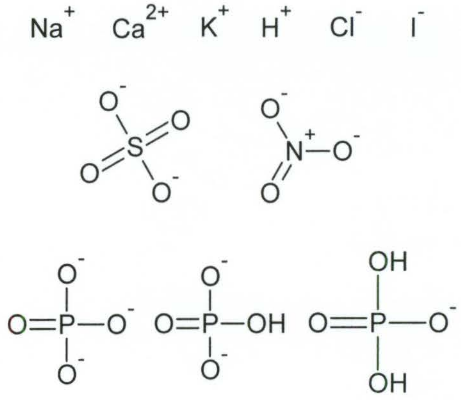


Figure 3.1: Structure of ions from salts used in experiments.

3.2 Solution Properties

Solution properties were investigated first due to the simplicity of analysis as compared to that of the ion properties. To begin the effect of salt concentration was determined which was then intended to ionic strength experiments. Lastly, the effect of solution pH was determined so that the effect of small pH changes in other studies can be accounted for.

3.2.1 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 ($CaCl_2$), varying between 0.0005M and 0.3M, were used (see Figure 3.2). 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).

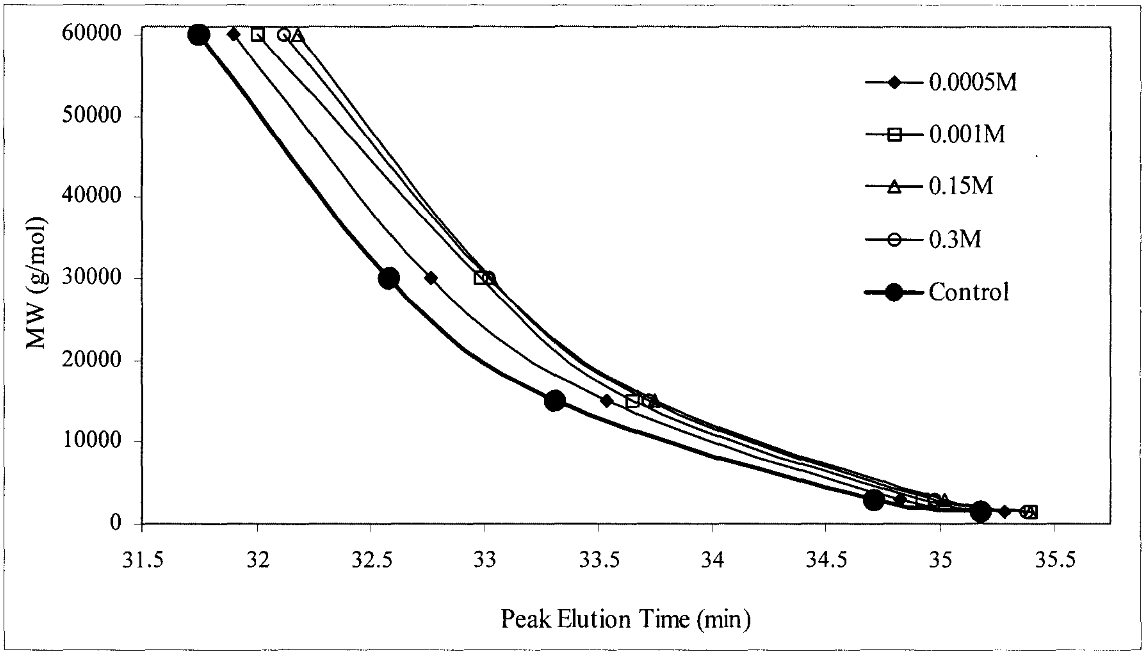


Figure 3.2: Effect of $CaCl_2$ salt concentration on elution time of PMPC.

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%$^{w}/_{v}$ of sodium azide (NaN_3). A comparison between the 0.0005M and the 0.001M curves shows that an increase in salt concentration will decrease the hydrodynamic volume of the polyelectrolyte. A further comparison of the three highest salt concentration leads to a conclusion that higher concentrations of salt have no greater effect on the hydrodynamic volume than the 0.001M concentrations. From this observation, it is believed that increasing the salt concentrations decreases the hydrodynamic volume up to a certain "saturation point" after which further increases in the salt concentration had no additional effect on the PMPC solution properties. The saturation point is believed to occur at a concentration at which all the charges present on the polyelectrolytes have been shielded/screened from other polyelectrolyte charge groups, either from the same polyelectrolyte or from other polyelectrolytes in solution. Without screening of repulsive forces between polyelectrolyte charge groups the polyelectrolytes will undergo chain expansion, resulting 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.

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. For example, one group of researchers examined the effect of solution conditions on the characterization of a phosphazene polyelectrolyte by aqueous gel permeation chromatography. One parameter that they examined was salt concentration, where they compared polyelectrolyte behavior in an aqueous solution of 0.42M NaCl to that in a solution of 0.14M NaCl (Andrianov & Le Golvan, 1996). They concluded that a change in concentration will not affect the solution properties but it is likely that this is not always the case. Had the researchers examined behavior at lower concentrations they would have expectedly observed some behavioral change and been able to conclude that by a 0.14M concentration the solution had already reached its saturation point. Therefore our finding provides a basis for other research and proves that extensive concentration ranges are particularly important when examining concentration effects.

3.2.2 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 (Murrell & Jenkins, 1994). 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 will carry the additional ionic strength from the sodium azide. In addition, since the sodium azide is in such low concentration it is believed not to significantly affect the solution properties.

For this investigation two salts were chosen, namely sodium sulfate (Na_2SO_4) and sodium nitrate ($NaNO_3$). Since ionic strength is a function of the ion valency and concentration both of these aspects need to be examined in order to make any conclusions about the effect of ionic strength on the hydrodynamic volume of PMPC. Curves of

Na_2SO_4 at concentrations of 0.1M and 0.3M were compared for the concentration component and curves of Na_2SO_4 at 0.1M and $NaNO_3$ at 0.3M were compared for the valency component. It should be noted that the ionic strength for the 0.1M Na_2SO_4, 0.3M Na_2SO_4 and the 0.1M $NaNO_3$ solutions are 0.3mol/L, 0.9mol/L and 0.3mol/L, respectively. These have all been graphed on Figure 3.3 for viewing clarity.

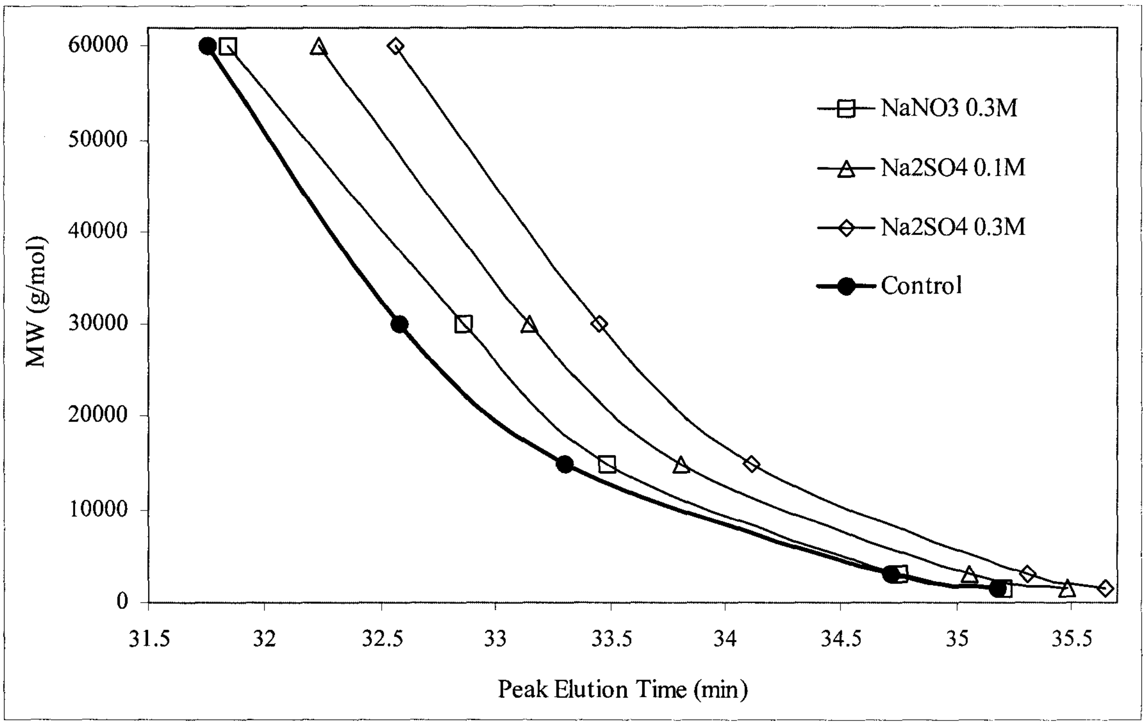


Figure 3.3: Graph shows effect of ionic strength. Ionic strength of $NaNO_3$ at 0.3M = ionic strength of Na_2SO_4 at 0.1M.

The comparison of the two Na_2SO_4 curves yields the same conclusion as the previous section, namely that higher concentrations decrease the hydrodynamic volume to a greater extent. Of greater importance is the comparison of Na_2SO_4 and $NaNO_3$ with equivalent ionic strengths. Comparison of the 0.3M $NaNO_3$ curve and the 0.1M Na_2SO_4

curve (both of 0.3M ionic strength) shows that different values of hydrodynamic volume are achieved. If ionic strength were an important parameter one would expect that solutions 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 large change in hydrodynamic volume. Once the parameter (or parameters) is determined it will likely be shown to be dominate over ionic strength. For this reason it is believed that although it cannot be concluded that ionic strength plays no role in the in 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. Although increasing the concentration of Na_2SO_4 (i.e. increasing the ionic strength of the solution) does bring about a greater change in hydrodynamic volume it is believed that the reason can be attributed to the concentration increase and not to the ionic strength increase due to the fact that the curves with the same ionic strength do not fall on to the same lines. In conclusion there is no substantial ionic strength effect on the hydrodynamic volume of PMPC.

3.2.3 Effect of Solution pH

All of the solutions produced throughout this work had pH values ranging from 7 to 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 will 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 0.1M NaH_2PO_4, 0.1M Na_2HPO_4 and 0.15M Na_2HPO_4. The first two salt solutions provide a comparison at the same salt concentration. The 0.15M 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 Figure 3.4 all three curves fall on the same values.

