Polyelectrolyte Determination in Drinking Water

Report to the Water Research Commission

by

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Umgeni Water

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Executive Summary

Polyelectrolytes such as poly-diallyldimethyl ammonium chloride (polydadmac) and epichlorohydrin-dimethylamine (epi-dma) are water soluble organic polymers that are used as primary coagulants in the treatment of drinking water.

Although it is desirable to remove traces of polyelectrolyte added during the treatment process, residual amounts of these polyelectrolytes may persist in solution after the filtration stage. Polyelectrolyte products used in the water supply industry may contain in addition to polyelectrolyte, measurable amounts of certain contaminants.

Evidence suggests that several of these contaminants could have an adverse effect on the health of water consumers if ingested in sufficient quantity over time. As they are established and efficient coagulants in water treatment, more emphasis is being placed on the impact of these organic polymers on health issues.

Consequently, water treatment plant operators are required to know the residual concentration of polyelectrolytes at various stages in the treatment process and the eventual quality of the treated water. The desired limit of detection for the polymers is 1 mg/L, which is 10% of the maximum permitted dose for polydadmac and epi-dma (Fielding et al., 1999).

A quantitative method for monitoring the concentration of polyelectrolyte dosed is of utmost importance particularly in the event of an overdose or an accidental spill. The quantitative method applied should thus satisfy the criteria of precision, high sensitivity and reproducibility to control polyelectrolyte dosage levels to drinking water. At present, no readily available, operator friendly method is available for this purpose.

OBJECTIVES

The objectives of this study were to:

- elucidate the advantages and disadvantages of selected non-specific analytical techniques applied to the quantification of residual polymers
- to extend these existing analytical techniques to obtain accurate and reliable results
- to obtain a detection limit at most of one tenth (i.e. 1.0 mg/L) of the maximum permitted dose of the actual polymer for both poly diallyldimethyl ammonium chloride and epichlorohydrin-dimethylamine

LABORATORY INVESTIGATION

The objectives were achieved by subjecting the polyelectrolytes, poly diallyldimethyl ammonium chloride and epichlorohydrin-dimethylamine to the analysis as described in the cited method and the performance of the method was then gauged in relation to these polyelectrolytes.

Thereafter, various experimental parameters were investigated to improve the efficiency of the method and to establish potential interferences. Analyses were conducted on both distilled and real water systems.

The methods investigated in this study were reported as having sensitive measurements of trace amounts of polyelectrolyte and consist of simple operations. The methods are:

- **Potassium polyvinylsulphate (KPVS) method**

  The thrust of this study extends on the investigations carried out by Wang et al. (1978), who applied a colloidal titrimetric method of analysis for the determination of polyelectrolytes in wastewater. The titration method is based on a colour change in the toluidine blue indicator. Initially, the cationic polyelectrolyte (analyte) and potassium polyvinylsulphate (titrant) form a preferential complex or colloid. When all of the analyte has been consumed, the excess titrant reacts with the indicator resulting in a colour change from blue to blue-violet. This colour change is then measured spectrophotometrically.

- **Ponceau S dye method**

  This investigation is based on the work of Fielding et al. (1999), who observed that a cationic polyelectrolyte (analyte) forms an insoluble complex when mixed with a fixed concentration of an anionic dye. The polycation-dye complex is insoluble in water and the organic solvent. The change in the light absorbance of the dye in the aqueous phase before and after complexation is then measured. This change in absorbance is proportional to the mass of the polyelectrolyte that has complexed with the dye.

- **Tannic acid method**

  This spectrophotometric determination of the polymer is based on the complexation of the polymer with tannic acid. Since tannic acid solution is a negatively charged colloid, it is considered to form ionic bonds with cationic flocculants and hydrogen bonds with non-ionic flocculants. The transmittance measurements were then recorded at 554 nm and 830 nm respectively. Hanasaki and co-workers (1985) have demonstrated this method to be effective in quantifying trace amounts of polymer in wastewater.

- **High pressure liquid chromatography (HPLC)**

  Normal phase HPLC

  The method investigated here was recommended for the determination of quaternary ammonium compounds in environmental matrices (HMSO, 1996). The procedure includes the concentration of the cationic polyelectrolyte in an environmental sample by a combination of simple evaporation, solvent extraction by both solid-liquid and liquid-liquid extraction and anion exchange chromatography. Separation of the cationic polyelectrolyte was achieved by normal phase HPLC using a refractive index detector and quantification of the polyelectrolyte by peak height integration.

  Size Exclusion Chromatography (SEC)

  SEC investigations of both polymers were examined using a gel filtration column. The polyelectrolyte samples were analysed in aqueous solutions using water as the mobile phase and an evaporative light scattering (ELS) detector. Nitrogen (N_2) gas was used as the nebulizing gas.

FINDINGS

From the methods investigated, the KPVS and ponceau S dye methods were most successful in quantifying the amount of residual polyelectrolyte. These methods showed good precision with linear calibration curves.

The colloidal titration method proved to be a simple and cost effective method with minimal interference compared to the ponceau S dye method. The automation of analyses was important because it enabled bench analyses to be carried out more efficiently and rapidly and with better precision. The adaptation of the titration to a photometric titration means that more dilute solutions can be analysed and that a degree of background absorbance or turbidity can also be tolerated. Continuous monitoring of the analyte in a production process is also possible.

The ponceau S dye method although successful, was subject to interferences from other constituents in the sample especially metal ions like calcium, magnesium, iron and manganese. Analyses using different raw water samples containing high concentrations of these metal ions illustrated variations in the calibration curves. A further analysis of the effect of each metal ion on the polymer determinations demonstrated that below 20 mg/L the determination is achievable with some error. Any concentration of the metal ion above 20 mg/L produces a serious error. These errors may be obviated by prior treatment of the sample which is time consuming and necessary and may prove to be a disadvantage since quick decisions and actions are sometimes required.

The tannic acid method described in cited literature was unsuccessful for the determination of polydadmac and epi-dma. Various experimental parameters were investigated using tannic acid that produced broad transmittance readings across the spectrum with no proper signals that could be used to construct a calibration curve. Variations in pH, ionic strength, tannic acid concentration as well as the polyelectrolyte concentration did not provide any tangible results.

Normal phase high pressure liquid chromatography (HPLC) determinations of both polymers using a refractive index detector were unsuccessful. The lack of response prompted further investigation of numerous variables including flow rate and the polarity of the mobile phase. The variables examined did not provide any single component peaks that could be used for the determination of either polymer.

HPLC using a size exclusion column and an ELS detector was able to identify both polyelectrolytes. A variation of polymer concentration and flow rate was conducted. A slower flow rate produced a well resolved single component peak. Lower concentrations of polyelectrolyte did not produce well resolved peaks. The limits of detection were 4300 mg/L and 3130 mg/L for polydadmac and epi-dma respectively.

Table 1: Summary of successful methods

CRITERIA FOR METHOD VALIDATION	METHOD		
	KPVS	**PONCEAU S**	**SEC**
Analytical Technique	Titration	Spectrophotometric	SEC/ELSD detector
Preparation Time	15 min	30 min	15 min
Sample Size	40 ml	50 ml	20 µL
Limit of Detection	1 mg/L	1 mg/L	4300 mg/L polydadmac 3130 mg/L epi-dma
Reproducibility	± polydadmac 12% ± epi-dma 11%	± polydadmac 13% ± epi-dma 3%	-
Linearity in calibration curve	Linear	Linear	-
Specificity	Non-specific (unblended coagulants)	Non-specific (unblended coagulants)	Specific (blended coagulants)
Interferences	Other cationic polyelectrolytes and Anionic content of raw water matrix (Cl^-, NO_3^-, SO_4^{2-} and HCO_3^-)	Other cationic polyelectrolytes and Cationic content f raw water matrix e.g. Metal ions (Ca^{2+}, Mg^{2+} Mn^{2+} and Fe^{2+})	None. Separation is based on sample size. Larger molecules elute first. Small molecules in water matrix will take longer to elute
Stability	Buffer to be prepared fresh daily Other reagents are stable	Dye stable up to a week. H_2SO_4 solution to be prepared daily	Reagents to be prepared daily
Robustness	Affected by change in pH	Affected by change in pH	Affected by change in pH and temperature
Level of Operation	Operator level	Operator level	Skilled level in lab

CONCLUSION

The quantification and identification of polydadmac and epi-dma was accomplished by the KPVS, ponceau S and SEC studies. The tannic acid method and normal phase HPLC were unsuccessful at quantifying both polyelectrolytes. A method validation was conducted for each investigation to ensure that the methods work well and are acceptable for their intended purpose.

Although the KPVS and ponceau S dye methods are subject to interference from other compounds that are present in the raw water matrix, they may be overcome by prior treatment. These methods are also operator friendly and can be performed at a treatment works on a daily basis.

SEC studies were excellent at identifying the polyelectrolytes. The technique is highly sophisticated and must be conducted in a laboratory with trained personnel. The detection limits are higher than one would expect on a treatment works making it useless for routine analyses. However, in the event of an overdose or an accidental spill; it is an excellent tool to quantify the polymers. This is advantageous as the range of detection using the former two methods limits the determination.

RECOMMENDATIONS

KPVS Method

- The method can be used for routine monitoring of polyelectrolyte samples on a treatment works.
- The method is capable of determining residual polyelectrolyte concentrations between 0 mg/L to 12 mg/L.
- The method can be used for raw water samples containing the following anions i.e. Cl^-, NO_3^-, HCO_3^- with some error in the calibration curve due to their interference. Pre-treatment of raw water samples is recommended to eliminate this error.
- Raw water samples containing high concentrations of SO_4^{2-} also require pre-treatment as their presence obstructs the absorbance maximum of the toluidine blue dye thus interfering with the determination and resulting in an error in the calibration curve.

Ponceau S Dye Method

- The method can be used for routine monitoring of polyelectrolyte samples on a treatment works.
- The method is capable of determining residual polyelectrolyte concentrations between 0 mg/L to 10 mg/L.
- For higher concentrations of polyelectrolyte (i.e. more than 10 mg/L), more dye is required for the determination to be successful.

- For large box plot spreads, more replicates are recommended in order to attain accurate results.
- The method can be used for raw water samples containing the following cations i.e. Ca^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+} with some error. For cation concentrations exceeding 20 mg/L, the determination is subject to gross error.

Size exclusion chromatography

- SEC using an ELS detector is an excellent technique for identifying polydadmac and epi-dma samples.
- The method can be used to quantify these polyelectrolytes in the event of an overdose or accidental spill of the polyelectrolyte sample on a treatment works.
- The determination is possible at detection limits of 4300 mg/L for polydadmac and 3130 mg/L for epi-dma. At concentrations below these, well resolved peaks are not observed.

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Glossary

$Al_2(SO_4)_3$	Aluminum sulphate
Al^{3+}	Aluminum (III) ion
$Al(OH)_{3(s)}$	Aluminum hydroxide
ANSI	American National Standard
AWWA	American water works association
$Ca(OH)_{2(s)}$	Calcium hydroxide
Ca^{2+}	Calcium (II) ion
Cl^-	Chloride ion
DBAS	Disulphine blue active substance
DBS	dodecylbenzenesulphonate
DDDPB	1.5-dimethyl-1,5-diazaundecamethlene polymethbromide
DHTDMAC	Dihardenedtallowdimethylammonium chloride
DTDMAC	ditallow dimethyl ammonium chloride
ELS	Evaporative light scattering
Fe^{2+}	Iron (II) ion
Fe^{3+}	Iron (III) ion
$FeCl_3$	Iron chloride
$Fe(OH)_{3(s)}$	Iron hydroxide
HCO_3^-	Bicarbonate ion
HPLC	High pressure liquid chromatography
KH_2PO_4	potassium hydrogen phosphate
KPVS	Potassium polyvinyl sulphate
M	molarity
Mg^{2+}	Magnesium (II) ion
$MgCO_3$	Magnesium carbonate
$Mg(OH)_{2(s)}$	Magnesium hydroxide

mg/L	milligram per liter
min	Minutes
Mn^{2+}	Manganese (II) ion
mV	milli volt
N_2	Nitrogen
Na^+	Sodium ion
NO_3^-	Nitrate ion
NSF	National sanitation foundation
ppb	part per billion
ppm	part per million
PVC	polyvinyl chloride
RI	Refractive index
SEC	size exclusion chromatography
SO_4^{2-}	Sulphate ion
TB	toluidine blue
TLC	Thin layer chromatography
UV	ultra violet
USEPA	United States Environmental Protection Agency
λ	Wavelength

Accuracy	The accuracy of a method is the closeness of the measured value to the true value for a sample.
Interferences	It is the proportion of measurement which arises from sources other than the analyte itself. Possible sources are from the instrument, added reagents or from the sample matrix.
Limit of detection	It is the lowest analyte concentration that produces a response above the noise level of the system. It is the smallest amount or concentration of an analyte that can be detected by a given procedure by a degree of confidence.