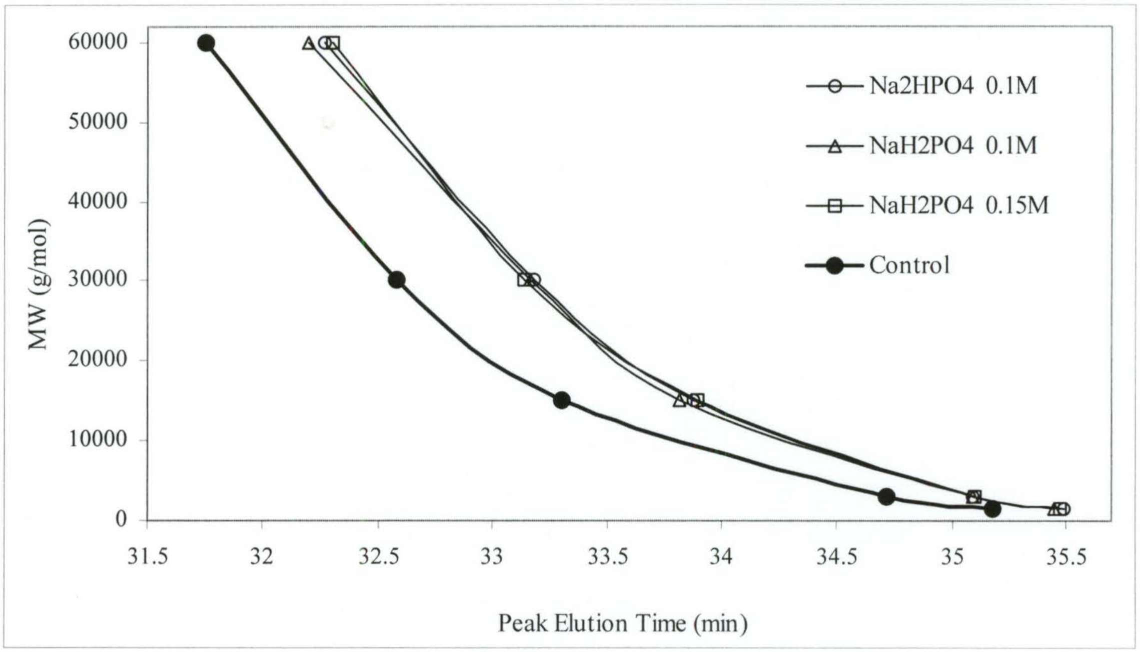


Figure 3.4: Graph to examine the effect of solution pH of PMPC. Note: pH values are: Na_2HPO_4 ~5 and NaH_2PO_4 ~9.

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 the difference observed was negligible with the large pH range (approximately 4 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) will not directly alter the hydrodynamic volume of PMPC. Therefore, it is similarly expected that a small pH range is not likely to affect other zwitterionic polyelectrolytes.

3.3 Ion Properties

With the exception of salt concentration (at low levels), no substantial evidence was found to show that solution properties were responsible for changes in the hydrodynamic volume of PMPC in the solution property section. Because of this one would believe that hydrodynamic volume changes must be attributed to other factors. This lack of understanding of the shifts involved with various salts and salt concentrations up to this point may be related to the fact that solution properties only directly affect intermolecular forces (i.e. forces between different polyelectrolyte chains) and not intramolecular forces (i.e. forces between charge groups found on the same polyelectrolyte). One reference, discussed in the introductory chapter, conducted a study on the effect of added salt on polyelectrolyte solutions using a small-angle scattering method. Although this study does not directly relate to this work nor does it examine more complex variables their conclusion is helpful in pointing to our pending results. Upon completion they were able to determine that changes in solution properties, as a result of added salt, can be accounted for by both inter- and intra-molecular functions. (Nishida et al., 2002). This further encourages us to investigate the ion properties to determine if they do in fact play a role in hydrodynamic volume change and possibly to determine if this is a result of changes occurring on the intramolecular level.

3.3.1 Cation Effect

In order to get a full picture of the salt interactions each salt type must be chosen so as to successfully examine the effect of the cation and the anion individually. In this section we will independently examine the cation component in terms of cation size/type and cation valency. Through initial brainstorming it was hypothesized that the salt anion would have a greater effect than the salt cation due to the geometry of PMPC. Since this polyelectrolyte carries the negative charge closer to the backbone (than the positive charge) it is expected that this charge would be more shielded and thus less involved in hydrodynamic volume changes. Therefore, it is anticipated that the salt cation will have less of an effect due to accessibility, among other variables.

3.3.1.1 Cation Size/Type

In order to determine the effect of cation size and/or type appropriate salts needed to be selected, which consisted of the same anion and differently-sized cations. For example, comparison of a sodium ion (22.99g/mol) and a magnesium ion (24.31 g/mol) would not be good choices, for two reasons. Firstly, the molecular masses of the two ions are not significantly different and any difference would most likely not be detectable. Secondly, the two cations have different valences, namely the sodium ion is monovalent and the magnesium ion is divalent. By comparing these two ions one would also be comparing valency and thus no clear conclusion could be made. Therefore, for the purpose of determining the cation size/type effect, we chose sodium chloride (NaCl) and potassium chloride (KCl) salts. The molecular mass of Na^+ and K^+ are 22.99g/mol and 39.10g/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 the 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 Figure 3.5, curves of both KCl and NaCl at 0.1M concentration are compared. Since the anion type, anion concentration, the salt concentration and the cation valency are the same for each curve any difference can be directly attributed to the cation size/type.

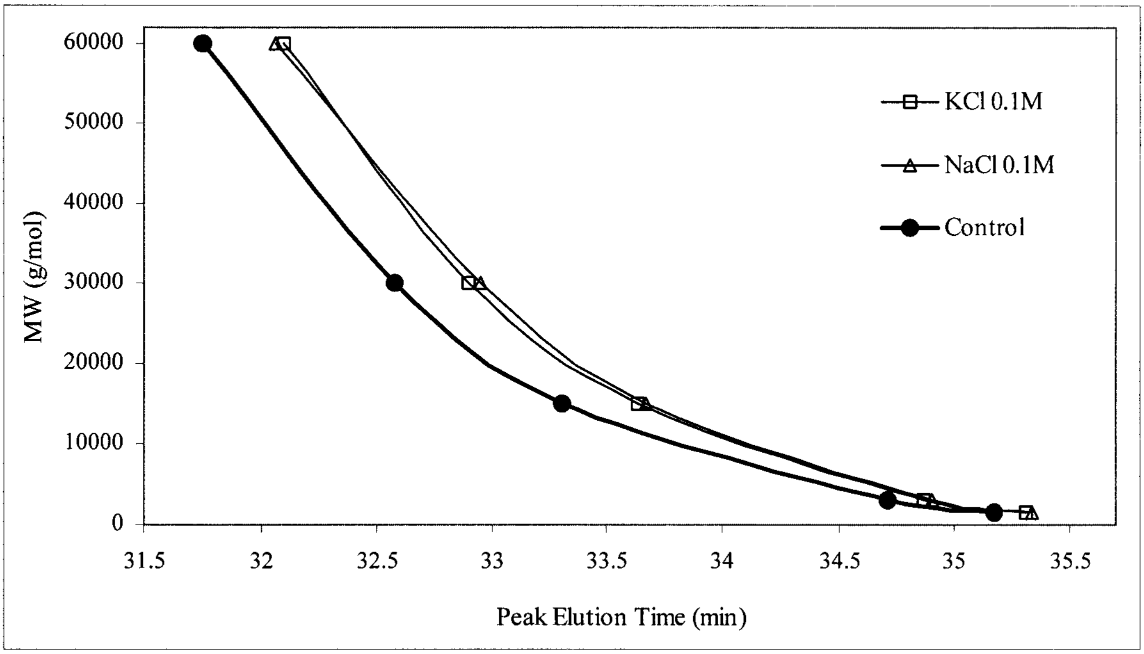


Figure 3.5: Graph examining the difference in effect between Na^+ and K^+ ions Note: $Na^+ < K^+$.

Examination of the two curves shows that they are relatively identical at each molecular weight. Any small differences for specific molecular weight points between the two salt solutions are well within the variance parameters stated in Chapter 2. It should be noted that in all the experiments presented the elution time span between adjacent molecular weight samples (ranging from approximately 0.5 to 0.8 minutes) is considerably larger than the variance value (0.03584). Due to the great similarity of the two curves, it can confidently be concluded 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 changes. Preliminary mechanistic conclusions would be that the cation has little-to-no effect due to the specific geometry of the PMPC molecule (i.e. the polyelectrolyte negative charge is located close to the backbone, while the positive charge is located on the end of the side chain) and related steric hindrances.

3.3.1.2 Cation Valency

Now that the effect of cation size/type can be ruled out the next logical step to take would be to do a similar comparison with different salts in order to determine whether a change in cation valency brings about a change in the hydrodynamic volume of PMPC. This will result in either validation or invalidation of the preliminary conclusion that the cations have no considerable effect due to the geometry of PMPC.

To examine the cation valency effect, salts needed to be selected that again contained the same anion and different cations. The salts chosen were NaCl, with monovalent Na^+ and $CaCl_2$, with divalent Ca^{2+}. The comparison of these two salts was considerably more complex due to the fact that a 1M $CaCl_2$ solution will contain twice as many Cl^- ions and three times the value of ionic strength as a 1M 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 3.6 shows the comparison of monovalent and divalent cations. In order to fully address all possible parameter variations three concentrations of $CaCl_2$ were compared to a 0.3M NaCl solution. $CaCl_2$ at a concentration of 0.3M directly compares to the 0.3M NaCl solution under constant salt concentration. The 0.15M $CaCl_2$ solution compares to the 0.3M NaCl solution under constant anion concentration and the 0.1M $CaCl_2$ solution compares to the 0.3M NaCl solution under constant ionic strength. On 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 visible effect on the hydrodynamic volume of PMPC.

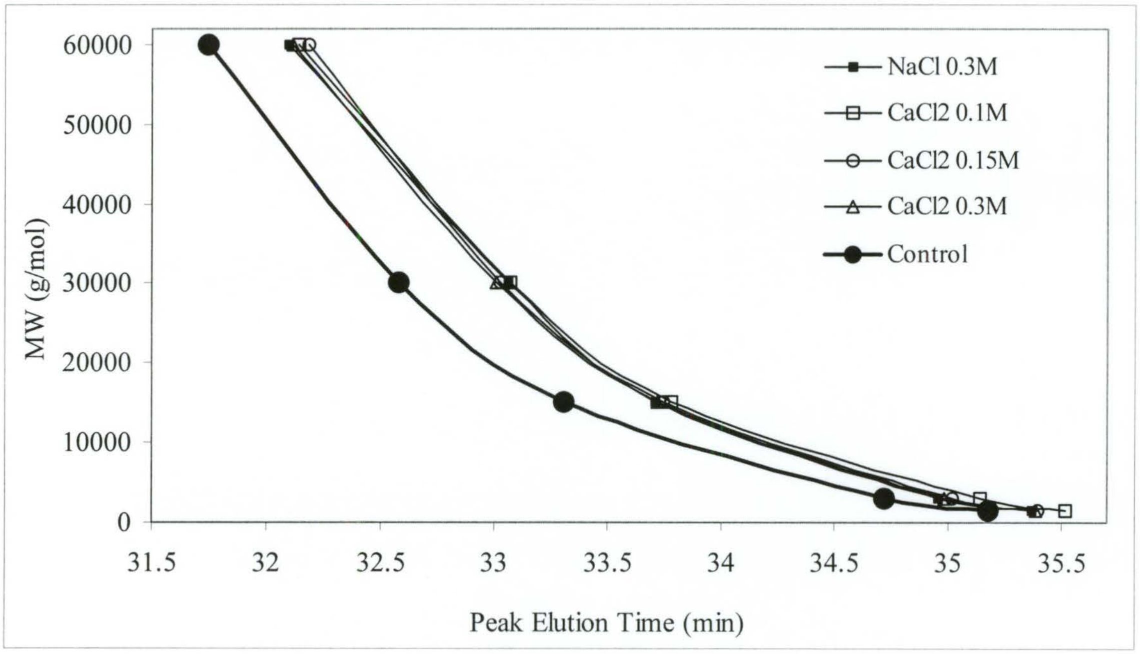


Figure 3.6: Graph examining the hydrodynamic volume difference associated with cation valency. Monovalent Na^+ is compared with divalent Ca^{2+}.

This verifies the preliminary conclusion stated in the last section, that the cation has no effect. Surprising though the conclusion that the cation valency does not affect the solution properties of PMPC contradicts previous research conducted in 2003. In that study the effect of monovalent NaCl and divalent $CaCl_2$ on polymer aggregates of phosphorylcholine (PC)-based copolymers using fluorescence spectroscopy was examined. It was found that the divalent salt had an effect on the solution properties resulting in the disruption of the aggregates while the monovalent did not (Miyazawa & Winnuk, 2003). The reason for the contradictory conclusion regarding whether or not the cation does affect the solution properties of zwitterionic polymers could be due to many reasons. First, the polymer that they used is a copolymer of poly (*N*-isopropylacrylamide) (PNIPAM), pyrene and PC, which will result in very different solution behaviors due to interaction between the different monomer types. Second, the study was examining aggregation not specifically hydrodynamic volume. Lastly, and possibly most importantly, the researchers did not address the fact that the divalent salt will contain twice as many Cl^- ions as the monovalent salt. As we have shown in the salt concentration section, Cl^- ion concentration can affect the solution properties (up to a certain point) and therefore should be considered. Closer examination of the data presented in that paper suggests that the last reason is quite plausible but further investigation would need to be conducted to completely verify this rationalization. Due to these reasons it will be taken as fact that there is no cation valency effect and thus all remaining experiments will be done under the assumption that the any cation valency effect is negligible and thus can be excluded when conducting analysis of other properties.

3.3.1.3 Cation Conclusion

Through the comparisons made we have found that by varying the cation type/size and the cation valency no change in the hydrodynamic volume of PMPC was observed. Upon returning to the original hypothesis, which stated that the cation would have a less effect than the anion, it was discovered that not only will the anion have a greater effect but also that the cation has no observable effect at all. This conclusion with its supporting data is likely to be applicable to other zwitterionic polyelectrolytes. In addition the interpretation may have implications for the copolymer research as discussed in the last section and possibly for other polyelectrolyte research.

3.3.2 Anion Effect

Now that it has been determined that the cation component of salts does not affect the hydrodynamic volume of PMPC further experiments can be carried out to examine the role of the anion component of the salt. In this section we will again examine the size/type and valency, and additionally, the ion configuration/geometry will be studied, which will compare monatomic anions to polyatomic anions.

3.3.2.1 Anion Size/Type

In order to examine the anion size/type effect on the hydrodynamic volume of PMPC two salts were selected that contained anion molecules of significantly different size. In this case KCl, with the Cl^- anion and potassium iodide (KI) with the larger I^- anion were used, where the molecular mass of the Cl^- anion and the I^- anion are 35g/mol and 128g/mol, respectively. In addition to mass differences, the volume of I^- is significantly larger than Cl^- due to the presence of extra orbitals (3d, 4s, 4p, 4d, 5s, 5p). 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 isolate the only variation to the anion size/type. This is also achievable since both anions are monatomic and monovalent in nature.

By comparing the KCl and the KI curves in reference to the control curves, found in Figure 3.7, one can see that clear conclusions 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, within the previously determined variance of 0.0358 minutes. 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.

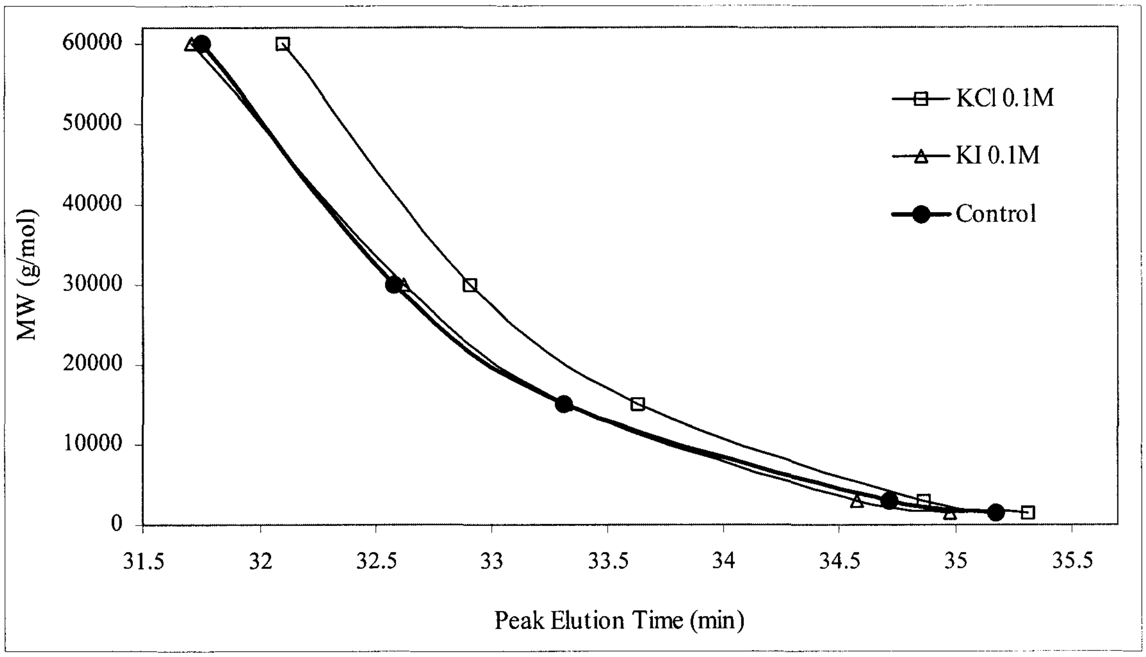


Figure 3.7: Graph examining the difference in effect between Cl⁻ and I⁻ ions Note: Cl⁻ < I⁻.

Therefore, in summary the anion has shown to have and not to have an effect on the hydrodynamic volume of PMPC, where I^- ions do not affect the solution properties of PMPC to any observable degree while Cl^- ions in solution can greatly decrease the hydrodynamic volume of PMPC. Therefore, a smaller salt anion will affect the hydrodynamic volume of PMPC more than the larger salt ion.

By giving particular and more detailed attention to the KI curve, with respect to the control 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 $0.1\%^{w}/_{v}$ of NaN_3. One may think that a possible reason for the lack of effect of the larger ion is due to the steric hindrance associated with the very large

I^- anion. A larger ion may not be able to penetrate the polyelectrolyte volume thus resulting in no screening of the polyelectrolyte charges. 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. The relative size of the anion compared to the polyelectrolyte void volumes is likely very small and therefore diffusion-related steric hindrance of the I^- is likely not occurring. This will be revisited later on in the chapter.

Another interesting observation of the KI curve with respect to the control curve, involves the cation. If it is agreed that the I- anion has no effect due to its size then the fact that this line falls on the control curve further proves that there is no cation effect. The mechanism responsible for the lack of effect due to the cation and the large anion will be discussed in further detail later on in this chapter once a complete set of parameters is presented.

3.3.2.2 Anion Valency

Now that it has been determined that the anion component of the salt does have an effect on the hydrodynamic volume of PMPC and that the effect can vary depending on what anions are present in the solution, the next step is to formulate a more complete picture of the degree of effect as a result of other anion characteristics. Therefore, we will now examine the effect associated with the anion valency. For this investigation, salts of sodium nitrate ($NaNO_3$) and sodium sulfate (Na_2SO_4) were chosen. 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 g/mol and 96 g/mol respectively. In addition both anions are polyatomic and thus both have polarity.

By comparing concentrations of 0.3M for $NaNO_3$ and 0.15M for Na_2SO_4 both the concentration of the cation and the number of negative charges in solution from the anion are equivalent. Figure 3.8 shows these two concentrations along with the control curve.

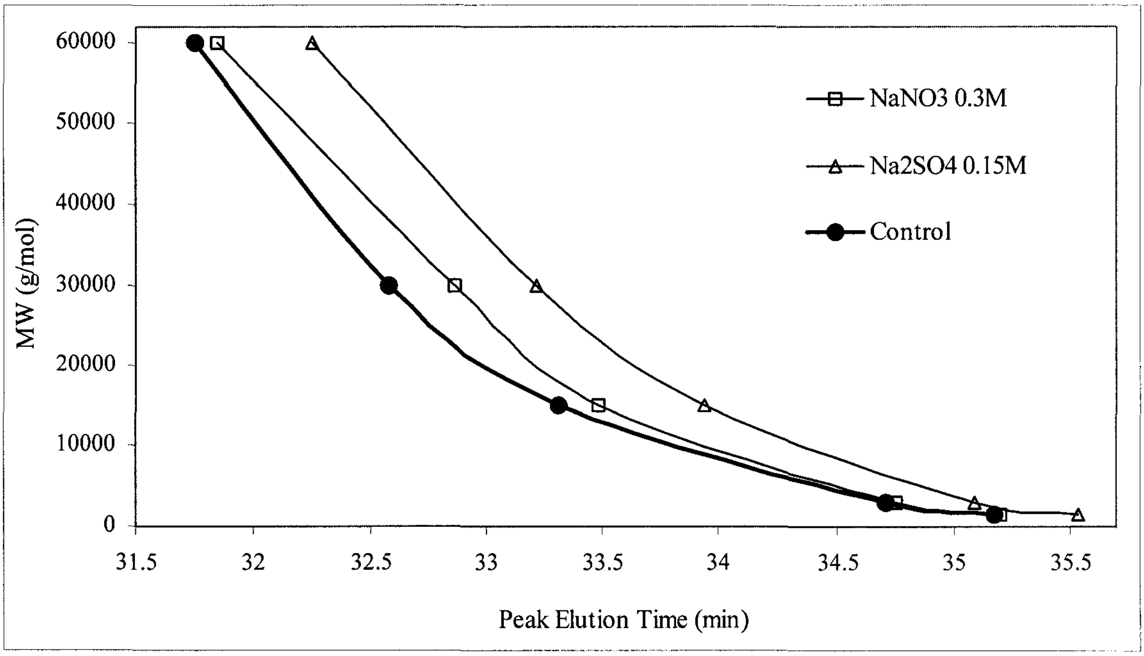


Figure 3.8: Graph examining the difference associated with anion valency. Monovalent NO_3^- is compared with divalent SO_4^{2-}.

Simple observation shows that both salt solutions cause a shift to a higher elution time as compared to the control curve. As before, this denotes a decrease in the hydrodynamic volume of the PMPC sample. Therefore it can be said that both salt have an effect on the PMPC through reduction of the hydrodynamic volume. It is also 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 minutes, which can be considered quite a substantial shift, especially when taking into account that the time lapse between the 1500g/mol and the 3000g/mol sample is only about 0.5 minutes. 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-} will decrease the hydrodynamic volume to a considerably greater extent than the monovalent NO_3^-.

Another interesting observance in the graph concerns the $NaNO_3$ curve in reference to the control curve. It is found that the difference between the $NaNO_3$ and the control peak elution times for each molecular weight value decreases as the molecular weight decreases. For example, when comparing the two points (one for the $NaNO_3$ and one for the control) for both the 3000 and 30000 g/mol molecular weight samples the shift is very much different. For the 3000 g/mol molecular weight samples the point only seems to shift by 0.04 minutes, while the shift is approximately 0.3 minutes for the 30000 g/mol molecular weight sample. In other words, higher molecular weight samples will go about a greater decrease in hydrodynamic volume than a lower molecular weight samples in the presence of certain salts. In the last section of this chapter the mechanism involved in this phenomenon will be elucidated but for now it seems that the next logical step is to examine how the anion configuration will alter the salt effect on the hydrodynamic volume of PMPC.

3.3.2.3 Anion Configuration

Anion configuration or anion geometry denotes molecular structure of the anion in solution. The two basic configurations examined in this work are monatomic, where the anion consists of a single atom and polyatomic, where the anion has more than one atom.

For this study NaCl and $NaNO_3$ salts were used to examine the difference between a monatomic Cl^- ion and a polyatomic (4) NO_3^- ion. Both salts have already been shown to have an effect on the hydrodynamic volume of PMPC in reference to the control curve. In Figure 3.9 we compare both salts at 0.3M, which allows for equivalency of other solution properties such as solution concentration, solution ionic strength, cation concentration and anion valency.