Linearity in calibration curve	It enables the response of an instrument to be related to the mass, volume or concentration of the analyte in the sample. A series of standards is used to prepare a calibration curve in which an instrument response is plotted as a function of mass, volume or concentration. If the plot is linear, a calibration factor (related to the slope of the curve) may be calculated. This facilitates a rapid computation of results without reference to the original curve.Reproducibility (Precision)The random or indeterminate error associated with the measurement or a result. It can be represented statistically by the standard deviation or relative standard deviation.
Robustness	Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters such as pH, percent organic content, temperature and buffer concentration.
Specificity	These refer to techniques that are said to work for only one analyte in a sample. It determines the ability of the method to accurately measure the analyte response in the presence of all potential sample components.
Stability	Stability of reagents, mobile phases, standards and sample solutions needs to be determined. Samples and standards should be tested over a 48 hour period and quantitation should be determined by comparison to a freshly prepared sample. If solutions are not stable over a 48 hour period, storage conditions or additives should be identified that can improve stability.

1. Literature Review

1.1. Role of Polyelectrolytes in Drinking Water

Polyelectrolytes are water soluble, high molecular weight polymers. The major functions of these polymers are manifested in the treatment of drinking water where they serve as primary coagulants or as coagulant aids. Cationic polyelectrolytes as sole coagulants are effective in treating low turbidity, coloured water by direct filtration (Edzwald *et al.*, 1987). Coagulant aids are normally used in conjunction with a conventional iron or aluminium coagulant (Packham, 1973). They may also be used as flocculants (Hargreaves, 1999; Narkis *et al.*, 1990), filter aids (Ghosh, Cox and Prakash, 1985) and as sludge conditioners (Kim, 1993). Polyelectrolytes play a vital role in removing many potentially harmful water contaminants which include suspended sediments, micro-organisms, inorganic and dissolved organics (Lurie and Rebhun, 1997). Their efficiency as coagulants can be seen in the fact that 75% of waterworks in South Africa have adopted polyelectrolytes as part of their water treatment process (Leopold, 2004).

1.2. Coagulation and Flocculation

Coagulation is a process that combines small particles into larger aggregates. In the coagulation and flocculation processes, solids that are unable to settle easily are converted into larger and heavier settleable solids. This is a physical-chemical change that occurs by the addition and mixing of a coagulant chemical into the raw water. The settleable solids can then be removed by sedimentation and filtration (AWWA, 1995). The particles to be removed from the raw water are usually negatively charged (AWWA, 1995; Narkis and Rebhun, 1977) and so the coagulant chemicals used in drinking water treatment are generally positively charged.

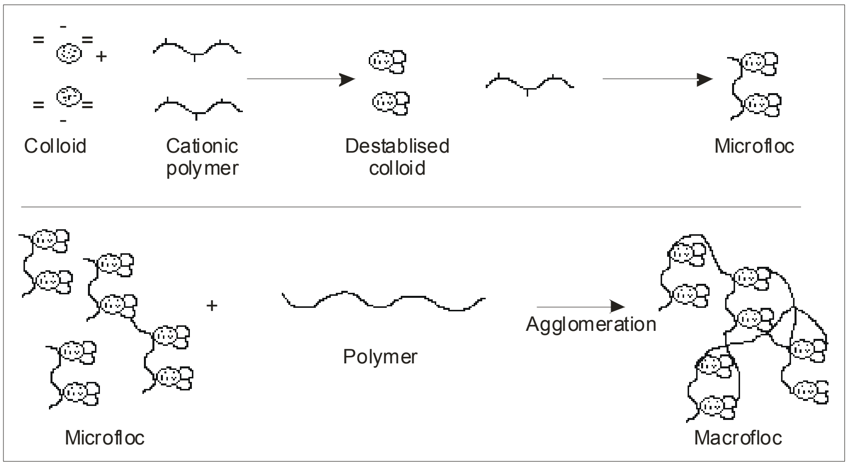

Figure 1: Diagram showing floc formation during coagulation and flocculation

(Source: AWWA, 1995)

Coagulation forms part of the conventional treatment process and may be considered as three sequential steps i.e. coagulant formation, particle destabilisation and interparticle collisions. Coagulant formation and particle destabilisation occur in rapid mixing tanks whilst interparticle collisions occur in flocculation tanks (Amirthrajah and O'Melia, 1990).

Colloidal particles carry an electric charge. This electric charge is the primary factor responsible for colloidal stability. To induce colloidal particles to aggregate, two distinct steps should occur: repulsion forces must be reduced (i.e. particles must be destabilized) and particle transport must be achieved to provide contacts between the destabilized particles (Yao, 1967).

O' Melia, (1969), reported that the destabilisation of colloids by different coagulants may occur in various ways i.e.

- Double layer compression – The addition of ions that have a charge opposite to that of the colloidal particles. Causes the compression of the electrical double layer surrounding the colloidal particle. The coagulating power of the cations increased as the valence of the ions increased from 1, 2 to 3.

- Adsorption and charge neutralization – Some chemical species are capable of being adsorbed at the surface of colloidal particles. If the adsorbed species carries a charge opposite to that of colloids, such adsorption causes a reduction of surface potential and a resulting destabilization of the colloidal particle. This is a different mechanism from double layer compression.

- Enmeshment in a precipitate – If certain metal salts are added to water in sufficient amounts, rapid formation of precipitates will occur. Colloids may serve as condensation nuclei for these precipitates or may become enmeshed as the precipitates settle. Coagulants such as $Al_2(SO_4)_3$, $FeCl_3$, $MgCO_3$ and $CaOH_2$ can induce coagulation through the formation of insoluble $Al(OH)_{3(s)}$, $Fe(OH)_{3(s)}$, $Mg(OH)_{2(s)}$ and $Ca(OH)_{2(s)}$. Removal of colloids in this manner is frequently referred to as sweep floc coagulation.

- Adsorption and inter-particle bridging – A polymer molecule will become attached to a colloidal particle at one or more sites. Attachment may result from the columbic attraction if the polymer and particle are of opposite charge or from ion exchange, hydrogen bonding and van der Waals Forces if they are of similar charge.

In some cases, an excessive dose of the polyelectrolyte may occur. This may lead to restabilisation of the colloids in the raw water where the total surface of each particle is converted to positive and leads to an increase in the turbidity of the water (AWWA, 1990). In such cases, inefficient coagulation of the particles will occur and will ultimately lead to the production of poor quality water. The full potential of coagulation may be achieved if the right coagulant is used at optimum dosage and with the correct combination of operating conditions (Rong-Jin, 1998; AWWA, 1988; Morrow and Rausch, 1974).

1.3. Types of Coagulants

The success of a coagulant is influenced by many physical and chemical factors, some of which include: mixing conditions, pH, alkalinity, turbidity level and water temperature. In some cases, ozone is used prior to coagulation as an oxidant (Saunier *et al.*, 1983; Pryor *et al.*, 2002). This has noticeably improved flocculation thereby increasing filter run-time, lowering cost of coagulant chemicals and reduced sludge disposal costs (Cheng-nan Wang *et al.*, 1986).

Coagulants may sometimes contain ions with more positive charges than others. Those that have trivalent ions like aluminium (Al^{3+})and iron (Fe^{3+}), are 50 to 60 times more effective than chemicals with bivalent ions, such as calcium (Ca^{2+}). Bivalent ions are in turn, 700 to 1,000 times more effective than coagulants with monovalent ions, such as sodium (Na^{+}) (AWWA, 1995). Coagulants may be classed into two categories i.e. inorganic coagulants and organic coagulants. Table 2 lists the most commonly used coagulants.

Table 2: Common chemical coagulants used in the treatment of drinking water

Common Name	Chemical formula	Comments
Aluminium sulphate	$Al_2(SO_4)_3 . 14(H_2O)$	Most common coagulant. Often used with cationic polymers
Ferric chloride	$FeCl_3$	May be more effective than alum in some cases
Ferric sulphate	$Fe_2(SO_4)_3$	Often used with lime softening
Ferrous sulphate	$Fe_2(SO_4)_3 . 7H_2O$	Less pH dependent than alum
Aluminium polymers	-	Includes polyaluminium chloride and polyaluminium sulphates
Cationic polymers	-	Synthetic polyelectrolytres, large molecules
Sodium aluminate	$Na_2Al_2O_4$	Used with alum to improve coagulation
Sodium silicate	$Na_2O . (SiO_2)_x$	x can range from 0.5-4.0, ingredient of activated silica coagulants aids

(Source: AWWA, 1995)

1.3.1. Inorganic Coagulants

1.3.1.1. Alum (Aluminium sulphate)

Alum is the most common coagulant used in water treatment. It reacts with the alkalinity of the water to form jelly-like floc particles of aluminium hydroxide, $Al(OH)_3$ (Licsko, 1997; Stumm and Morgan, 1962) at an optimal pH range of 5.8-8.5. The trivalent aluminum ion neutralises the negatively charged particles of colour and turbidity in the raw water by neutralisation (Dentel and Gossett, 1988).

1.3.1.2. Iron Salts

Iron salts such as ferric chloride and ferric sulphate are also used as coagulants (Johnson and Amirtharajah, 1983) and can operate effectively over a wider range of pH values than alum. The metal ions of these coagulants hydrolyse to form positively charged iron (III) hydroxide complexes that aid in the removal of suspended solids in raw water (Licsko, 1997). They are quite corrosive and require special facilities for storage and handling. The efficiency of the iron coagulants is also dependent on the alkalinity of the water. If the alkalinity is outside of the optimal range, floc will not form. It will re-form at a later stage in the treatment process when the pH is altered or in the distribution system resulting in a build-up of sediments in the piping system (AWWA, 1995).

1.3.1.3. Polymeric inorganic coagulants

Polymeric compounds with species of iron, (Fe^{3+}) and aluminium, (Al^{3+}) have been developed and used as coagulants (AWWA, 1990). Other alternative aluminium based coagulants have also been developed for water treatment applications e.g. polyaluminium chloride, aluminium chlorohydrate and polyaluminium chlorohydrate. These polyaluminium coagulants, with polyaluminium chloride in particular have been used successfully in the potable water industry.

Gebbie and Stewart (2002) reported that these polyaluminium coagulants have numerous advantages when compared to alum. Some of these are:

- a lower level of alkalinity is consumed
- smaller doses are required
- less sludge is produced
- low levels of residual aluminium in the treated water are achieved
- they have ability to work efficiently at lower temperatures

1.4. Organic Coagulants

These are extremely large molecules (polymers), that when dissolved in water produce highly charged ions (Schwoyer, 1981). The charge density of the polyelectrolyte is important in determining its optimum dosage as the rate of adsorption and flocculation is dependant on the charge density and molecular mass of the polyelectrolyte (Shun-Yun and Gregory, 1990). Three basic organic polyelectrolytes are:

- Cationic polyelectrolytes
- Anionic polyelectrolytes
- Non-ionic polyelectrolytes

1.4.1. Cationic polyelectrolytes

Cationic polyelectrolytes are polymers that produce positively charged ions when dissolved in water. They are widely used because the suspended and colloidal solids found in water are generally negatively charged. They can be used as primary coagulants or coagulant aids (Edzwald *et al.*, 1987; Packham, 1973). Some advantages of using cationic polyelectrolytes are (Jindal and Singh, 1986; Narkis and Rebhun, 1997):

- the amount of coagulant used can be reduced
- floc particles settle better
- sensitivity to pH is reduced and the
- flocculation of living organisms e.g. bacteria and algae is improved

1.4.2. Anionic polyelectrolytes

These are polymers that produce negatively charged ions when dissolved in water. They are used to remove positively charged solids present in the raw water and are used primarily with inorganic or organic coagulants (Kleber, 1973). They produce larger flocs and they are not materially affected by pH, alkalinity, hardness or turbidity.

1.4.3. Non-ionic polyelectrolytes

These polyelectrolytes have a balanced or neutral charge. Upon dissolving, they release both positively and negatively charged ions. They are aids to primary coagulation (Kleber, 1983) and are added in larger doses than cationic or anionic polyelectrolytes. They are also less expensive than cationic and anionic polyelectrolytes.

1.5. Application of Coagulation Chemicals

The jar test is used experimentally to determine the optimal conditions for the coagulation, flocculation and sedimentation processes (AWWA, 1976). This is done by trying various combinations of chemical dosage (singly and in combination), mixing speed and settling interval (John *et al.*, 2002; Benedek, 1976). Jar tests results are expressed in mg/L and are converted to the equivalent daily use on the plant. Once used on the plant, streaming current detectors are used to monitor and control the coagulant feed rate especially during variable water quality or flow conditions (Dental and Kingrey, 1989; Bernhardt and Schell, 1993). Polyelectrolyte removal from the water treatment process is uncertain so the best practice is to consider any toxic hazard from these polyelectrolytes on the basis of the applied dose of the chemicals. The optimised coagulant dosage then aids in reducing the impacts of water treatment on the environment (Bernhardt and Schell, 1996).

1.6. Organic Polyelectrolytes used in Drinking Water Treatment

Polydiallyldimethyl ammonium chloride (polydadmac) and epichlorohydrin- dimethylamine (epi-dma) are cationic polymers commonly used as coagulants in the treatment of drinking water in South Africa. They are used singly or in combination with inorganic polymeric coagulants (Kerdachi *et al.*, 1993). When used with aluminium containing coagulants, the overall amount of aluminium added is less, (below 10%), than when using aluminium sulphate as a single coagulant (Nozaic *et al.*, 2001). The concern about the amount of aluminium in the finished water which is thought to be a possible cause of Alzheimer's disease, (Letterman and Driscoll, 1988), is thus eliminated. Organic polyelectrolytes are advantageous, especially from an economic perspective (Nozaic *et al.*, 2001).