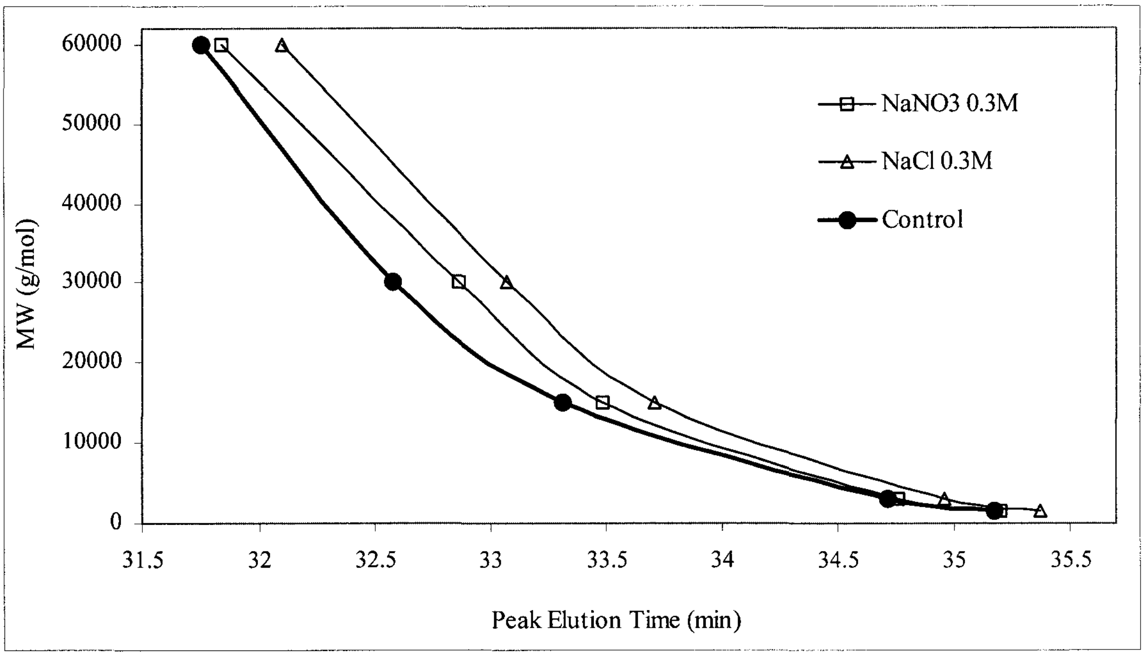


Figure 3.9: Graph examining the effect of anion configuration through comparison of the monatomic Cl^- ion and the polyatomic NO_3^- ion.

It is clearly observed that the Cl^- ion decreases the hydrodynamic volume of PMPC to a greater extent than the NO_3^- ion, as denoted by a greater shift to the right. Again these curves were examined while accounting for the pre-determined variance of 0.03584 minutes. Due to the equivalency of the other solution and ion properties, in can be concluded, at this point, that a monatomic anion will have a greater effect on the hydrodynamic volume of PMPC than a polyatomic anion. In 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, where the molecular masses of Cl^- and NO_3^- are 35g/mol and 62g/mol, respectively, this conclusion cannot be fully verified at this time. In a later section an examination of combination properties will be conducted and it is expected at that time a more qualified conclusion will be made.

3.3.2.4 Anion Conclusion

Unlike the conclusion made in the cation investigation, there does appear to exist an anion effect on the hydrodynamic volume of PMPC. Again we return to the original hypothesis, which states that the cation would have a less effect than the anion, and find that the anion does in fact have a greater effect to the point where any cation effect present is so minimal that it is simply not observed. From this general conclusion, more specific conclusions can now be made about the anion size/type, valency and configuration.

It was discovered that the smaller Cl^- anion had a greater effect than the larger I^- anion, that the divalent SO_4^{2-} anion had a greater effect than the monovalent NO_3^- anion, and that the monatomic Cl^- anion had a greater effect than the polyatomic NO_3^- anion with respect to the hydrodynamic volume of the PMPC. From these discoveries it can be concluded that a greater decrease in hydrodynamic volume is expected when smaller, higher valency and/or monatomic anions are present in solution. Now that a full picture of the individual and solution properties has been drawn a more detailed attempt can be made at explaining the mechanism involved. It is believed that the mechanism of polyelectrolyte-salt interaction will clearly show why certain properties are more important than others.

3.3.3 Polyelectrolyte-Salt Interaction Mechanism

Although there may be cation presence within the polyelectrolyte volume, results have shown that the cation is not responsible for changes in hydrodynamic volume. Furthermore, it has been shown that the anion component of the salt has a considerable effect on the hydrodynamic volume of PMPC. The reasoning behind the absence of the cation effect and the presence of the anion effect can be visualized through Figure 3.10 and Figure 3.11.

Figure 3.10 shows how the polyelectrolyte likely behaves in the presence of salt ions. The left diagram shows how a polyelectrolyte will adopt a linear configuration in solution due to repulsive forces between charge groups on different side chains. The right diagram shows how, with the addition of salt, the polyelectrolyte is able to change to a more random coil configuration, effectively decreasing the hydrodynamic volume. 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 outlying 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.

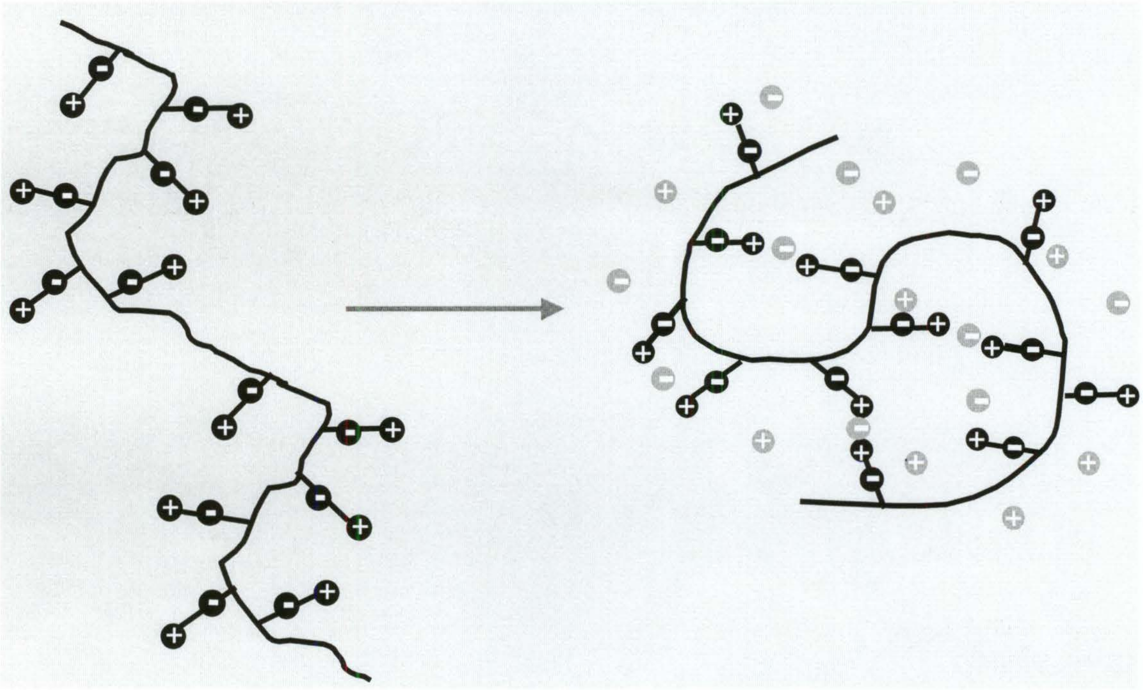


Figure 3.10: Schematic showing how salt ions in solution can decrease the PMPC hydrodynamic volume.

The lack of influence of the cation is related to the structure of PMPC in terms of side chain flexibility, steric hindrance and repulsive forces. Due to the closer proximity of the polyelectrolyte negative charge (anionic group) to the backbone it is believed that interaction of solution cations with negative charges from two side chains will not be 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 Figure 3.11.

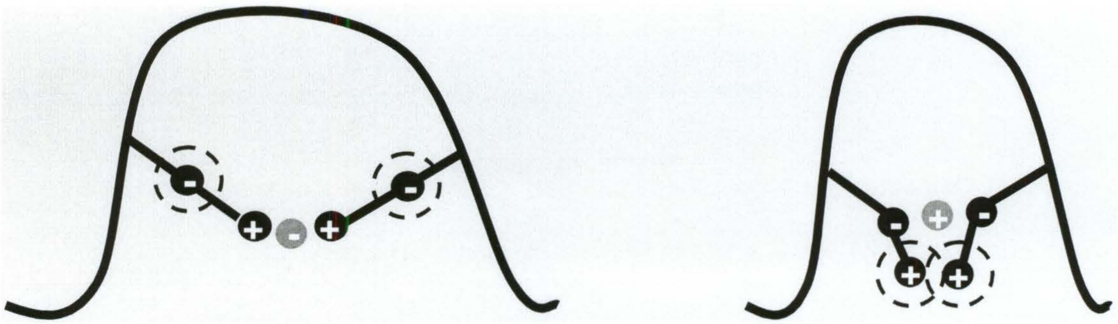


Figure 3.11: Schematic showing why the salt anion has an effect and the salt cation does not. Insufficient chain flexibility, repulsive forces and steric hindrance impede ionic interaction between the polyelectrolyte and the salt cation.

Convergence of two anionic charge groups of the polyelectrolyte to one single salt cation will require that the side chain bend and flex, likely to implausible degrees. In addition, it is believed that the portion of the side chain following the negative charge (i.e. the $CH_2CH_2N^+(CH_3)_3$ component) will cause 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 will 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 of Figure 3.11, which denote the area of charge presence. Here the two dotted rings are likely to overlap resulting in strong repulsive forces that will 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. Therefore, due to the geometry of PMPC, where the anionic charge group is situated in closer proximity to the backbone than the cationic charge group, there exists a considerable anion effect but no discernable cation effect from the salt.

This mechanism can be extended to other polyelectrolyte geometries. For example, when examining polybetaines with reverse placement of the positive and negative charges (i.e. the cationic charge group is closer to the backbone than anionic charge group) one would expect that the salt cation would be of greater influence on the hydrodynamic volume than the salt anion. An extension of this would involve the examination of cationic and anionic polyelectrolytes. It would be likely that only the anion component of the salt would significantly alter the hydrodynamic volume of a cationic polyelectrolyte and that only the cation component of the salt would significantly alter the hydrodynamic volume an anionic polyelectrolyte. Of course this cannot be considered conclusive without the appropriate experimentation but it can be stated as a highly reasonable hypothesis.

Some previous studies have been conducted regarding these phenomena and therefore our type of study could be used to clarify or verify previous data obtained. One such study was conducted in 2005 that examined the effect of salt on a cationic polyelectrolyte by SEC methods. They observed that the salt anion and the salt cation produced different effects with respect to the solution properties of the polyelectrolyte. It was concluded that the anion had a greater effect on the polyelectrolyte and that it was possibly due to the anion's ability to get closer to the polyelectrolyte because of the positively charged nature of the polymer. Interestingly, they found that Br^- ions reduced the hydrodynamic volume to a greater extent than the smaller Cl^- ions, which is in contrast to our observed trend for zwitterionic PMPC. Due to the complex nature of zwitterionic polyelectrolytes it is quite possible that the trends observed are not applicable to single-charged polyelectrolytes (cationic or anionic). It should however be noted that they did suggest that observed differences were within experimental uncertainties and thus additional tests were required, such as viscosity and conductivity measurements (Marcelo et al., 2005). Similar studies for other cationic or anionic polyelectrolytes have been conducted and it would again be interesting to verify their results using our method.

3.3.4 Combination Properties

Up to this point many conclusions have been made about the ion properties, all of which have been considered independent of one another. The distinct conclusions include: 1) there is no observable cation effect on the hydrodynamic volume, 2) there is an anion effect on the hydrodynamic volume, 3) a smaller anion decreases the hydrodynamic volume more than a larger anion, 4) a divalent anion decreases the hydrodynamic volume more than a monovalent anion and 5) a monatomic anion decreases the hydrodynamic volume more than a polyatomic anion. From these conclusions one would assume that a complete picture has been drawn but upon observation of Figure 3.12 this seems not to be the case.

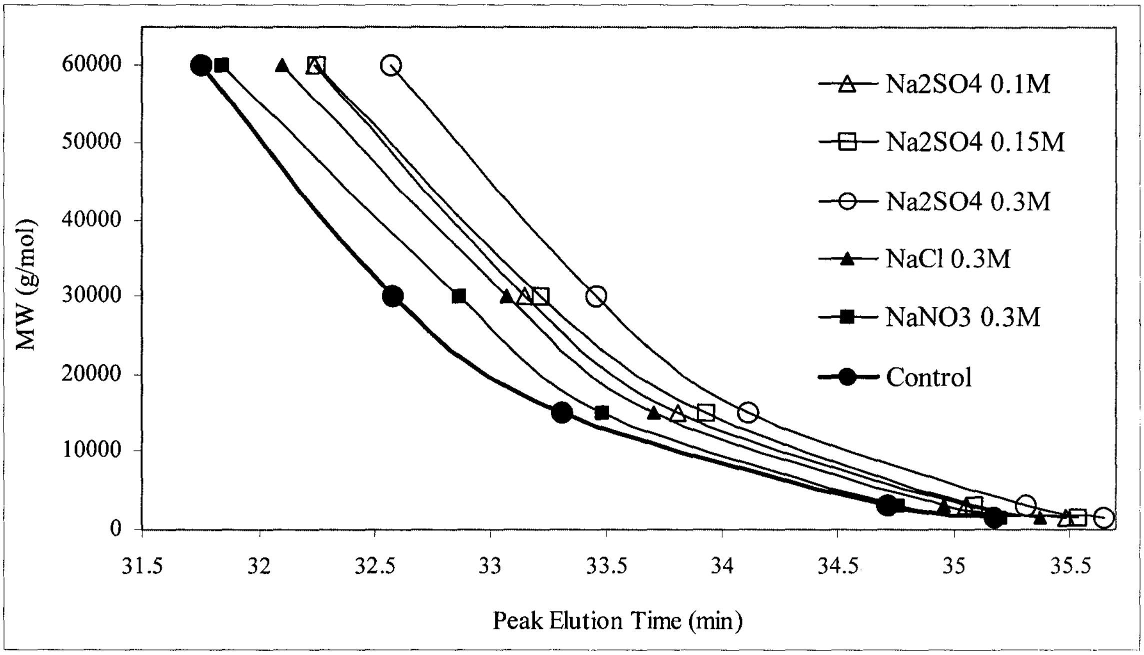


Figure 3.12: Graph examining the combination of effects from anion size/type, valency and configuration.

The graph compares the control curve to both NaCl and $NaNO_3$ at a concentration of 0.3M as well as Na_2SO_4 at various concentrations. Although both the NaCl and the Na_2SO_4 curves are located to the right of the $NaNO_3$ curves (denoting a greater effect), as observed before, the placement of the NaCl and the Na_2SO_4 curves relative to one another is peculiar. This placement follows the conclusion that a divalent anion has a greater effect on the hydrodynamic volume of PMPC than a monovalent anion but it contradicts another conclusion. In the anion configuration section it was concluded that a monatomic anion has a greater effect on the hydrodynamic volume of PMPC than a polyatomic anion. Therefore, following this the NaCl curve would be to the right of the Na_2SO_4 curve. When comparing divalent SO_4^{2-} and monovalent Cl^- the opposite seems to be true since the Na_2SO_4 curve is a higher elution times than the NaCl curve, denoting a larger reduction in hydrodynamic volume. Specifically, even though the SO_4^{2-} is a polyatomic anion it has more effect than the monovalent Cl^-. This finding suggests that the divalent nature of the SO_4^{2-} anion more than compensates for the fact that it is a polyatomic anion. Due to this observation it would seem that anion effect is a combination of various anion characteristics and that the degree of hydrodynamic volume reduction results from some weighted addition of these properties.

Now that all of the properties have been investigated the different anions can be organized in reference to one another according to their ability to decrease the hydrodynamic volume of PMPC. The following shows the sequence of increasing anion effect on the hydrodynamic volume: $I^- < NO_3^- < Cl^- < SO_4^{2-}$. In order to understand the balance of properties it is important to investigate other characteristics, other than size, valency and configuration, which may further explain the balance of properties. Only once all related properties are addressed can the complete methodology of salt effects on the solution properties of polyelectrolytes be formulated.

3.3.5 Other Properties Related to the Anion Effect

In addition to the structural mechanism displayed in section 3.3.3 further factors may also point to the reasoning behind the effect of the various anion characteristics; type/size, valency, and configuration. In this section several areas of molecular chemistry and physics will be examined to see if other molecular properties lead to the different salt effects on the hydrodynamic volume of PMPC. By examining other properties we should be able to explicitly account for and elucidate the combination effects of the solution and ion properties investigated experimentally.

3.3.5.1 Dissociation Constant and pKa

Another means of measuring the anions ability to interact with the polyelectrolyte cationic group is by examining the relation of interaction with respect to dissociation constants. The dissociation constant or the pKa value of a molecule measures it ability to attract and hold onto a H^+ ion. For each acid, there exists a conjugate base, such as hydrochloric acid (HCl) and its conjugate base the chloride ion (Cl^-). An acid that is very strong will have a very weak conjugate base. This is important in our work since a conjugate base will be the anion salt components, such as Cl-, I-, etc. By examining the strength of the anion's acid conclusions can be made about the strength of the anion and its ability to attract and hold the cationic group of PMPC.

It is known that the pKa values for the anion-related acids increase in the following order: $H_2SO_4 < HCl < HNO_3 < HI$. Hence, the strength of the conjugate bases in increasing order is as follows: $I^- < NO_3^- < Cl^- < SO_4^{2-}$. These trends are particularly interesting since they follow the same order as the anion's ability to decrease the hydrodynamic volume of PMPC, where a strong conjugate base will decrease the hydrodynamic volume to a greater extent. This outcome is particularly exciting since it gives a basis to our data.

It should also be noted that anion parameters such as ionization energy, electronegativity and electron affinity all follow similar trends to the dissociation constant. This is expected as all these parameters are directly or indirectly related and therefore all help to verify that the experimental data obtained through this work is accurate and probable.

Extension of this investigation to examinations of ionic potential and charge density will serve to explain why these chemical properties follow our observed trends, in terms of ion characteristics.

3.3.5.2 Ionic Potential and Charge Density

The most obvious explanation of the combined property phenomenon involves the ionic potential of the anion or in other words, 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 are occurring that decrease the hydrodynamic volume. Distinguishing between these processes is important when remarking on the effect of solution properties versus the effect of ion properties. The first process involves screening of repulsive forces. As explained earlier, when PMPC is in solution the charge groups of equivalent sign will repel each other resulting in an expanded, linear configuration. Salt ions in solution are able to screen the repulsive forces that occur between like-charged groups on the polyelectrolyte, effectively depleting the repulsive forces and allowing for the polyelectrolyte to attain a more random-coil configuration. This mechanism is linked to the solution properties discussed at the beginning of this chapter, namely ionic strength and concentration. The second process is significantly more complex and involves specific ionic interactions between the polyelectrolyte charge groups and the anions in solution. As shown in Figure 3.10 and Figure 3.11 an anion can situate itself in close proximity to two (or more) cation charge groups and in actual fact bring the two groups closer together, resulting in a overall decrease of hydrodynamic volume. It is this process which 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 (Sata & Nojima, 1999) 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 2.31Å (Sata & Nojima, 1999), 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 both 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; the surface charge density of SO_4^{2-} is less than that of both Cl^- and NO_3^- and therefore we find that the surface charge density trend does not apply to all of our data. Further reflection produced two possible reasons for discrepancy between the experimental data and theoretical values.

The first reason is 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 and not so much the surface charge density. The divalent nature of the SO_4^{2-} ion requires that it "bind" with two positive charges (in other words two cationic groups) to achieve neutrality. Due to the fact that this neutralization will result in a lower energy state, the anions ability to "pull" two cationic groups together is very 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).

The other rationale associated with the discrepancy between empirical and theoretical data is related to the accuracy of the surface charge density calculations. A better means of comparing charge density may be to utilize anion volumes. Previous calculations of surface charge density were conducted under the assumption that the anions were all spherical in nature. Since this is not necessarily the case (especially with polyatomic ions) the use of predetermined anion volumes may be more accurate in examining surface charge density. 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, respectively (Jenkins, H. Donald B., Roobottom, Passmore, & Glasser, 1999). For the SO_4^{2-} anion the volume per negative charge becomes 0.0455 nm^3. By comparing the values of volume per negative charge for the four anions used is this work we find that the charge density increases as follows: $I^- < NO_3^- < Cl^- < SO_4^{2-}$. Recalling the order of increasing effect on the hydrodynamic volume of PMPC ($I^- < NO_3^- < Cl^- < SO_4^{2-}$), we find that both trends are the same. 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 will determine 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.

3.4 Salts Remaining from Polymerization and Counterion Binding

When a polyelectrolyte solution sample is prepared solution ions can be present from three different sources; those leftover from polymerization, polyelectrolyte counterions and ions from added salt. The last source is what we have been examining in this work but it is important to address the other two sources in order to get a complete understanding of the ionic interactions occurring in our polyelectrolyte samples.

Some concerns that have become apparent in the literature involve the presence of salts leftover from polymerization. During polymerization salts such copper bromide are used. Different methods are utilized to remove these ions, but without sufficient time scales or good methods some still remain, especially those that are situated within the polyelectrolyte volumes. In some cases the salt ions can complex with the polyelectrolyte. However, unlike polyelectrolytes such as poly(methacrylic acid), PMPC does not complex and therefore methods such as dialysis can successfully remove the remaining ions. For our polyelectrolyte samples, dialysis was run for five days upon which time no color remained (which denotes the presence of salt), indicating the removal of the majority of the salt ions. Due to the lengthy dialysis procedure run for our samples and the fact that the copper bromide is not likely to complex with PMPC, we believe that ions from the polymerization are not present. It should however be noted that studies on salt effects, which are conducted without sufficient removal of the polymerization salts, are likely to have inconclusive and/or inaccurate results.

The other possible source of ions in solution, other than those from added salt is polyelectrolyte counterions. Counterions in solution can be distributed in three ways; moving outside polyelectrolyte volume, bound to the polyelectrolyte but able to move within the polyelectrolyte volume, and bound to a specific polyelectrolyte charge group and unable to move (Oosawa, 1971). Due to the highly distributed nature of counterions they can greatly alter the solution behaviors of the polyelectrolyte. Fortunately, in the case of zwitterionic polybetaines there are no counterions from the polyelectrolyte. Only with cationic, anionic and sometimes amphiphilic polyelectrolytes, are there counterions present in solution. For example, cationic poly (dimethylaminoethyl methacrylate) contains Cl^- ions in solid form for neutralization, which are released when the polyelectrolyte is put into solution. With polybetaines (dually charged), the charge groups will be neutralized by each other and thus no counterions exist or are released into solution. Therefore without the presence of polymerization salts or counterions it can be concluded that the only ions that are of concern in our work are the ions from the added salt.