Despite this, some western countries like Switzerland and Japan (Letterman and Pero, 1990) do not use synthetic polyelectrolytes in their water treatment processes due to health concerns. This is underlined by the fact that there are inadequate techniques available for measuring residual organic polyelectrolytes in potable water (Kerdachi *et al.*, 1993; Fielding *et al.*, 1998; Freese *et al.*, 2002).

1.6.1. Polydiallydimethyl ammonium chloride

Polydadmac is manufactured in two sequential steps: formation of the monomer and its polymerisation. The monomer is formed by the reaction of the stoichiometric excess of allyl chloride with dimethylamine in aqueous solution (Nozaic and Freese, 2004).

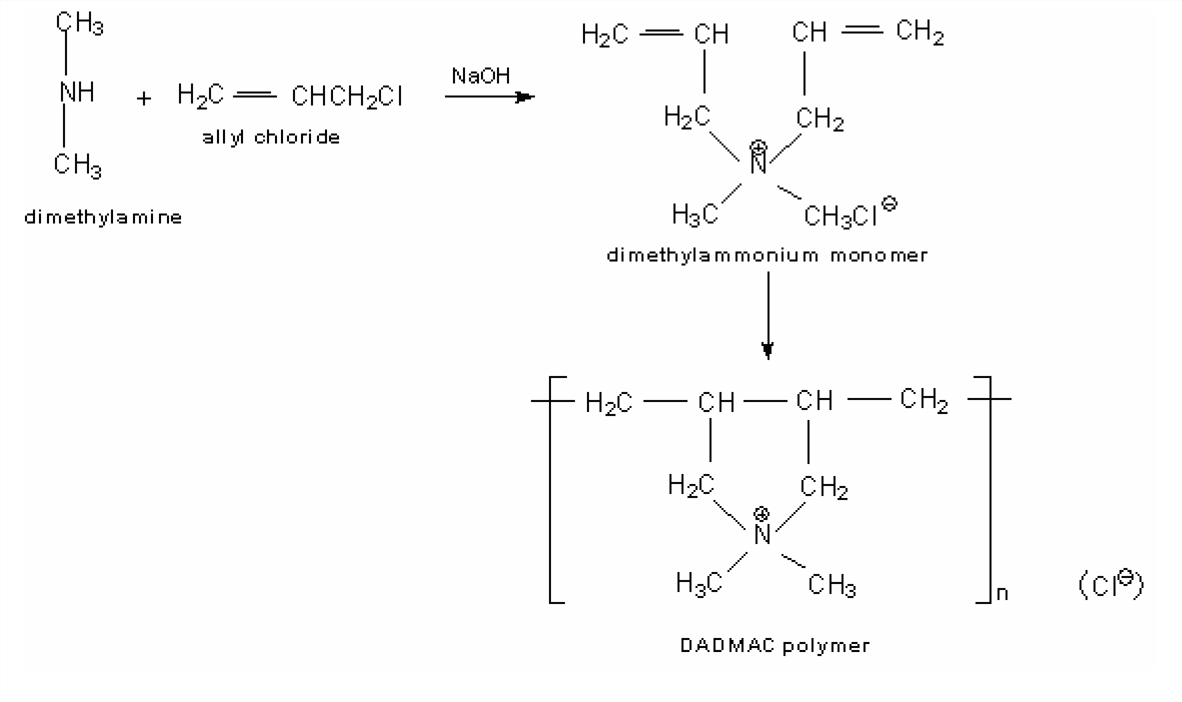

Figure 2: Reaction pathway for the formation of polydiallyldimethyl ammonium chloride
(Source: Nozaic and Freese, 2004)

1.6.2. Epichlorohydin-Dimethylamine

Epi-Dma co-polymer is a polyamine type polyelectrolyte. It is formed by the step reaction synthesis of 2-hydroxy-3-dimethylaminopropyl, a monomer formed by the reaction of epichlorohydrin and dimethylamine. This process tends to produce a linear quaternary ammonium compound of low to moderate molecular weight (Nozaic and Freese, 2004).

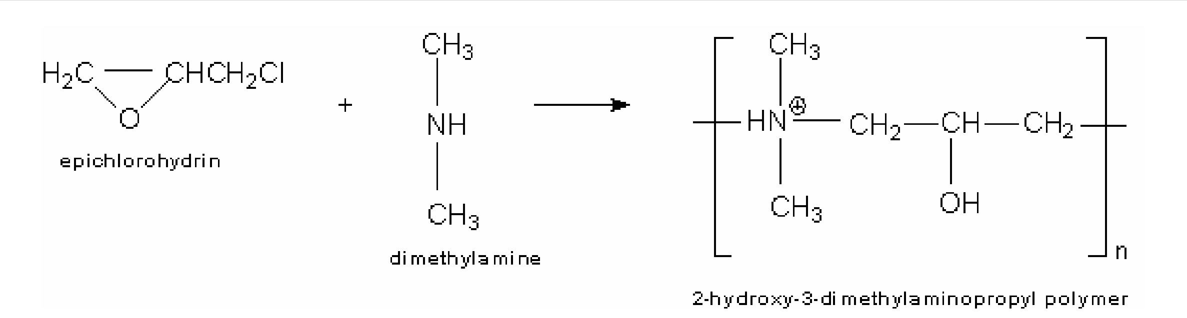

Figure 3: Reaction pathway for the formation of epichlorohydrin-dimetylamine
(Source: Nozaic and Freese, 2004)

1.7. Polyelectrolyte Products and their Contaminants

Despite the fact that most polyelectrolyte added during treatment will be removed with the floc, there is a small possibility that low concentrations may remain in the water after the filtration stage (Fielding *et al.*, 1999). Studies show that polyacrylamide (synthetic polyelectrolyte) still persisted in the filtered water after being used as a coagulant aid (Goppers and Straub, 1976).

Polyelectrolyte products used in the water supply industry may contain in addition to polyelectrolyte, measurable amounts of certain “contaminants”. These “contaminants” are essentially unreacted raw material from the polyelectrolyte manufacturing process for example: the monomers, unreacted chemicals used to form the monomer units, initiators, quenchers etc.

Evidence suggests that certain of these contaminants (Table 3) could have an adverse effect on the health of water consumers if ingested in sufficient quantity over time (Letterman and Pero, 1990). Continued exposure to low concentrations of contaminants such as epichlorohydrin is an important concern in potable water treatment as it is an animal carcinogen (WHO, 1996).

Table 3: List of contaminants found in polyelectrolyte products

Contaminant	**Polyelectrolyte**
Diallyldimetylammonium chloride	Polydadmac
Dimethylamine	Polydadmac/Epi-Dma
Allylchloride	Polydadmac
Diallylether	Polydadmac
5-Hexanal	Polydadmac
Epichlorohydrin	Epi-Dma
glycidol	Epi-Dma
1,3-dichloro-2-propanol	Epi-Dma
2,3-dichloro-1-propanol	Epi-Dma
3 chloro-1,2-propanediol	Epi-Dma
2-hydroxy-3-dimethylaminopropyl chloride	Epi-Dma
1,3-Bis(dimethylamino)-2-propanol	Epi-Dma

(Source: Letterman and Pero, 1990)

Different reactants and manufacturing processes can be used to prepare what is essentially the same polymer. Process monitoring and control must therefore be an important consideration in polyelectrolyte manufacture if contaminant levels are to be managed. This subject can be addressed by national standards and regulations on the quality of the product which is something South Africa lacks (Freese *et al.*, 2002; Leopold, 2004).

Polyelectrolytes and their contaminants may also react with treatment chemicals added from other water treatment processes like ozonation and chlorination to form undesirable by-products (Klaus and Lawrence, 1977; Mallevialle and Bruchet, 1984). A liberal dilution of products that usually occurs in treatment applications tends to make acute poisoning an unlikely event. Consequently, polyelectrolytes and their reaction by-products should be identified and measured especially if there is and overdose or accidental spill (Fielding *et al.*, 1999).

An example of this occurred in July 2000, where 17 tonnes of primary coagulant accidentally spilled into the raw water canal during a coagulant transfer from an underground bulk storage tank at the Amanzimtoti Waterworks (supplying the south of Durban). An abnormally high dose of polyelectrolyte (polyamine/aluminium chlorhydrate blend) of approximately 4500 mg/L had resulted in the water supply system (Dlamini *et al.*, 2002). The draining of clarifiers and filters to the sludge plant and to a nearby stream resulted in fish kill due to the polyamine fraction of the polyelectrolyte (Dlamini *et al.*, 2002). The acute toxicity of polyelectrolytes on several aquatic organisms as well as various algal species was investigated by Biesinger and Stokes (1986). The authors found that non-ionic and anionic polymers were not acutely toxic to the species tested however, cationic polymers were very toxic and their toxicity varied widely with their chemical structure.

1.8. Health Effects

Polymers are not well adsorbed from the gastrointestinal tract although some movement may occur into the lymphoid tissue. They are relatively inert biologically, with little effect on health, but the monomers present in the polymers could elicit toxicity (Health effects, 1989). The epichlorohydrin monomer can be acutely toxic. Its ingestion leads to a rapid and complete adsorption from the gastrointestinal tract and distribution to many tissues. Epichlorohydrin poses as a health hazard in several ways e.g. it may cause extreme irritation when applied topically, may result in respiratory failure through depression of the central nervous system, sub-acute exposure may cause abnormalities in the blood and the internal organs and infertility in male animals has been noted (Letterman and Pero, 1990).

Synthetic polyelectrolytes may present a toxic risk based on the presence of unreacted monomer residues and not necessarily with the polymer itself. Due to the uncertainty of the removal of polyelectrolytes from the water treatment process the best practice is to consider any toxic hazard on the basis of the applied dose of the chemicals (Goppers and Straub, 1976). AWWA has recommended in its standards (AWWA Standard B451-92, AWWA Standard B452-90) that the maximum allowable concentration of polydadmac and epi-dma be 10 mg/L.

The presence of polymer in the finished water has encouraged attempts to quantify the amount of polyelectrolyte in various aqueous environments i.e. finished water, sludge, backwash waters etc. The information derived from these methods may enable water researchers to evaluate the potential hazard of these substances on the health of water consumers.

1.9. South African Legislation on the Manufacture and use of Polyelectrolytes

The South African approval system for the manufacture of polyelectrolytes was initially structured according to that of the United States Environmental Protection Agency (USEPA) (Roodt and McNally, 2002). A health clearance certificate was awarded following a comparison of South African manufacturers' product formulation to that of products accepted by the USEPA (AWWA Standard B451-92, AWWA Standard B452-90). It did not include testing of the product or inspection of the manufacturing facilities. A maximum dosage level for the product was given based on that used in the United States (Roodt and McNally, 2002).

The USEPA has since then upgraded its approval system by working in partnership with the National Sanitation Foundation (NSF). The South African National department of health in 1994 also became aware of the need for a more rigid approval system like that of the NSF. A task group was set up to propose a new South African approval system for water treatment chemicals which to date has not materialized (Freese *et al.*, 2002; Leopold, 2004).

This means that some South African manufacturers have no approval system for their products as not all of them may have the affordability to outsource to the NSF. For those that are using the NSF approval system, the product is certified to be of a good standard to use in water treatment without posing a potential health hazard.

1.10. NSF Standards for Polyelectrolytes

The United States National Sanitation Foundation (NSF) is a neutral third party that serves in the development of consensus standards for industries, regulatory agencies, and users of product, equipment, and services related to public health and environment.

http//:www.nsf.org

NSF also provides a product certification services to manufacturers of products covered by its standards (ANSI/NSF 60-1996a, ANSI/NSF 61-1997a) on a voluntary basis. The ANSI/NSF standard 60 (1999) was developed to establish health effects requirements for chemicals and contaminants added to drinking water from drinking water treatment chemicals. It includes chemicals added and intended to be present in the finished water as well as those that are added and not intended to be present in the finished water. Compounds responsible for non- compliances from 1991-1999 are listed in the ANSI/NSF Standard 60 (1999), of which the dadmac monomer and dimethylamine appear here.

The dadmac monomer has resulted in non-compliance in polydadmac products of section 4 (coagulation and flocculation). Standard 60 restricts polydadmac polymer application not to exceed 25 mg/L with a carryover of not more than 50 µg/L. of polydadmac into the finished water. Non-compliance from dimethylamine also occurs in section 4 products. The NSF has supported the use of epi-dma up to a maximum allowable level of 120 µg/L.

1.11. Analytical Methodologies

A number of methods have been devised for the quantitative determination of polyelectrolytes in water. Some of these include:

- colloidal titration
- extraction-spectrophotometry
- chromatography
- polarography
- fluorometry
- potentiometry
- flow injection analysis

1.11.1. Titrimetric methods

A titration comprises of a controlled reaction by a standard reagent in known amounts with a solution of analyte so that the stochiometric or equivalence point for the reaction between the reagent and the analyte may be located (Fifield and Kealy, 1990). Titrimetric methods of analysis have become widespread for precise routine and non-routine analyses in both industry and research laboratories. They consist of fast solution reactions between the analyte and titrant with relative precision values that lie between 0.1% to 1% being reported (Fifield and Kealy, 1990). A reproducible identification of the endpoint is essential for a precise and accurate titration.