3.5 Additional Concentration Effects: Result of "Over-Saturation"

To get a comprehensive understanding of the effect of salt concentration, additional salt solution sample sets were run, so as to examine the validity of our previous observations. Although the preliminary conclusions were found to be somewhat correct, a peculiar phenomenon was observed which led to a new evaluation of the data. In the original salt concentration study it was determined that an increase in the salt concentration resulted in a more pronounced effect on the hydrodynamic volume of PMPC. Specifically it was found that at higher salt concentrations the hydrodynamic volume was minimized. It was also preliminarily determined that additional increases in salt eventually leads to a so-called "saturation point" at which further increases in salt concentration no longer decreased the hydrodynamic volume of the polyelectrolyte.

The new data is presented in Figure 3.13, which shows the effect of seven different calcium chloride salt concentrations on the 1500g/mol and 3000g/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 1500g/mol to 60000g/mol. Error bars representing the variance of 0.03584 minutes have been used to show that the trends are not a result of experimental error.

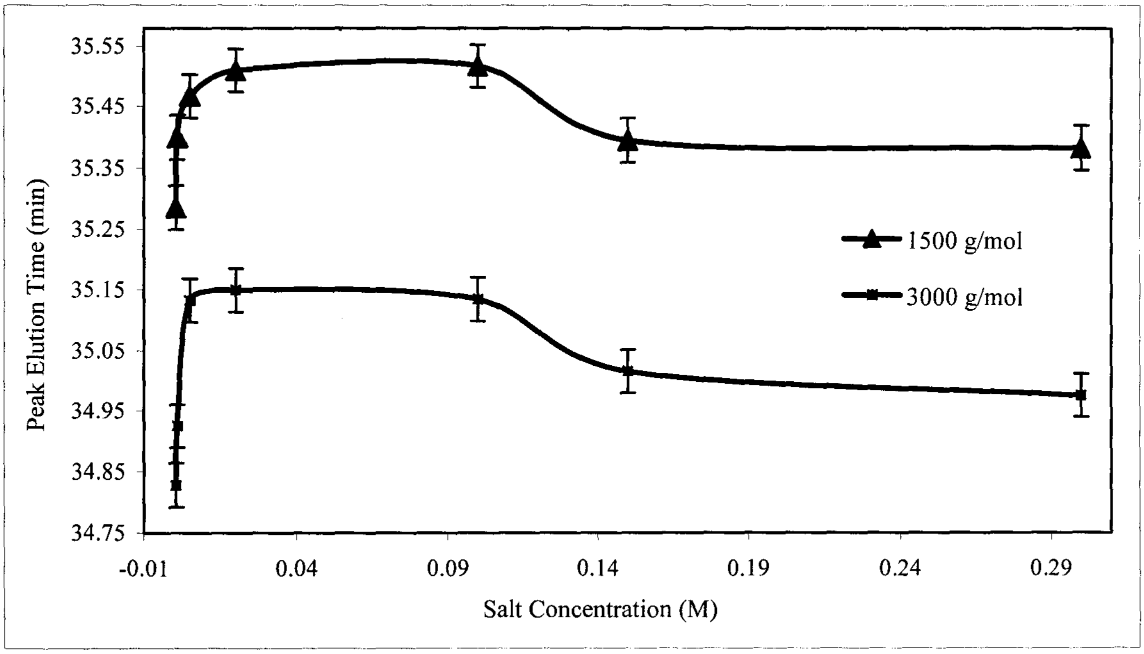


Figure 3.13: $CaCl_2$ concentration effect past the "saturation point", as seen by two different molecular weights.

The two curves each contain four regions; 1) the hydrodynamic volume decreases with increasing salt concentration, 2) the hydrodynamic volume does not change with increasing salt concentration, 3) the hydrodynamic volume 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.

The originally unobserved shift back to a lower elution time (i.e. larger hydrodynamic 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 phenomena 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 0.001M and 0.02M 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 below (Figure 3.14).

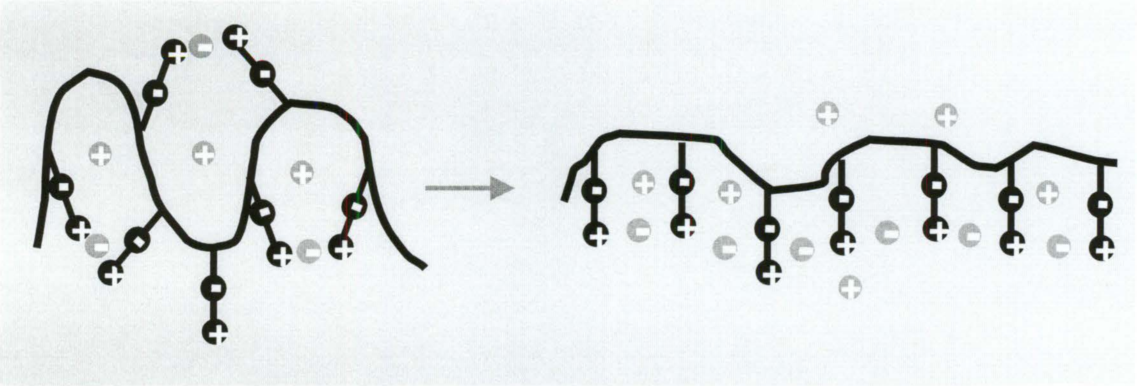


Figure 3.14: Schematic showing how increases in salt concentration can increase the hydrodynamic volume of PMPC through increases in the number of ionic interactions.

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 will be excluded from more column packing pores than the coiled polyelectrolyte, resulting in an earlier elution time and a larger hydrodynamic volume.

The fourth region is believed to occur when the adjacent cation charge groups have been fully saturated with the salt anions and undergo no further chain expansion. Theoretically this saturation point occurs when all adjacent pairs has been "pulled apart" and therefore further increases in salt concentration have no greater effect on the hydrodynamic volume.

With the $CaCl_2$ salt solution it was found that the transition from the first to second region occurs at a concentration of approximately 0.01M, the transition from the second to third region occurs at a concentration of 0.1M and the transition from the third to fourth region occurs at a concentration of 0.15M. Although the transition from one region to another seems to occur at a certain concentration it is believed that these values are very much dependant on the salt type. Therefore, this study should be considered to be a qualification of phenomenon not quantification of concentration data when applying to other systems.

3.6 Comparison of Effect of Properties on the Different Molecular Weights

As mentioned in an earlier section the time shift (relative to the control curve) associated with the addition of salt varies by molecular weight of the sample. Further investigation is required to draw conclusions about the possible mechanism associated with the variation in hydrodynamic volume change. In order to do this a bar graph was constructed for all molecular weight samples under various salt conditions (see Figure 3.15). For simplicity all salt solutions were used at 0.3M concentration.

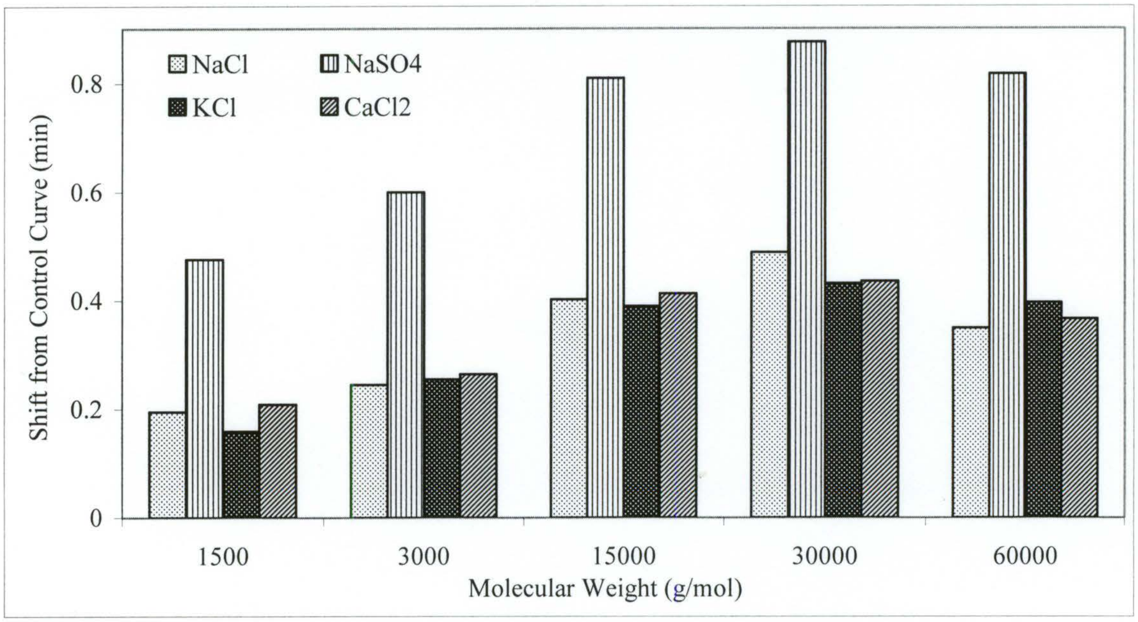


Figure 3.15: Graph to show time lapse between the control curve and the salt solution set for all molecular weight values. All salt solutions are at 0.3M concentration.

For all the salt solutions the time shift seems to increase as the molecular weight increases from 1500g/mol to 30000g/mol. This would suggest that a higher molecular weight undergoes a more drastic change in hydrodynamic volume than a lower molecular weight. This is of course assuming that the samples are within the linear section of the calibration curve. A linear section on the calibration curve denotes a linear relationship between the hydrodynamic volume and the elution time. The portion that is linear is specific to each set of columns and in our case it was found that all five molecular weight samples are within this linear region. A logical explanation for the variation in hydrodynamic volume changes is that the polyelectrolyte with a higher initial hydrodynamic volume (i.e. higher molecular weight) has a greater ability to in fact "shrink" in the presence of salt because it contains more void volume within the polyelectrolyte solution volume. In other terms, the effect on the hydrodynamic volume could be linked to the number of monomeric units in the polyelectrolyte (i.e. the number of charges present), which is related to the repulsive forces. The presence of more repulsive forces will result in a greater degree of chain extension and thus a greater hydrodynamic volume of the polyelectrolyte. When salt is added to the solution it depletes the repulsive forces and the hydrodynamic volume decreases. The degree of this decrease is relative to the number of charges shielded/depleted and therefore with a higher molecular weight sample there are more charges present that can be shielded which leads to more possibilities for decrease in the hydrodynamic volume.

An exception to the trend of increased hydrodynamic effect for increasing molecular weight seems to occur for the largest molecular weight samples, namely 60000g/mol. In this case there seems to be a slight decrease in the time shift compared to the 30000g/mol molecular weight samples for all the salt solutions. Although no clear explanation is evident this phenomenon may be related to the omission of salt ions from the polyelectrolyte volume or chain entanglements. If the polyelectrolyte is tightly wound up on itself it may be difficult for the salt ions to diffuse into the polyelectrolyte volume. In addition, if the salt ions are able to penetrate the polyelectrolyte volume there may still be the occurrence of chain entangles which restrict the polyelectrolyte from adopting a more tightly coiled configuration. This is quite plausible since higher molecular weight polymers are often associated with greater chain entanglements. Regardless of the mechanism this aspect is not of any great importance since the decrease in time shift is very minimal. The decrease in the time shift, from the 30000 g/mol to the 60000 g/mol molecular weight samples ranges from 0.03 to 0.07 minutes. Comparison of these values to the predetermined variance value of 0.03584 minutes shows that the change is very minimal. When comparing the 1500 g/mol to the 30000 g/mol molecular weight samples the time shift ranges from 0.2 to 0.4 minutes, a considerably greater difference. Therefore, although the decreased effect on the 60000g/mol molecular weight sample is somewhat noticeable the more important observation is the positive deviation trend evident for the molecular weight samples ranging from 1500g/mol to 30000g/mol.