The colloidal titration has demonstrated to be an excellent method for determining the concentration of natural and synthetic polyelectrolytes. The titration is based on the reaction between a positively charged polyelectrolyte (polycation) and a negatively charged polyelectrolyte (polyanion). When the oppositely charged polyelectrolytes are mixed, they tend to associate to form a neutral complex and eventually precipitate out of solution (Schwoyer, 1981).

Wang and Schuster (1975) developed an accurate, quantitative determination of cationic and anionic polyelectrolytes by the direct titration technique in the concentration range varying from 0.0002 N to 0.005N. Potassium polyvinylsulphate (KPVS) is used as the standard anionic titrant for the determination of cationic polyelectrolytes and 1.5-dimethyl-1,5-diazaundecamethlene polymethbromide (DDDPB) as the standard cationic titrant for anionic polyelectrolyte determination. The technique is based on a direct neutralization reaction between the cationic and anionic forms of the polyelectrolyte (Wang and Schuster, 1975).

Wang and Schuster (1975) recommended the preparation of a calibration curve for routine analyses. This formed the groundwork for later work proposed by Wang and colleagues (1978), who once again, studied the quantification of anionic and cationic polyelectrolytes by the regular direct titration method and the dilute titration method. In all instances, linear calibration curves were achieved (figure 4). Wang *et a.l* (1978) concluded that the colloid titration method could be used for chemical coagulation process control and in the determination of optimum coagulation conditions.

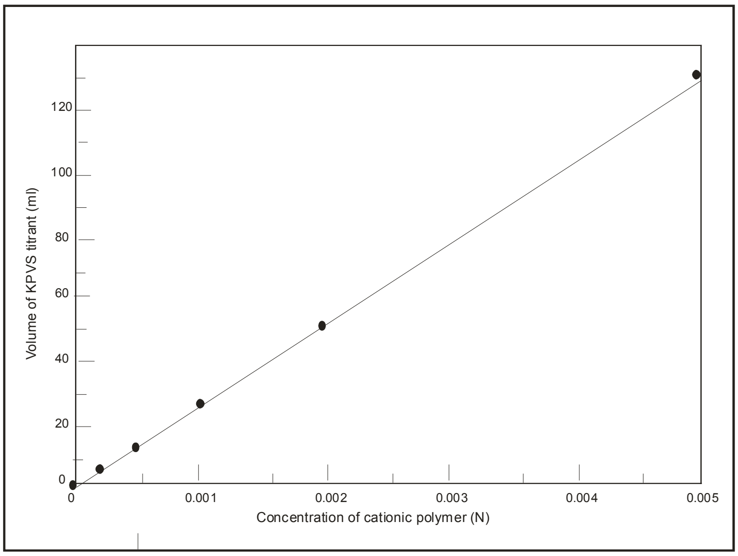

Figure 4: Calibration curve for the direct titration of Wang et al. (1978)

The success of the colloidal titration for the determination of cationic polyelectrolytes prompted Hutter and colleagues (1991) to extend this method to measure the adsorption of cationic polymers by bleached hair. Their extensions to the original method included the use of a buffer of pH 6 prepared from mono and dibasic sodium phosphate and the titration carried out by a semi-automated system. This allowed the endpoint volume of the titration to be read to the nearest 0.025 mL. In conclusion Hutter *et al.* (1991) stipulated that the colloid titration method is readily adaptable to the determination of cationic polymers in bleached hair and that the results are reproducible within closely defined parameters.

The use of other cationic dyes as indicators by the colloid titration method is also possible. Masadome and Hoshi (2003) recently studied the determination of an anionic polyelectrolyte by a photometric titration using crystal violet dye as the cationic indicator. The reaction of crystal violet with anionic polyelectrolyte such as KPVS and carrageenan using polydadmac as a titrant resulted in the decrease of absorbance of crystal violet at the maximum absorbance wavelength of 590 nm. Masadome and Hoshi, (2003) established that calibration curve generated from this study showed that a linear relationship exists between the concentration of anionic polyelectrolyte and end-point volume of titrant.

Polyelectrolytes may also be quantified by potentiometric titrations as investigated by Masadome and colleagues (1999), who used a plasticized polyvinyl chloride (PVC) membrane electrode sensitive to dodecylbenzenesulphonate (DBS) ion for the determination of anionic polyelectrolytes. KPVS is used as the anionic polyelectrolyte and polydadmac as the titrant. The end point of the reaction is determined by the potential jump of the plasticized PVC membrane electrode. This is a result of the decrease in concentration of the DBS ion added to the sample solution as a marker ion, due to the ion association reaction between DBS and polydadmac. Masadome *et al.* (1991) observed a linear relationship between the concentration of anionic polyelectrolyte and the end point volume of the titrant in the concentration range between 2 x 10^{-5} and 4 x 10^{-4} M for KPVS, alignate and carrageenan.

Potentiometric methods of analysis were also extended to cationic polyelectrolyte determination by means of a potentiometric flow injection utilizing a flow through type tetraphenylborate-selective electrode detector (Masadome and Asano, 1999). The method is based on detecting any concentration decrease of the tetraphenylborate ion by the formation of an ion associate between the cationic polyelectrolyte, polydadmac and the tetraphenylborate ion. Masadome and Asano (1999) concluded that a linear relationship exists between the peak height and the logarithmic concentration of polydadmac with a slope of 17 mV/decade over a concentration range of 5 x 10^{-5} to 1 x 10^{-3} mol/L. The detection limit for polydadmac was 1 x 10^{-5} mol/L.

The success of colloidal titrations of zirconium solutions by KPVS via potentiometric endpoint detection using a toluidine blue selective electrode has recently been reported (Sakurda et al., 2004). Since visual detection is often difficult for the direct titration of polyanion, the toluidine blue (TB) concentration was monitored by potentiometry using a TB selective electrode. Zirconium hydrolyzed polymer was found to react with polyvinylsulphate to form a stochiometric polyion complex. The determination could be performed with a 1% of relative standard deviation and the stochiometry at pH ± 2 was one mol of zirconium per equivalent mol of polyvinylsulphate (Sakurda *et al.*, 2004).

1.11.2. Spectrophotometric methods

Spectrophotometric methods of analyses are an extensive technique for quantitative trace analysis. It is also is one of the most sensitive techniques available with relative precision values between 0.5 - 5% being reported (Fifield and Kealy, 1990).

Fifield and Kealy (1990) have recognized the following fundamental aspects when carrying out spectrophotometric determinations:

- Stability of the absorbance with respect to time (30 minutes should be the minimum) and to minor variations in pH, ionic strength and temperature
- Degree of selectivity of a complexating agent including the effect of other species likely to be present and the effect of an excess of reagent
- Conformity to Beer-Lambert law

Anionic dye complexation has been used to determine the concentration of cationic polyelectrolytes in water. A direct spectrophotometric method is used since it is more sensitive considering the low concentrations of polyelectrolyte being evaluated. The cationic polyelectrolyte forms an insoluble complex when mixed with a fixed concentration of an anionic dye. The polycation-dye complex is insoluble in water and in organic solvents and can be removed from the test mixture. Consequent changes in the light absorbance of a standard dye solution are proportional to the mass of the polyelectrolyte complexed with the dye.

Polyethyl-4vinyl pyridinium iodide, a cationic polyelectrolyte, was quantified by the method described above using 2 different anionic dyes, i.e. rose bengal and eosin A (Dey and Palit, 1968). The dye-electrolyte complex that formed was precipitated by centrifuging.

The peak height of the difference spectra (obtained by subtracting the absorbances before and after precipitation of the dye-polyelectrolyte complex) was found to increase linearly with an increase in concentration of the polyelectrolyte and reached a maximum when the concentration of the polyelectrolyte equaled the concentration of the dye. A linear plot passing through the origin was achieved (plot of maximum absorbance of the difference spectra of the dye-polyelectrolyte complex) (Dey and Palit, 1968). At higher concentrations, the polyelectrolyte and the dye-polyelectrolyte complex went completely into solution, thus drastically decreasing absorbance of the different spectra (figure 5).

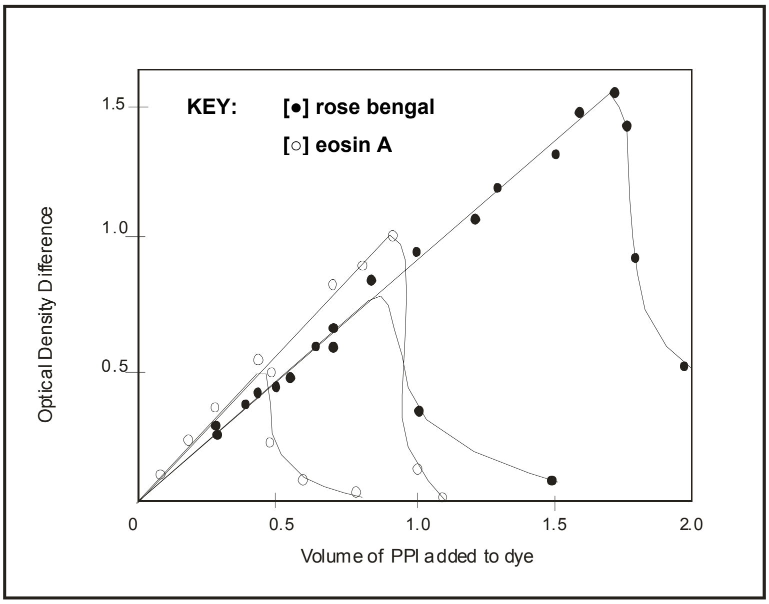

Figure 5: Plot of optical density difference as a function of volume of added polyelectrolyte (PPI) in rose bengal [●] and eosin A [○] (Dey and Palit, 1968)

Parazak and collegues (1987) took advantage of the natural hydrophobic character of the polycation-dye complex and facilitated its removal with an organic solvent (freon), as the complex interfered with the spectrophotometric measurements. Ponceau s dye was used and the need for centrifugation to remove the colloid was eliminated as it was too time consuming. The polyelectrolytes investigated were polydadmac and epi-dma.

The polycation-dye complex was trapped between the aqueous and organic phases. The absorbance of the aqueous phase was measured at 520 nm in 1 cm cuvettes (Parazak *et al.*, 1987). The plot of absorbance vs. concentration of polyelectrolytes yielded negative slopes. By using the correct sample size, the method is sensitive down to 0.5 – 1 mg/L. Other dyes examined by were eriochrome black T, anthraquinone-1,5-disulphonic acid disodium salt, eosin B and alizarine sodium monosulphate.

The method of Parazak and collegues (1987) described above, was further modified by Fielding *et al.* (1999) who substituted dichloromethane for freon as the latter was not considered to be suitable for use in routine analysis. Dichloromethane formed a defined interface on which the dye/polymer complex could precipitate and collect. Experimental results using dichloromethane compared with that of freon, showed little difference on the performance of the method.

1.11.3. Miscellaneous methods

Jungreis (1981) described a simple micro-determination of polymer flocculants in mine water. It was shown that the laminar flow rates of dilute polyacrylamide and guar solutions through membrane filters under vacuum are inversely proportional to their concentrations. Some concerns raised by Jungreis (1981) were that entrance and exit effects and differences in radius of gyration of polyacrylamide may give rise to some deviations.

Also, the solution viscosity is a strong function of the molecular weight distribution of the long chain branching so if the sample molecules were not nearly as identical in this respect; this would logically result in a potential error. Jungreis (1981) concluded that due to these reservations, the following method would not succeed for polymer solutions in general.

According to Yateman and Bark (1991), fluorometry may be used for the determination of trace amounts of non-ionic, cationic and anionic polyelectrolytes. It was shown that when polyacrylamides are reacted with hypochlorite to form either sodium hypochlorite or chloramine-T to produce amines. These are then reacted with fluorescamine to give intensely fluorescent compounds. Good linear correlations between the concentration and the fluorescent intensity were observed (Yateman and Bark, 1991). The overall time of determination is approximately 2 hours and the relative standard deviation at the 5 mg/L level is approximately 5%.

Water-soluble polymers have also been quantified by turbidity and complexation with metal ions. Ruchova and co-workers (1984) found that after the addition of methacrylic acid to a polymer solution, the resulting turbidity could be measured. The turbidification of the solution is stable for up to 10 minutes, which provides good reproducibility of the method. The other method is based on the property of acrylic polymers to form a blue precipitate with copper salts, the amount of copper that has already reacted being proportional to the content of polymers in water (Ruchova *et al.*, 1984).

Wee (1984), investigated the quantification of ditallow dimethyl ammonium chloride (DTDMAC), a cationic surfactant in river water, sewage influent and sewage effluent by using three different methods i.e.

- high performance liquid chromatography (HPLC) coupled with conductimetric detection,
- colorimetric procedure commonly referred to as disulphide blue active substance (DBAS) and
- a combination of DBAS and thin layer chromatography (TLC)

The results from these comparisons showed that the DBAS procedure was less specific and less sensitive than either DBAS-TLC or HPLC and therefore tended to overestimate the concentration of DTDMAC in environmental samples. It also showed that DBAS-TLC and HPLC to be comparable in specificity although HPLC is more sensitive and requires specifically less time to perform than either DBAS-TLC or DBAS (Wee, 1984).

Attia and Rubio (1975) reported a method for the direct determination of polyacrylamide and polyethylene oxide flocculants by nephlometry in dilute solution. They reported that the method could be used for the determination of cationic, anionic and non-ionic flocculants. Hanasaki and co-workers (1985) developed this method for determining the levels of flocculent in real wastewater. Tannic acid solution is used as a reagent for this method since it forms ionic bonds with cationic flocculants and hydrogen bonds with non-ionic flocculants because of its negative charge.

Transmittance values of for polyaminocarbonate cation were found at 554 nm and 830 nm (figure 6) and linear calibration curves of tannic acid with the polyaminocarbonate cation were obtained (figure 7). Temperature, pH and salt concentration were investigated, as they are known to affect turbidity. Transmittance was found to be independent of temperature whilst for pH experiments transmittance values were found to be high on the acidic side and rapidly decreased with a shift from neutral to alkaline pH (Hanasaki *et al.*, 1985).

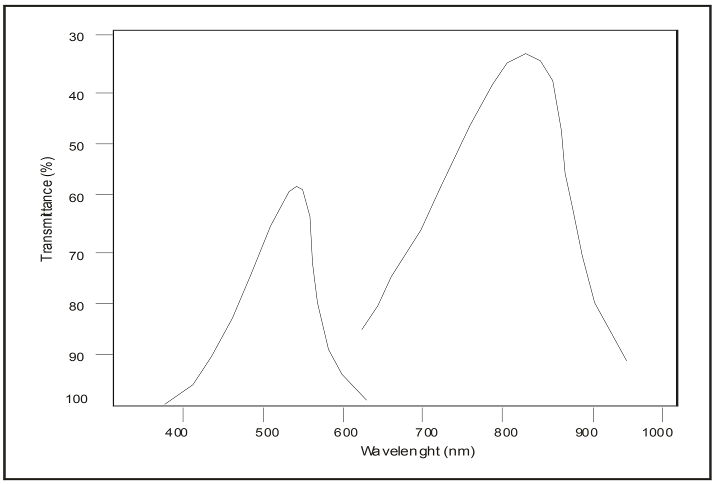

Figure 6: Transmittance of tannic acid solution with cationic polyelectrolyte

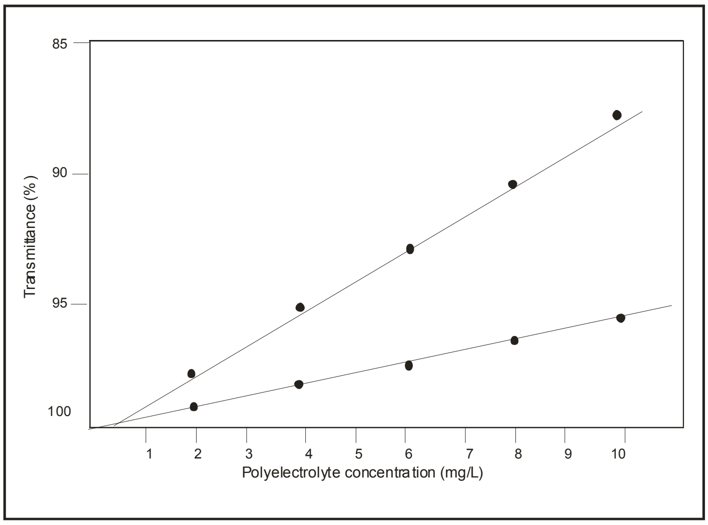

Figure 7: Calibration curve for polyelectrolyte determination at 554 nm and 830 nm as obtained by Hanasaki *et al.* (1985)

Attia and Rubio (1975) carried out their experiments with different concentrations of 0.1 M $NaNO_3$ and NaCl, whilst Hanasaki *et al.* (1985) used $NaNO_3$ and $NaClO_3$. A comparison of the calibration curves obtained by Hanasaki and collegues (1985) appeared to be the same as those of Attia and Rubio (1975). Where no salt was used, no calibration curve was obtained, as a suspensoid was not produced.

The methods described thus far, are non-specific methods for the determination of trace amounts of polyelectrolyte. Ideally, classical methods such as gravimetry and titrimetry are favoured due to fact that they are simple to perform and will allow unskilled operators to achieve precise results (Fifield and Kealy, 1990).

1.11.4. High pressure liquid chromatography

High pressure liquid chromatography (HPLC) is a simple, rapid, sensitive and specific analytical technique that is capable of providing an accurate estimate of compounds. HPLC has already proven to be a convenient method to quantify high molecular weight quaternary ammonium compounds like polyacrylamide in environmental matrices. HPLC requires fewer extraction processes than indirect methods and therefore goes through less product loss.

During the selection and development of a chromatographic method various important factors need to be considered:

- The method should be adjusted to be stability indicating and selective. If not, the new method should be able to display this.

- The chromatographic method from literature must be first choice and have the freedom of column choice.
- Potential impurities and degradation products should be used with the sample medium.
- A single component peak must be identified.
- The analysis time range should be kept practical, no more than 40 min.
- Recovery from sample preparation procedures (dilution, extraction or filtration) should be established.
- The stability of samples should be tested.
- An analysis of repeatability and quantification limit using both sample and standard must be considered.
- Robustness of the chromatographic method should be determined (the following variables can be tested e.g. solvent strength in mobile phase, temperature, flow rate, injection volume, pH of mobile phase, ionic strength in mobile phase, sample diluent and detector response).

1.11.4.1. HPLC for monomer detection

Epichlorohydrin monomer detection has been investigated by a combination of various instrumental techniques with much success. Neu and Sprenger (1997) described using solid phase extraction for the enrichment of epichlorohydrin from water samples with subsequent determination by gas liquid chromatography with electron capture detection. The authors achieved detection limits of 0.1 µg/L using a sample volume of 100 mL and have shown the method to be successfully applied to the determination of epichlorohydrin in waste water.

Aguilar *et al.* (2004) have investigated the analysis of a group of pollutants varying widely in polarity and volatility in aqueous samples at trace levels by on-line coupling of equilibrium-sorptive gas chromatography. The performance of the method was tested on various environmental samples and showed within and between day precision of the absolute peaks varied between 3 - 16%. Detection limits achieved were between 0.1 and 1 µg/L with a sample volume of 200 mL being required to reach equilibrium with the stationary phase.

Epichlorohydrin has also been determined by sulfite derivatization and ion chromatography (Bruzzoniti, 2004). An ion chromatography – mass spectrometry (IC-MS) coupling system was employed for the separation and the identification of products of epichlorohydrin when reacted with a nucleophilic agent SO_3^{2-}. The high capacity column used for the separation provided a high resolution and allowed the mass spectrometric identification of epichlorohydrin sulphite derivatives. This study demonstrated that chlorine atoms are displaced from epichlorohydrin during the reaction while mass spectrometry confirmed that none of the products formed contained chlorine atoms.

A reversed phase HPLC technique has been developed for the biological samples (Teerlink *et al.*, 1997). Dimethylamine is converted to a stable fluorescent product by pre-column derivatisation with fluoromethylchloroformate. The DMA derivative is then resolved from other derivatives of other amines and amino acids by gradient elution with a total run-time of 15 minutes. The lower limit of determination in biological samples is 0.1 µmol/L. Recoveries from spiked serum samples were 97-107%.

1.11.4.2. HPLC for polymer detection

Dihardenedtallowdimethylammonium chloride (DHTDMAC), a cationic surfactant found in river water was determined by HPLC coupled with conductimetric detection (Wee, 1984). The clean up procedure included addition of linear alcohol ethoxylate and linear alkyl sulphonate to prevent adsorption of cationic surfactants onto the walls of the container and to facilitate extraction of the cationic surfactant respectively. Solvent extraction was performed by methylene chloride. Wee (1984) concluded that the method was specific for the determination of DHTDMAC with a precision of ± 10% or less.

A similar method was described in the HMSO Publication (1996), for the determination of DHTDMAC as well as other monoakyltrimethyl ammonium and diakyldimethyl ammonium compounds from sewage, sewage effluents, river waters, potable water, sewage sludge's, river sediments and sludge amended soils. This method is based on the concentration and clean-up of cationic surfactant by a combination of simple evaporation, solvent extraction (solid-liquid and liquid-liquid) and anion exchange chromatography. Separation of the cationic surfactant occurs by normal phase HPLC. Detection is by conductometry and quantification by peak height integration. The limits of detection that were established are 2.5 µg/L for liquor samples and 0.5 µg/L solid samples.

A more promising and direct method for detecting the presence of polyelectrolytes and for assessing their nature is size exclusion chromatography (SEC). SEC separates high molecular weight polyelectrolytes from the matrix and is immune to interferences from colour as well as other low molecular weight compounds.

Soponkanaporn and Gehr (1986) used SEC to quantitatively determine the concentrations of high molecular weight cationic, anionic and non-ionic acrylamide based polyelectrolytes. A series of columns were used together with an Ultra Violet (UV) detector at 200 nm. An aqueous solution of KH_2PO_4 was use as the mobile phase to control the non-SEC mechanisms.

A linear response was achieved in the calibration curve with concentration range from 5 - 100 mg/L with reasonably consistent retention times. Investigations of the effects of a high salt concentration of 0.25 M did not interfere in the determination.

Soponkanaporn and Gehr (1989) later investigated the degradation of polyelectrolytes due to ageing, ozone, chlorine and biological processes as residual polyelectrolyte discharged into drinking water or into natural water bodies could be subjected to one or more of the above mentioned degradation processes.The authors (Soponkanaporn and Gehr, 1989) showed that the degradation rate increased with decreasing concentration of polyelectrolyte, increasing pH, and increasing temperature. SEC showed that complete degradation into carbon dioxide (CO_2) and other organic monomers occurred during biodegradation. Ozonation and chlorination increased degradation rates but also produced refractory compounds. Chloroform was produced as a result of interaction with chlorine.

Soponkanaporn and Gehr (1990) later assessed the behaviour of polyelectrolytes by SEC measurements. The authors showed that the higher molecular weight fractions of cationic polyelectrolytes are adsorbed partially and more than lower molecular weight fractions. They concluded that SEC can be used to study the behaviour of polyelectrolytes during the treatment process and hence to design more effective polyelectrolytes and to predict flocculation efficiency of existing polyelectrolytes.

Leung *et al.* (1987) used a combined technique of ultrafiltration and aqueous SEC by UV detection for measuring the residual polyelectrolyte in coal washery process water. Prior to chromatography, fractionation and concentration of the polyacrylamide in effluents was achieved by ultrafiltration with a hollow fibre cartridge having a nominal molecular weight cut-off of 100 000. Leung and co-workers (1987) found that precision studies gave a relative standard deviation of 5.8% and a precision of 2.2% at a 95% confidence interval at a concentration range of 20 ppm. Ultrafiltration improved the resolution of the peaks with no adsorption effects, improved sensitivity and longer column life.

Beazly (1996) showed that partially hydrolyzed polyacrylamide can be determined by SEC in oil field production water. The concentration range evaluated was from 1 ppm to 10 ppm with and gave a precision of ± 1% and a lower limit of detection of 1ppb. Beazley (1996) concluded that SEC proved to be an attractive technique for polyelectrolyte determination as it eliminated many of the interferences in oil field production water.

The disadvantage of HPLC is that compounds not separated by the clean up procedure can ionize under non-aqueous conditions and have similar HPLC retention times to cationic surfactants under investigation. SEC is advantageous to HPLC as it separates high molecular weight polyelectrolytes from other low molecular weight compounds and can eliminate overlapping of peaks.

2. Methodology

2.1 KPVS Method

2.1.1 Preparation of reagents

2.1.1.1 Toluidine Blue Solution 0.1% (w/v)

A 0.1% solution of toluidine blue dye solution was prepared by weighing out 0.1 g of the dye (Fluka) and dissolving it to 100 mL with doubly distilled water. This dye solution served as the indicator.

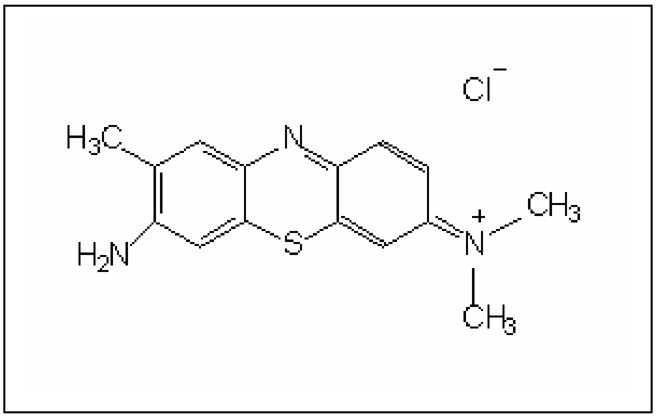

Figure 8: Structure of toluidine blue

(Source: Merck Chemical Database ChemDAT, 2003)

2.1.1.2 Phosphate buffer pH 6

A KH_2PO_4/NaOH buffer of pH 6 (Kenkel, 1988) was made by mixing 50 mL of a 0.1 M solution of KH_2PO_4 with 5.6 mL of a 0.1 M NaOH solution and diluting to 100 mL with doubly distilled water.