4 Conclusions and Recommendations

4.1 Contributions and Conclusions

Throughout this thesis we have gained some valuable insight into the effect of salt on the hydrodynamic volume of polyelectrolytes. Through utilization of a systematic methodology, individual properties were isolated so that a series of conclusions could be made about how they alter the solution properties of PMPC. From this we were able to compare the distinct properties to each other and to explain the balance of effects from different sources. By separating the properties into two categories, namely solution and ion properties, we were able to examine both intra- and inter-molecular polyelectrolyte forces and to address the alternate mechanisms transpiring. In addition to the study of salt properties, initially planned, we have succeeded in explaining other phenomena that was observed from the study, such as the presence of "saturation" points associated with salt concentration and the variation in the degree of hydrodynamic volume change with different molecular weight samples.

Through the extensive data obtained from the various salt types and concentrations, the following conclusions were made from both the solution and ion properties:

1) The salt concentration has an effect up to a certain "saturation" point.
2) The ionic strength has no visible effect (any effect is related to the concentration component).

3) There is no observable solution pH effect.
4) There is no observable cation effect. This includes no cation type/size or cation valency effect.
5) There is an anion effect.
6) A smaller anion has a greater effect than a larger anion.
7) A divalent anion has a greater effect than a monovalent anion.
8) A monatomic anion has a greater effect than a polyatomic anion.

By expanding the study to look at various properties concurrently we were able to determine that changes in hydrodynamic volume were related to a combination of different salt properties. Isolating for the anion component we found that the order of increasing effect on the hydrodynamic volume of PMPC was $I^- < NO_3^- < Cl^- < SO_4^{2-}$.

This sequence was found to follow other properties, such as pKa values, electron affinity, etc. One of the most important discoveries involved the evaluation of charge densities of the salt anion. Through comparison of the volume per negative charge for all four anions we found that the sequence was the same as for the sequence of increased effect on hydrodynamic volume; $I^- < NO_3^- < Cl^- < SO_4^{2-}$. Therefore, it could be concluded that a salt anion will have more of an effect on the solution properties of PMPC if its charge density is higher.

Further examination of the salt concentration effect led to the observance of four distinct regions; 1) the hydrodynamic volume decreases with increasing salt concentration, 2) the hydrodynamic volume does not change with increasing salt concentration, 3) the hydrodynamic volume increases with increasing salt concentration and, 4) the hydrodynamic volume again does not change with increasing salt concentration. Each region could be explained by the variety of ionic interactions taking place in solution. Interactions between both distant and near polyelectrolyte chains are considered to be responsible for the different behaviors.

The phenomenon observed regarding the different shifts associated with the different molecular weight samples could again be explained by considering the polyelectrolyte solution configuration. It was determined that a polyelectrolyte with a higher initial hydrodynamic volume (i.e. higher molecular weight) would be able to shrink to a greater degree, in the presence of salts. Chain entanglements and salt exclusion from the polyelectrolyte volume were able to account the slight decrease in the time shift associated with the highest molecular weight sample (60000g/mol).

4.1.1 Applications

The information that can be attained from salt studies, such as the one presented here can be extremely beneficial in many areas of application. Polyelectrolytes have long been used in various areas, such as cosmetics, ceramics, papermaking, and more recently, biomedical applications. In cosmetics, ceramics and papermaking polyelectrolytes have often been used as thickeners, emulsifiers, rheology modifiers, solubilizers and colloids, all of which can be altered by the presence of salt. In the newer biomedical field, polyelectrolytes have been utilized as drug carriers, protective barriers, and stabilizers, among others. Another technical application is the use of polyelectrolytes as models for naturally occurring polyampholytes (i.e. those found in the body), which can increase our understanding of various biological functions. The specific study of salt effects is important for all biological applications due to the presence of salt in the body.

Current advancements of polyelectrolyte application are often stalled by the lack of understanding surrounding their complicated solution behavior. Therefore, studies such as ours can make big strides toward further advancements of these potentially valuable materials. One recent and very exciting application of polyelectrolytes in the biomedical field involves complexation with proteins and nucleic acids. With recent discoveries involving the benefits of protein- and DNA-based drug therapies, a means of successful delivery is desired. Polyelectrolytes have been one of the top candidates for this objective. By complexing the proteins or DNA with polyelectrolytes many of the desired functions are achieved, namely drug carrier, protective barrier from environment and drug stabilizer. In order to achieve successful complexation and eventual drug release it is necessary to fully understand the solution properties of the polyelectrolyte utilized. We have shown here that solution properties are greatly modified by the presence of salts. Therefore, studies such as ours can help increase the success of both complexation and drug release. In complexation, appropriate salt conditions can be used to ensure complete and stable interaction between the polyelectrolyte and the drug. For delivery in the body, salt studies will help to predict the release kinetics in various biological environments (i.e. salt conditions, pH, etc.) thus resulting in better polyelectrolyte specification.

Furthermore, this type of study can be used to improve polymerization grafting techniques. For example, it would be beneficial to understand polymer configurations (coiled versus expanded) in the presence of certain salts, when trying to achieve a specified grafting density on a surface. Theoretically, by altering the type and concentration of the salts in solution one can vary the resultant grafting density, since certain polymer configurations can lead to higher grafting densities. This is but one example of how important polyelectrolyte solution properties are in the materials preparation stage.

4.1.2 Method Development

In addition to constructing a comprehensive profile of salt effects we have successfully developed a detailed methodology that can be utilized for various other polyelectrolyte studies. By developing a system that consists of commonly used instruments (GPC, DRI), salt effect studies can easily be applied to many other polyelectrolyte systems. The application of aqueous GPC (with DRI detection) principles to the study of polyelectrolyte solution effects minimizes the detection equipment required, and provides sufficient resolution, accuracy, and time effectiveness. The discoveries have shown that size exclusion chromatography 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. Therefore this is a novel and highly applicable method for measuring ionic interactions between polyelectrolytes and salts, and for determining the resultant solution properties. It should also be noted that this method could be extended to organic GPC systems for non-water soluble polymers.

4.2 Recommendations

From the conclusions made for PMPC, hypotheses can be made concerning other types of polyelectrolytes. It is believed that a zwitterionic polyelectrolyte, which has the opposite geometry as PMPC (i.e. the positive charge group is closer to the backbone than the negative charge group) will be effected similarly for solution properties, but differently in terms of ion properties. It is likely that no anion effect will occur while the cation effect will be significant. Analogous hypotheses can be made for cationic and anionic polyelectrolytes, even though the effects are likely less complex. It is expected that the cationic polyelectrolyte will only experience an effect from the salt anion and that the anionic polyelectrolyte will only experience an effect from the salt cation. These hypotheses can be verified by examining previous literature and by further testing using our methods. Comparison of previous observances found in the literature to those obtained through our methods would be a very interesting study.

In contrast, polyampholytes (zwitterionic polymers consisting of separate anionic and cationic monomer units) are more difficult to hypothesize on, partly due to the fact that a polyampholyte can exhibit either the polyelectrolyte or the anti-polyelectrolyte effect in solution. The ensuing effect will depend on the monomers used to construct the polyampholyte. Due to this no hypothesis will be made, except to say that the effect will greatly vary depending on the polyampholyte composition.

As it has already been discussed, the method used in this work can be expanded to other polyelectrolyte types. It would be very interesting and useful to test if our hypotheses about cationic, anionic and other zwitterionic polyelectrolytes are in fact correct. This would require similar studies on polyelectrolytes such as poly(dimethylaminoethyl methacrylate), poly(methylmethacrylic acid) and poly[3-(2-(N-methylacrylamido)-ethyl-dimethyl-ammonio) propane sulfonate], which are cationic, anionic and zwitterionic (opposite charge placement of PMPC) in nature, respectively. This could also be expanded to zwitterionic polyelectrolytes of similar geometry to PMPC, such as poly(N-isopropylacrylamide phosphorylcholine), as a means of verification. Lastly, it would likely be very beneficial to test polyampholytes since hypotheses cannot be easily made about this type of polyelectrolyte.

Another interesting field of study could involve the use of higher valency salts (i.e. > 2, for example aluminum chloride ($AlCl_3$)) to see if the trend of surface charge density still holds true, as is expected. In addition more complex configuration salts (i.e. number of atoms > 5, for example sodium acetate ($NaC_2H_3O_2$)) could be utilized to further test the observed trends. The advantage of our technique/method is that it can also be used to examine the effect of other additives on the hydrodynamic volume of polyelectrolytes. This could include buffers, organic solvents, etc.; basically anything that is compatible with the column packing and the equipment could be tested.

To end, it may be beneficial to examine the effects of salt on the hydrodynamic volume of polyelectrolytes using another method, such as light scattering. This will allow for a comparison of the two techniques, in terms of accuracy, temporal efficiency and reproducibility. It is however believed that in terms of accuracy, availability and ease of analysis our method is on par if not better than other techniques.

REFERENCES

Abad, C., Braco, L., Soria, V., Garcia, R., & Campos, A. (1987a). Solution properties of polyelectrolytes. I. exclusion chromatography of sodium polystyrene sulfonate in salt-free water as eluent. *British Polymer Journal, 19*(6), 489-500.

Abad, C., Braco, L., Soria, V., Garcia, R., & Campos, A. (1987b). Solution properties of polyelectrolytes. II. comparison of the effects of both polyion and eluent salt concentrations on the calibrations in aqueous exclusion chromatography. *British Polymer Journal, 19*(6), 501-508.

Andrianov, A. K., & Le Golvan, M. P. (1996). Characterization of poly[d(carboxylatophenoxy)- phosphazene] by an aqueous gel permeation chromatography. *Journal of Applied Polymer Science, 60*(12), 2289-2295.

Bennemann, K. -H., Brouers, F., & Quitmann, D. (1982). *Ionic liquids, molten salts and polyelectrolytes : Proceedings of the international conference held in berlin (west), june 22-25, 1982.* Berlin: Springer-Verlag.

Blagodatskik, I. V., Sutkevich, M. V., Sitnikova, N. L., Churochkina, N. A., Pryakhina, T. A., & Philippova, O. E. et al. (2002). Molecular mass characterization of polymers with strongly interacting groups using gel permeation chromatography-light scattering detection. *Journal of Chromatography. A, 976*(1-2), 155-164.

Borukhov, D. A., & Orland, H. (1998). Random polyelectrolytes and polyampholytes in solution. *European Physics Journal B, 5*, 869-880.

Bruessau, R. J. (1992). Size exclusion chromatography of polyelectrolytes with aqueous elution solvents. *Makromolekulare Chemie-Macromolecular Symposia, 61*, 199-218.

Campos, A., Garcia, R., Porcar, I., & Soria, V. (1994). Solution properties of polyelectrolytes. XI. adsorption effects in aqueous size-exclusion chromatography of polyanions. *Journal of Liquid Chromatography, 17*(14&15), 3261-3283.

Dobrynin, A. V., Colby, R. H., & Rubinstein, M. (2004). Polyampholytes. *Journal of Polymer Science Part B: Polymer Physics, 42*(19), 3513-3538.

Feng, W., Zhu, S., Ishihara, K., & Brash, J. L. (2005). Adsorption of fibrinogen and lysozyme on silicon grafted with poly(2-methacryloyloxyethyl phosphorylcholine) via surface-initiated atom transfer radical polymerization. *Langmuir, 21*, 5980-5987.