2.1.1.3 Sodium hydroxide solution (0.1 M)

Sodium hydroxide pellets (BDH) was readily dissolved in doubly distilled water and diluted to a known volume.

2.1.1.4 HCl solution (0.1 M)

0.1 M HCl solution was prepared by diluting approximately 5.3 mL of a 30% solution (BDH) to 500 mL with doubly distilled water.

2.1.1.5 Potassium Polyvinylsulphate stock solution (0.01 M)

Potassium polyvinylsulphate salt (Aldrich) was accurately weighed, dissolved in doubly distilled water and made up to a 1000 mL. Further dilution of the stock potassium polyvinylsulphate solution was carried out to obtain working solutions of 0.00004 M and 0.00009 M.

2.1.1.6 Polydadamac standard solutions

A 1000 mg/L stock solution of polydadmac (Zetachem) was prepared by readily dissolving 1 g of the polymer with doubly distilled water and then diluting to 1 L. A series of polydadmac standard solutions, ranging from 2 mg/L to 12 mg/L (in 2 mg/L increments), was then prepared by diluting a corresponding volume of the stock solution to 1 L.

2.1.1.7 Epi-dma standard solutions

A 1000 mg/L stock solution of epi-dma (Zetachem) was prepared by readily dissolving 1 g of the polymer with doubly distilled water and then diluting to 1 L. A series of epi-dma standard solutions, ranging from 2 mg/L to 12 mg/L (in 2 mg/L increments), was then prepared by diluting a corresponding volume of the stock solution to 1 L.

2.1.2 Procedure

- 0.5 mL of toluidine blue indicator was added to 20 mL of the $KH_2PO_4/KHPO_4Na$ buffer.
- The pH of the polymer sample was measured and adjusted accordingly to pH 6 using either HCl or NaOH.
- 40 mL of this sample was then added to the indicator-buffer solution and titrated by an automated system. (Figure 9)
- A known concentration of titrant, potassium polyvinylsulphate, was dispensed at a constant rate of 4.21 mL per/min into the reaction vessel with constant stirring.
- As the titrant was added, the resulting solution was circulated into the flow cell of a UV-Visible spectrophotometer at a rate of 140 mL per/min.
- The change in relative absorbance was monitored by the spectrophotometer at a fixed wavelength of 631 nm.

- As the titration progressed, the titration curve was simultaneously generated on a recorder and the endpoint calculated as the point lying midway between the lines drawn tangent to the baselines. The instrumentation enabled the endpoint to be read to the nearest 0.05 mL.

- Raw water samples were initially filtered by suction filtration using a Whatman filter paper No.1 (Ø = 5.5 cm). Thereafter, the experiments were conducted in a similar manner as with the doubly distilled water samples.

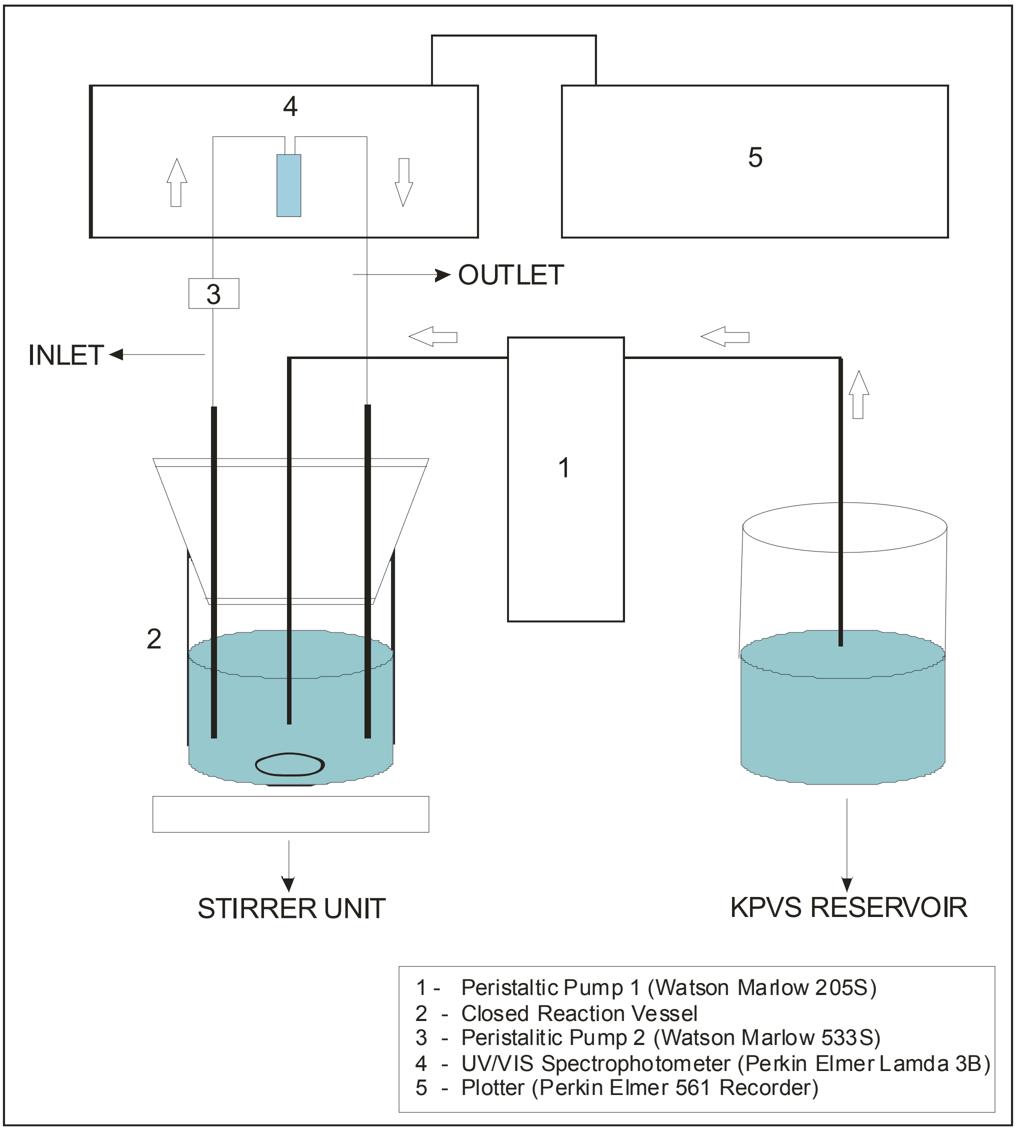

Figure 9: Automated system developed for the photometric titration

2.2 Ponceau S Dye Method

2.2.1 Preparation of reagents

2.2.1.1 Ponceau S dye (200 mg/L)

A 200 mg/L solution of ponceau S dye (Aldrich) was prepared by accurately weighing out 50 mg of the salt, dissolving it in doubly distilled water and then diluting to 250 mL.

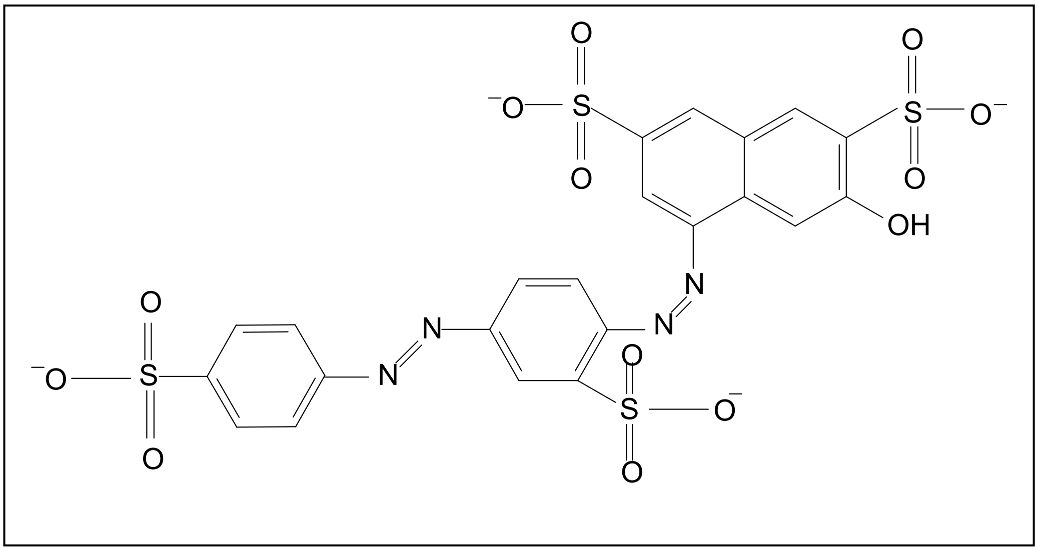

Figure 10: Structure of ponceau S dye

(Source: Merck Chemical Database ChemDAT, 2003)

2.2.1.2 0.5M Sulphuric acid (H2SO4)

0.5 M solution of H_2SO_4 was prepared by diluting 2.7 mL of the concentrated acid (BDH) to 100 mL.

2.2.1.3 Polydadmac standard solutions

A 1000 mg/L stock solution of polydadmac (Zetachem) was prepared by readily dissolving 1 g of the polymer with doubly distilled water and then diluting to 1 L. A series of polyDADMAC standard solutions, ranging from 0, 0.5, 1, 2, 5 and 10 mg/L was then prepared by diluting corresponding volumes of the stock solution to 1 L.

2.2.1.4 Epi-dma standard solutions

A 1000 mg/L stock solution of epi-dma (Zetachem) was prepared by readily dissolving 1 g of the polymer with doubly distilled water and then diluting to 1L. A series of epi-dma standard solutions, ranging from 0, 0.5, 1, 2, 5 and 10 mg/L was then prepared by diluting corresponding volumes of the stock solution to 1 L.

2.2.2 Procedure

- 50 mL of the polymer sample was placed in a stoppered tube
- 2 mL of 0.5 M sulphuric acid, 2.5 ml of ponceau S and 10 mL of dichloromethane was added to the tube respectively.
- The tube was shaken by hand for 2 minutes and left to stand for 15 minutes.
- Approximately 20 mL (a sufficient amount to perform 2 spectrophotometric determinations) of the aqueous phase was transferred to a cuvette using a pasteur pipette.
- The absorbance of the aqueous phase was measured at 520 nm by a spectrophotometer.
- A 50 mm quartz cuvette was used for polydadmac and a 10 mm quartz cuvette for epi-dma.
- Raw water samples were initially filtered by suction filtration using a Whatman filter paper No. 1 (Ø = 5.5 cm). Thereafter, the experiments were conducted in a similar manner as with the doubly distilled water samples.

2.3 Tannic Acid Method

2.3.1 Preparation of reagents

2.3.1.1 Tannic acid solution 0.1% (m/v)

Tannic acid salt (Saarchem) was readily dissolved in doubly distilled water and diluted to a known volume.

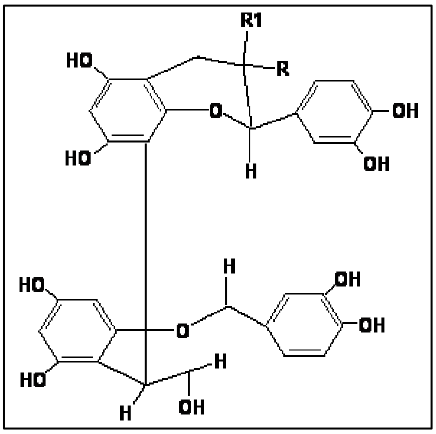

Figure 11: General structure of a tannin molecule

2.3.1.2 NaCl solution (0.5 M)

Sodium chloride salt (Saarchem) was accurately weighed, dissolved in doubly distilled water and diluted to a known volume.

2.3.1.3 Polydadmac standard solutions

A 1000 mg/L stock solution of polydadmac (Zetachem) was prepared by readily dissolving 1 g of the polymer with doubly distilled water and then diluting to 1 L. A series of polydadmac standard solutions, ranging from 2 mg/L to 12 mg/L (in 2 mg/L increments), was then prepared by diluting a corresponding volume of the stock solution to 1 L.

2.3.1.4 Epi-dma standard solutions

A 1000 mg/L stock solution of epi-dma (Zetachem) was prepared by readily dissolving 1 g of the polymer with doubly distilled water and then diluting to 1 L. A series of epi-dma standard solutions, ranging from 2 mg/L to 12 mg/L (in 2mg/L increments), was then prepared by diluting a corresponding volume of the stock solution to 1 L.

2.3.2 Procedure

- 15 mL of the polyelectrolyte standard was dispensed into a 50 mL volumetric flask.
- 20 mL of NaCl was then added to the flask followed by 5 mL of the tannic acid solution.
- The volumetric flask was then made up to volume with distilled water and the contents stirred for 1 minute.

- The solutions were left to stand for an hour at room temperature. Thereafter, transmittance was measured with a spectrophotometer from 200 - 900 nm (using a 50 mm quartz cuvette) to determine the wavelength/s of optimum transmittance.

2.4 High pressure liquid chromatography (HPLC)

2.4.1 Normal phase HPLC

2.4.1.1 Preparation of mobile phase

A mobile phase with an 89:10:1 mixture of chloroform/methanol/glacial acetic acid was prepared. (All reagents used were of HPLC grade).

2.4.1.2 Standard sample preparation

A 1000 mg/L of the polyelectrolyte sample (Zetachem) was made with water. 5 mL of this solution was heated to dryness and the remaining residue re-dissolved in 5 ml of mobile phase (5 mg / 5 mL). The polyelectrolyte sample was then injected onto the machine (Thermo separation – SMM Instruments). Concentration of sample is equivalent to its volume i.e. 20 µL is equivalent to 20 µg polymer.

2.4.1.3 Instrument and column requirements

Column:	Whatman Partisil 5 PAC (amino/cyano bonded silica) , 250 mm x 4.6 mm ID x 5 µm packing particle size x 85 Å pore size
Guard column:	Whatman Partisil PAC guard cartridge
Solvent programme:	Isocratic
Flow rate:	1 mL/min
Detector:	Refractive index (Shodex RI – 71)

The refractive index (RI) detector is the only universal detector in HPLC. The detection principle involves measuring of the change in refractive index of the column effluent passing through the flow-cell. The greater the RI difference between sample and mobile phase, the larger the imbalance will become. Thus, the sensitivity will be higher for the higher difference in RI between sample and mobile phase.

2.4.2 Size Exclusion Chromatography

2.4.2.1 Standard sample preparation

A 1000 mg/L of the polyelectrolyte sample (Zetachem) was made with water (mobile phase). The polyelectrolyte sample was then injected onto machine (Waters 600 E). Concentration of sample is equivalent to its volume i.e. 20 µL is equivalent to 20 µg polymer.

2.4.2.2 Instrument and column requirements

Column:	TOSOH TSK-Gel G5000PW$_{XL}$, 300 mm x 7.8mm ID x 10 µm particle size x 1000 Å pore size
Guard Column:	TOSOH TSK-Gel G5000PW$_{XL}$, 40mm x 6mm ID
Flow rate:	0.6 mL/min
Detector:	ELS (Sedre – Sedex 75)
Nebulizing gas:	N_2
Temperature:	40^oC

Evaporative Light Scattering (ELS) detectors involves nebulization of the column effluent to an aerosol, followed by solvent vaporization to produce small solute droplets. These droplets are then detected in the light scattering cell.In the cell, the sample particles scatter the laser light, but the evaporated mobile phase does not.

2.5 Sample Collection

Real water samples were obtained by the grab sample technique i.e. water was collected at one time from a single place from an area were the water was flowing continuously and representative of the total water quality. The water samples were collected in glass bottles, stored in a refrigerator and analysed the next day. Samples that required the determination of metal ions were collected in treated, high density polyurethane bottles and analysed at the Umgeni Water Laboratory Services Department by the Inductively Coupled Plasma technique. The specific sampling area was selected due to the varying water quality and the sample points were chosen in order to monitor polyelectrolyte concentration along the treatment process. The polyelectrolyte concentration is at its highest at the chemical treatment stage where it is first added to the water and decreases as the treatment process progresses.

2.6 Construction of Calibration Curve

During analytical determinations, some physical property (X) of an analyte is measured. The physical property varies in a known and reproducible way with the concentration (c_A) of the analyte. Skoog *et al.* (1996) have described a relationship between the concentration (c_A) of the analyte and the physical property (X):

$$c_A = k X$$

where, k is the proportionality constant and the physical property (X) is directly proportional to the concentration (cA). The physical property measured for all experiments in this study was absorbance.

The calibration curve was constructed by the standard addition method i.e. a known amount of analyte was added to the sample in order to determine the relative response of the detector to the analyte within the sample matrix. This method is advantageous in that all measurements of the analyte are made in the same matrix. This eliminates interference effects that may arise from differences in the overall composition of the sample and in the standards (Fifield and Kealy, 1990).

A series of standard solutions having known concentrations of the analyte were made. The amount of polymer in each solution was then determined by measuring the absorbance of each solution with a spectrophotometer. The absorbance of each of the standard solutions were then plotted to produce a calibration curve of absorbance vs. concentration. The curve was then used to determine the concentration of unknown solutions by finding the absorbance on the absorbance axis and reading the corresponding concentrations on the concentration axis.

By the method of standard addition, provided Beer's Law is followed, the absorbance of the solution is described by (Skoog *et al.*, 1996):

$$A_s = kV_sc_s + kV_xc_x$$

where: V_x is the volume of the unknown solution

c_x is the concentration of the unknown solution

V_s is the volume of the known solution

c_s is the concentration of the known solution

k is the constant

then, a plot of A_s as a function of V_s should yield a straight line of the form:

$$A_s = mV_s + b$$

where: m (kc_s) is the slope

b (kV_xc_x) is the y intercept

3. Results and Discussion

3.1. Potassium Polyvinylsulphate (KPVS) Method

Previous colloid titration work used for the quantification of cationic polymers was performed by means of a calibration curve using the direct titration technique. In the present study, a calibration curve was generated by an automated system. The automation of the titration permitted previous studies by the direct titration method to evolve into a photometric titration.

The automation of the titration included the dispensing of the titrant (potassium polyvinylsulphate) at a constant rate into a closed reaction vessel containing the analyte (polyelectrolyte). The thoroughly stirred solution from the reaction vessel was then circulated through a flow cell of a spectrophotometer where the absorbance value at 631 nm was measured. As the titration progressed, a titration curve (figure 12) was simultaneously generated by a recorder. The endpoint was calculated as the point lying midway between the lines drawn tangent to the baselines (Schwarzenbach and Flaschka, 1969). A closed system for the titration was developed to eliminate atmospheric and heat interferences as these interferences appeared to influence a decrease in absorbance of the indicator before the actual titration commenced.

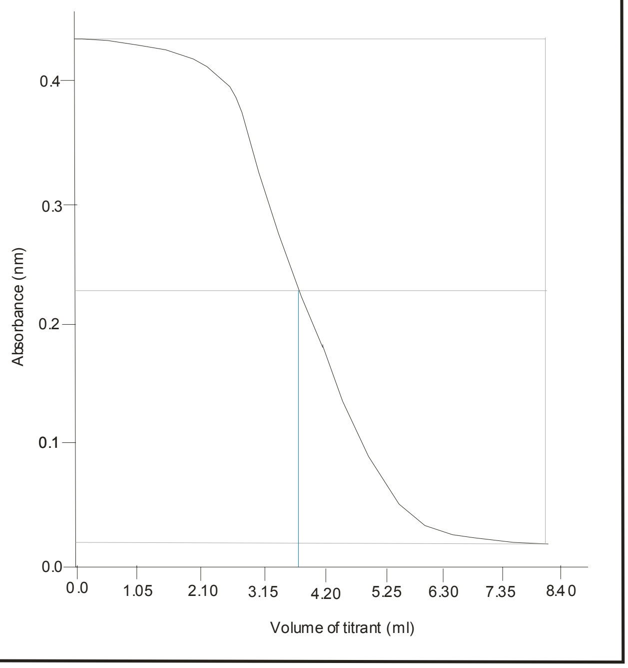

Figure 12: Titration curve and endpoint determination from the titration with toluidine blue indicator and KPVS titrant

The adaptation of the direct titrimetric method to a photometric titration was crucial to the reproducibility of the method. It allowed for the elimination of visual detection problems in the end point determination which gave a very subtle change from blue to blue- violet.

Six repetitions of the titration were conducted using the original method and the 'new' photometric method for each polymer. The concentration range chosen for this investigation remained constant for both methods although the concentration range of interest was much lower than that of Wang *et al.* (1978).

For the direct titrimetric method, the pH of the sample was measured initially. pH values were found to be approximately 6 and so adjustment according to the method of Wang *et al.,* (1978) to the desired range of 3 - 9 was not necessary. It was found that once the direct titration was complete the pH of the resulting solution decreased by approximately 0.5 pH units.

The raw data and statistical analyses for the direct titration can be seen in Tables 4 and 5 respectively for polydadmac.

Table 4: Raw data obtained from six repetitions of the direct titration of polydadmac

Concentration (mg/L)	Volume of titrant (mL)					
Blank	16.52	16.52	16.52	16.52	16.52	16.52
2	24.90	25.68	22.69	24.65	24.65	22.50
4	31.89	32.31	31.08	31.56	31.60	30.10
6	38.01	32.90	37.50	34.45	35.30	36.00
8	45.81	41.9	45.40	43.00	42.50	42.65
10	51.90	48.65	56.00	50.00	47.95	50.30
12	56.10	53.30	57.01	54.90	56.00	56.40

Table 5: Results of statistical analyses of the six repetitions for polydadmac using the direct titrimetric method

	Concentration (mg/L)					
	2	**4**	**6**	**8**	**10**	**12**
Mean	24.18	31.42	35.69	43.54	50.80	55.62
Median	24.65	31.58	35.65	42.825	50.15	56.05
Standard Deviation	1.28	0.76	1.91	1.64	4.87	1.33
Co-efficient of Variation (%)	5.31	2.43	5.35	3.77	9.59	2.38

A typical example of the raw data and statistical analyses for the photometric titration can be seen in Tables 6 and 7 respectively for polydadmac.

Table 6: Raw data obtained from six repetitions of the photometric titration of polydadmac

Concentration (mg/L)	Volume of titrant (mL)					
Blank	3.15	3.15	3.15	3.15	3.15	3.15
2	4.10	4.10	4.10	3.78	4.10	4.10
4	4.620	4.88	4.57	4.88	4.83	4.62
6	6.19	5.78	6.41	6.04	6.04	6.04
8	6.98	6.98	6.98	7.09	6.98	7.08
10	7.61	7.56	7.77	7.88	7.67	7.88
12	8.51	8.40	8.51	8.51	8.51	8.51

Table 7: Results of statistical analyses of the six repetitions for polydadmac using the photometric titration

	Concentration (mg/L)					
	2	**4**	**6**	**8**	**10**	**12**
Mean	4.04	4.73	6.08	7.02	7.73	8.49
Median	4.09	4.75	6.04	6.98	7.72	8.51
Standard Deviation	0.13	0.15	0.21	0.05	0.13	0.04
Co-efficient of Variation (%)	3.18	3.09	3.42	0.77	1.74	0.50

The statistical analyses were used to assess the performance of both methods that included the mean, median standard deviation and co-efficient of variation. These are given in Table 5 and Table 7 for polydadmac using the direct titration and the photometric titration respectively.

The standard deviation was calculated and used as a comparative study to determine the reproducibility of the 'new' photometric method to the original method. The standard deviations from the photometric titration were tabulated in conjunction with those of the direct titration. Table 8 indicates that the standard deviations of polydadmac showed a decrease when compared to the original method thus emphasizing the reproducibility of the new method.

Table 8: Standard deviations of the direct titrimetric method reported by Wang *et al.* (1978) and the photometric method for polydadmac

Concentration of polymer (mg/L)	2	4	6	8	10	12
Direct Titrimetric Method	1.28	0.76	1.91	1.64	4.87	1.33
Photometric Method	0.13	0.15	0.21	0.05	0.13	0.04

The calibration curve is presented (figure 13) with the minimum and maximum values of each standard in the data set. This illustrates that the calibration curve generated from the mean all values in the data set is an accurate representation of six replicate analyses. The calibration curve is linear for the concentration range of interest with a correlation coefficient of 0.9932.

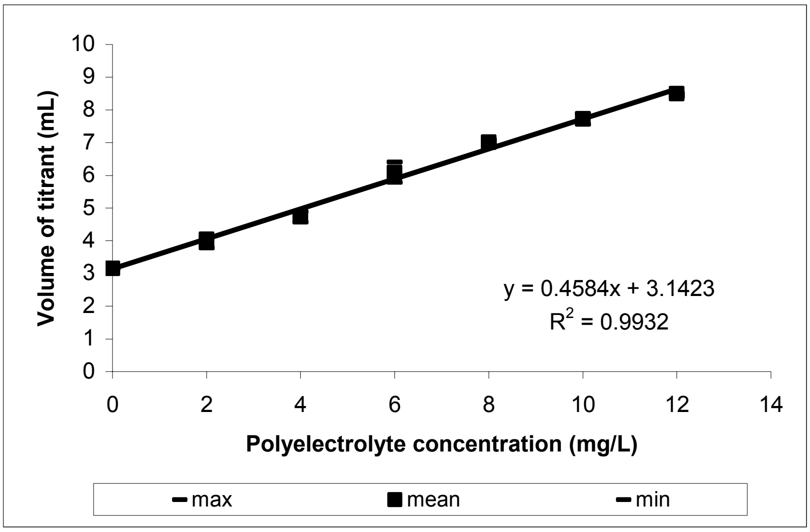

Figure 13: Mean calibration curve for polydadmac using the photometric method

The interval estimate calculations for the mean at a 95% confidence level for each concentration is specified below (Table 9) i.e. the true population parameter in approximately 95% of the cases may be found within these intervals.

Table 9: Interval estimates for population mean at 95% confidence interval for polydadmac

Concentration (mg/L)	Interval Estimate (mL)
2	3.94 - 4.15
4	4.62 - 4.85
6	5.92 - 6.25
8	6.97 - 7.06
10	7.62 - 7.83
12	8.45 - 8.52

The volume of titrant required to reach the end point of the titration for both polyelectrolytes is much lower for the photometric titration. The smaller consumption of chemicals means that there is less wastage and the cost of chemical purchases are reduced.