Feng, W., Brash, J. L., & Zhu, S. (2006). Non-biofouling materials prepared by atom transfer radical polymerization grafting of 2-methacryloloxyethyl phosphorylcholine:

Separate effects of graft density and chain length on protein repulsion. *Biomaterials, 27*(6), 847-855.

Feng, W., Brash, J., & Zhu, S. (2004). Atom-transfer radical grafting polymerization of 2-methacryloyloxyethyl phosphorylcholine from silicon wafer surfaces. *Journal of Polymer Science Part A: Polymer Chemistry, 42*(12), 2931-2942.

Fevola, M. J., Bridges, J. K., Kellum, M. G., Hester, R. D., & McCormick, C. L. (2004). pH-responsive polyzwitterions: A comparative study of acrylamide-based polyampholyte terpolymers and polybetaine copolymers. *Journal of Applied Polymer Science, 94*(1), 24-39.

Finch, C. A. (1983). *Chemistry and technology of water-soluble polymers*. New York: Plenum Press.

Garcia, R., Porcar, I., Campos, A., Soria, V., & Figueruelo, J. E. (1994). Solution properties of polyelectrolytes. X. influence of ionic strength on the electrostatic secondary effects in aqueous size-exclusion chromatography. *Journal of Chromatography A, 662*, 61-69.

Garcia, R., Porcar, I., Campos, A., Soria, V., & Figueruelo, J. E. (1993a). Solution properties of polyelectrolytes. IX. quantitative dependence on eluent ionic strength of elution volumes in aqueous size-exclusion chromatography. *Journal of Chromatography A, 655*, 3-9.

Garcia, R., Porcar, I., Campos, A., Soria, V., & Figueruelo, J. E. (1993b). Solution properties of polyelectrolytes. VIII. A comparative-study of the elution behavior on 2 organic-based packings. *Journal of Chromatography A, 655*, 191-198.

Garcia, R., Porcar, I., Figueruelo, J. E., Soria, V., & Campos, A. (1996). Solution properties of polyelectrolytes. XII. semi-quantitative approach to mixed electrostatic and hydrophobic polymer-gel interactions. *Journal of Chromatography A, 721*, 203-212.

Hann, N. D. (1977). Effects of lithium bromide on the gel-permeation chromatography of polyester-based polyurethanes in dimethylformamide. *Journal of Polymer Science, 15*, 1331-1339.

Hara, M. (1993). *Polyelectrolytes : Science and technology*. New York: Marcel Dekker.

Hunt, B. J., & Holding, S. R. (1989). *Size exclusion chromatography*. Glasgow; New York: Blackie; Chapman and Hall.

Ishihara, K., Nishiuchi, D., Watanabe, J., & Iwasaki, Y. (2004). Polyethylene/ phospholipid polymer alloy as an alternative to poly(vinylchloride)-based materials. *Biomaterials, 25*(6), 1115-1122.

Ishihara, K., Nomura, H., Mihara, T., Kurita, K., Iwasaki, Y., & Nakabayashi, N. (1998). Why do phospholipid polymers reduce protein adsorption? *Journal of Biomedical Materials Research, 39*(2), 323-330.

Jarm, V., Kovac-Filipovic, M., & Šegudovic, N. (1995). Some properties of aqueous solutions of poly[(maleic acid)-alt-(p-sulfostyrene, styrene)]. *Journal of Applied Polymer Science, 58*, 1967-1972.

Jarm, V., Sertic, S., & Šegudovic, N. (1995). Stability of aqueous solutions of poly[(maleic acid)-alt-styrene] sodium salts in the presence of divalent cations. *Journal of Applied Polymer Science, 58*, 1973-1979.

Jenkins, H. Donald B., Roobottom, H. K., Passmore, J., & Glasser, L. (1999). Relationships among ionic lattice energies, molecular (formula unit) volumes, and thermochemical radii. *Inorganic Chemistry, 38*, 3609-3620.

Kostanski, L. K., Keller, D., M., & Hamielec, A. E. (2004). Size-exclusion chromatography-a review of calibration methods. *J. Biochem. Biophys. Methods, 58*, 159-186.

Kuhn, A., Forster, S., Losch, R., Rommelfanger, M., Rosenauer, C., & Schmidt, M. (1993). Size-exclusion chromatography of cationic and anionic polyelectrolytes in aqueous solution. *MAKROMOLEKULARE CHEMIE-RAPID COMMUNICATIONS, 14*(7), 433-438.

Lowe, A. B., & McCormick, C. L. (2002). Synthesis and solution properties of zwitterionic polymers. *Chem. Rev., 102*(11), 4177-4190.

Malfait, T., Sloot, A., D., & Van Cauwelaert, F. (1990). High performance size-exclusion chromatography of anionic polymers in aqueous solution. *Journal of Applied Polymer Science, 39*, 571-581.

Marcelo, G., Tarazona, M., & Saiz, E. (2005). Solution properties of poly (diallyldimethylammonium chloride) (PDDA) *Polymer, 46*(8), 2584-2594.

Miyazawa, K., & Winnuk, F. M. (2003). Solution properties of hydrophobically modified phosphorylcholine-based polymers in water and in the presence of surfactants. *J. Phys. Chem. B, 107*, 10677-10682.

Miyazawa, K., & Winnuk, F. M. (2002). Solution properties of phosphorylcholine-based hydrophobically modified polybetaines in water and mixed solvents. *Macromolecules, 35*, 9536-9544.

Mori, S., & Barth, H. G. (1999). *Size exclusion chromatography*. Berlin ; New York: Springer.

Murrell, J. N., & Jenkins, A. D. (1994). *Properties of liquids and solutions* (2nd ed.). Chichester England ; New York: Wiley & Sons.

Natz, R. R., & Andelman, D. (2002). Polyelectrolytes in solution and at surfaces. In A. J. Bard, M. Stratmann, E. Gileadi & M. Urbakh (Eds.), *Encyclopedia of electrochemistry: V. 1. thermodynamics and electrified interfaces* (pp. 282-322). Weinheim: Wiley-VCH.

Nishida, K., Kaji, K., Kanaya, T., & Shibano, T. (2002). Added salt effect on the intermolecular correlation in flexible polyelectrolyte solutions: Small-angle scattering study. *Macromolecules, 35*, 4084-4089.

Nishio, T., & Minakata, A. (2003). Effects of ion size and valence on ion distribution in mixed counterion systems of a rodlike polyelectrolyte solution. 2. mixed-valence counterion systems. *J. Phys. Chem. B, 107*, 8140-8145.

Oosawa, F. (1971). *Polyelectrolytes.* New York: Marcel Dekker, Inc.

Parets, M., J. Garcia, R., Soria, V., & Campos, A. (1990). Solution properties of polyelectrolytes-V. viscometric study of mixed polyanions in pure water. *European Polymer Journal, 26*(7), 767-773.

Patra, C. N., & Yethiraj, A. (2000). Density functional theory for the nonspecific binding of salt to polyelectrolytes: Thermodynamic properties. *Biophysical Journal, 78*(2), 699-706.

Perez-Paya, E., Braco, L., Abad, C., Soria, V., & Campos, A. (1991). Solution properties of polyelectrolytes. VII. nonideal mechanisms in size-exclusion chromatography of synthetic polyions, peptides and proteins. *Journal of Chromatography, 548*, 93-104.

Perez-Paya, E., Braco, L., Campos, A., Soria, V., & Abad, C. (1989). Solution properties of polyelectrolytes. IV. use of a new hydrophilic size-exclusion chromatographic packing for the sepapartion of anionic and cationic polyions. *Journal of Chromatography, 461*, 229-242.

Rojas, O. J., Claesson, P. M., Muller, D., & Neuman, R. D. (1998). The effect of salt concentration on adsorption of low-charge-density polyelectrolytes and interactions between polyelectrolyte-coated surfaces. *Journal of Colloid and Interface Science, 205*(1), 77-88.

Ruiz, L., Hilborn, J. G., Leonard, D., & Mathieu, H. J. (1998). Synthesis, structure and surface dynamics of phosphorylcholine functional biomimicking polymers. *Biomaterials, 19*(11-12), 987-998.

Sata, T., & Nojima, S. (1999). Transport properties of anion exchange membranes prepared by the reaction of crosslinked membranes having chloromethyl groups with 4-vinylpyridine and trimethylamine. *Journal of Polymer Science Part B: Polymer Physics, 37*(15), 1773-1785.

Seery, T., & Dobrynin, A. E. (2004). Special issue on charged polymers. *Journal of Polymer Science Part B: Polymer Physics, 42*(19), iii-iii.

Šegudovic, N., Sertic, S., Kovac-Filipovic , M., & Jarm, V. (1995). Salt effect on size-exclusion chromatography of partially sulfonated alternating copolymers of maleic acid and styrene in a polar solvent. *Journal of Chromatography A, 704*(1), 149-156.

Soria, V., Campos, A., Garcia, R., Parets, M. J., Braco, L., & Abad, C. (1990). Solution properties of polyelectrolytes. VI. secondary effects in aqueous size-exclusion chromatgraphy. *Journal of Liquid Chromatography, 13*(9), 1785-1808.

Soria, V., Garcia, R., Campos, A., Braco, L., & Abad, C. (1988). Solution properties of polyelectrolytes. III. effect of sodium poly(styrene sulfonate) concentration on viscometric and size-exclusion chromatographic behavior at different ionic strengths. *British Polymer Journal, 20*(2), 115-123.

Tripathy, S. K., Kumar, J., & Nalwa, H. S. (2002). *Handbook of polyelectrolytes and their applications: vol.2-3*. Stevenson Ranch, Calif.: American Scientific Publishers.

Uchiyama, T., Kiritoshi, Y., Watanabe, J., & Ishihara, K. (2003). Degradation of phospholipid polymer hydrogel by hydrogen peroxide aiming at insulin release device. *Biomaterials, 24*(28), 5183-5190.

Ueda, T., Watanabe, A., Ishihara, K., & Nakabayashi, N. (1991). Protein adsorption on biomedical polymers with a phosphorylcholine moiety adsorbed with phospholipid. *Journal of Biomaterials Science. Polymer Edition, 3*(2), 185-194.

Van Oss, C. J. (1994). *Interfacial forces in aqueous media*. New York: M. Dekker.

Vlachy, V. (1999). Ionic effects beyond poisson-boltzmann theory. *Annual Review of Physical Chemistry, 50*, 145-165.

Volet, G., & Lesec, J. (1994). Non-exclusion effects in aqueous SEC: Behaviour of some polyelectrolytes using on-line mass detectors. *Journal of Liquid Chromatography, 17*(3), 559-577.

Wandrey, C., Bartkowiak, A., & Hunkeler, D. (1999). Partial molar and specific volumes of polyelectrolytes: Comparison of experimental and predicted values in salt-free solutions. *Langmuir, 15*, 4062-4068.

Wang, H., Miyamoto, A., Hirano, T., Seno, M., & Sato, T. (2004). Radical polymerization of 2-methacryloyloxyethyl phosphorylcholine in water: Kinetics and salt effects. *European Polymer Journal, 40*(10), 2287-2290.

Wu, C. (2004). *Handbook of size exclusion chromatography and related techniques* (2nd , rev. and expand ed.). New York: Marcel Dekker.

Wu, C. (1999). *Column handbook for size exclusion chromatography*. San Diego, Calif. ; London: Academic.

Zhao, X., Zhang, Z., Pan, F., Ma, Y., Armes, S. P., & Lewis, A. L. et al. (2005). Solution pH-regulated interfacial adsorption of diblock phosphorylcholine copolymers. *Langmuir, 21*(21), 9597-9603.