Epi-dma experiments showed a similar trend with the results. pH values measured were approximately 6 and so the pH did not need to be adjusted to the desired range of 3 - 9 according to the method of Wang *et al.* (1978). It was observed that once the titration was complete the pH of the resulting solution decreased by approximately 0.3 pH units.

The raw data and statistical analyses for the direct titration can be seen in Tables 10 and 11 respectively for epi-dma.

Table 10: Raw data obtained from six repetitions of the direct titration of epi-dma

Concentration (mg/L)	**Volume of titrant (mL)**					
Blank	12.70	12.70	12.70	12.70	12.70	12.70
2	17.08	19.95	20.40	17.80	18.40	21.15
4	24.60	26.98	27.15	26.00	25.92	27.43
6	36.30	34.68	35.00	35.82	34.39	36.20
8	43.50	43.60	42.90	42.15	43.05	42.59
10	53.55	51.20	52.10	51.95	52.70	51.46
12	57.40	58.05	63.40	60.85	58.80	57.90

Table 11: Results of statistical analyses of the six repetitions for epi-dma for the direct titrimetric method

	Concentration (mg/L)					
	2	**4**	**6**	**8**	**10**	**12**
Mean	19.13	26.35	35.39	42.97	52.16	59.40
Median	19.17	26.49	35.41	42.98	52.03	58.43
Standard Deviation	1.60	1.05	0.82	0.55	0.86	2.33
Co-efficient of Variation (%)	8.39	4.00	2.30	1.27	1.65	3.93

A typical example of the raw data and statistical analyses for the photometric titration can be seen in Tables 12 and 13 respectively for epi-dma.

Table 12: Raw data obtained from six repetitions of epi-dma using the photometric titration

Concentration (mg/L)	**Volume of titrant (mL)**					
Blank	2.31	2.31	2.31	2.31	2.31	2.31
2	2.57	2.63	2.78	2.52	2.63	2.52
4	3.15	3.36	3.36	3.57	3.47	3.47
6	4.41	4.57	4.67	4.73	4.67	4.57
8	5.46	5.36	5.36	5.46	5.46	5.67
10	6.83	6.67	6.83	6.98	7.09	6.83
12	7.72	7.83	7.67	7.72	7.72	7.67

Table 13: Results of statistical analyses of the six repetitions for epi-dma using the photometric method

	Concentration (mg/L)					
	2	**4**	**6**	**8**	**10**	**12**
Mean	2.61	3.40	4.60	5.46	6.87	7.72
Median	2.60	3.41	4.62	5.46	6.83	7.72
Standard Deviation	0.10	0.14	0.11	0.12	0.15	0.06
Co-efficient of Variation (%)	3.75	4.22	2.46	2.11	2.13	0.76

The statistical analyses of the mean, median standard deviation and co-efficient of variation, were once again used to assess the performance of both methods (table 11 and table 13). The standard deviation was calculated and used as a comparative study to determine the reproducibility of the 'new' photometric method to the original method.

Table 14 indicates that the standard deviations of epi-dma showed a decrease when compared to the direct titration method which once again emphasized the reproducibility of the 'new' photometric method.

Table 14: Standard deviations of the direct titrimetric method reported by Wang *et al.* (1978) and the photometric method for epi-dma

Concentration of polymer (mg/L)	2	4	6	8	10	12
Direct Titrimetric Method	1.60	1.06	0.82	0.55	0.86	2.33
Photometric Method	0.10	0.14	0.11	0.12	0.15	0.06

The calibration curve is presented (figure 14) with the minimum and maximum values of each standard in the data set. This illustrates that the calibration curve generated from the mean all values in the data set is an accurate representation of six replicate analyses. The calibration curve is linear for the concentration range of interest with a correlation coefficient of 0.9802.

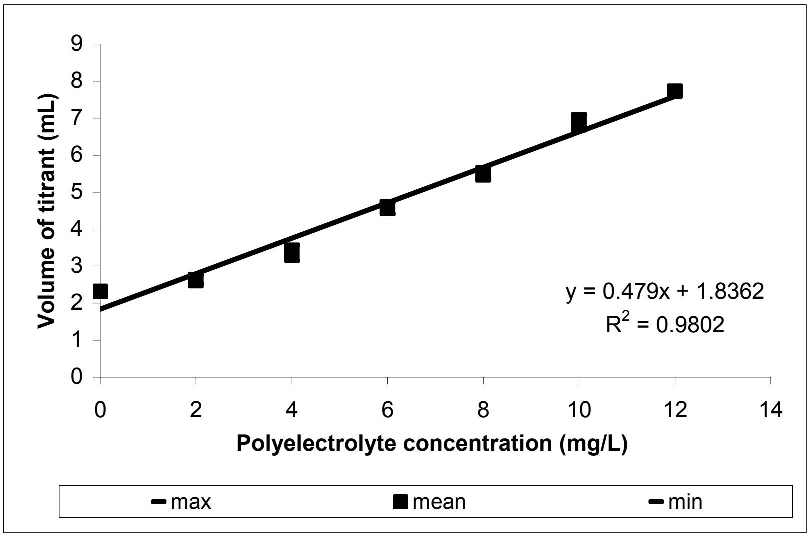

Figure 14: Mean calibration curve for epi-dma using the photometric method

The interval estimate calculations for the mean at a 95% confidence level for each concentration is specified below (Table 15) i.e. the true population parameter in approximately 95% of the cases may be found within these intervals.

Table 15: Interval estimates for population mean at 95% confidence interval for epi-dma

Concentration (mg/L)	Interval Estimate (mL)
2	2.53 - 2.69
4	3.28 - 3.51
6	4.51 - 4.69
8	5.37 - 5.55
10	6.75 - 6.98
12	7.67 - 7.76

The 'new' method has displayed good reproducibility and higher sensitivities than earlier studies. The success of this can be attributed to the fact that the photometric method developed was carried out by closely maintaining parameters in the experimental procedure and automation of the titration. These are:

- The burette was replaced by a titrant reservoir and was dispensed at a constant rate of 4.21 mL/min. This eliminated systematic errors associated with the burette e.g. diffusion from the burette tip that occurs as the height of the solution in the burette decreases. In addition, the inner surface of the burette may contain contaminants and will need to be thoroughly washed before each new experiment commences.

- The pH was maintained at 6. Initial studies reported that a pH range anywhere between 3 and 9 was sufficient for quantifying the amount of polymer. Preliminary tests of pH by maintaining all parameters and varying the pH at pH 3 pH 6 and pH 9 indicated that pH does have an effect on the end point volume (Figure 15). This test emphasized the dependency of maintaining the pH in order to obtain accurate results. The titration curve with minimal error was obtained at a pH of 6.

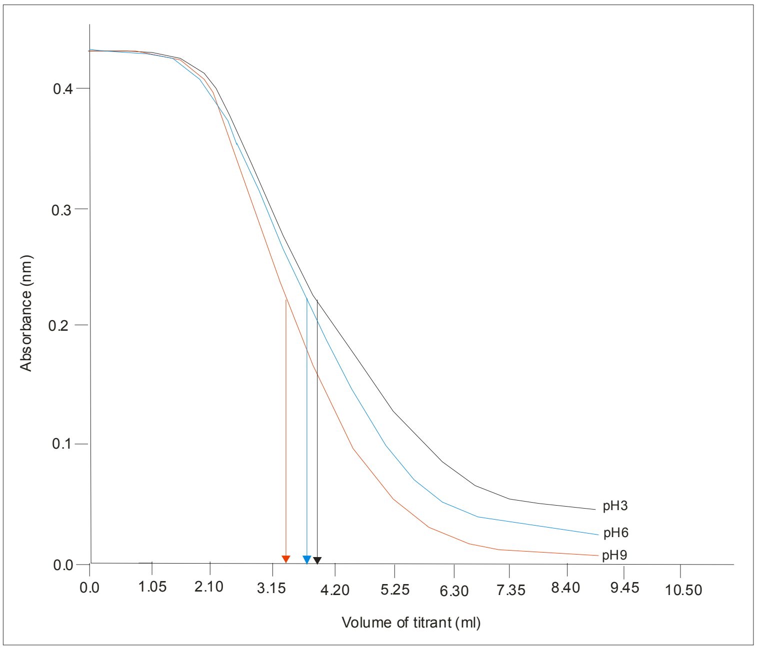

Figure 15: Titration curves for varying pH (pH 3, pH 6, pH9)

- The amount of indicator (toluidine blue) added was kept constant. This provided consistency in the indicator blank. Initial studies used 3 drops of toluidine blue which can lead to small inaccuracies in the determination. Variation of the dye volume showed that as the volume of dye increased, the absorbance increased proportionally. A constant dye volume of 0.5 mL at a concentration of 0.1% (w/v) was chosen.

- The wavelength of the spectrophotometer was set at 631 nm. This wavelength was chosen because it is the absorbance maximum of the dye toluidine blue which makes it an ideal wavelength to monitor the titration. The presence of any other absorbing species in solution did not necessarily interfere with the titration curve as the wavelength was fixed.

- The solution from the reaction vessel was continuously circulated through the flow cell at a rate of 140 mL/min. This together with the constant addition of the titrant gave a consistent time delay from the time the titrant was added to the time the titration curve was recorded. Both peristaltic pumps used in the experiments were calibrated thus eliminating systematic instrument errors.

- The formation of colloidal particles during the titration accumulated on the walls of the flow cell leading to inaccuracies in the end point determination. The flow cell was washed with a solution of concentrated nitric acid followed by a large amount of distilled water to remove the colloid build-up that formed along the walls of the flow cell.

The photometric method was then applied to a raw water system (sampled from the head of works), to determine matrix effects on the performance of the 'new' method. The experimental procedure and parameters remained the same as previous analyses only distilled water was substituted for raw water.

Raw data and statistical analyses are tabulated below (table 16 and table 17). The standard deviation was calculated and used as a comparative study to determine the compliance and reproducibility of the 'new' photometric method to raw water samples where matrix effects are present.

Table 16: Raw data obtained from six repetitions of the photometric titration of poly-diallyldimethyl ammonium chloride (raw water)

Concentration (mg/L)	Volume of titrant (mL)					
Blank	2.73	2.73	2.73	2.73	2.73	2.73
2	2.940	2.730	2.940	2.888	2.940	2.888
4	3.465	3.780	3.880	3.150	3.570	3.465
6	4.830	4.987	4.305	4.830	4.200	5.040
8	5.355	5.825	5.513	6.143	5.250	6.143
10	6.143	6.825	6.195	6.195	6.195	6.930
12	7.350	7.718	7.350	7.700	8.085	7.350

Table 17: Results of statistical analyses of the six repetitions for polydadmac (raw water)

	Concentration (mg/L)					
	2	**4**	**6**	**8**	**10**	**12**
Mean	2.89	3.55	4.70	5.70	6.41	7.59
Median	2.91	3.52	4.83	5.67	6.20	7.53
Standard Deviation	0.09	0.26	0.43	0.39	0.36	0.30
Co-efficient of Variation (%)	3.22	7.31	9.14	6.85	5.60	3.90

Table 17 displays the standard deviations of polydadmac on a raw water system. These are still considerably lower compared to the direct titration method (Table 18).

Table 18: Standard deviations of the direct titrimetric method reported by Wang *et al.* (1978) and the photometric method for polydadmac with a raw water sample

Concentration of polymer (mg/L)	**2**	**4**	**6**	**8**	**10**	**12**
Direct Titrimetric Method	1.28	0.76	1.91	1.64	4.87	1.33
Photometric Method	0.09	0.26	0.43	0.39	0.36	0.30

The calibration curve is presented (figure 16) with the minimum and maximum values of each standard in the data set. This illustrates that the calibration curve generated from the mean all values in the data set is an accurate representation of six replicate analyses. The calibration curve was once again linear within the concentration range of interest with a correlation coefficient of 0.9752.

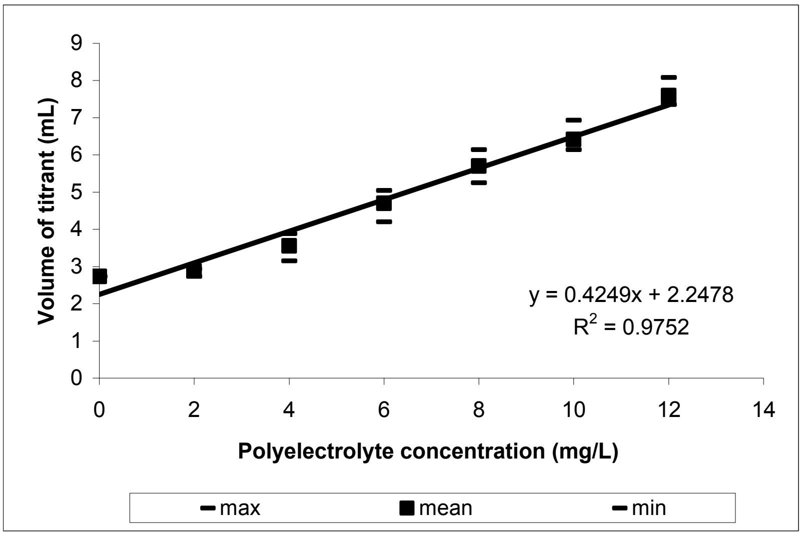

Figure 16: Mean calibration curve for polydadmac using the photometric method

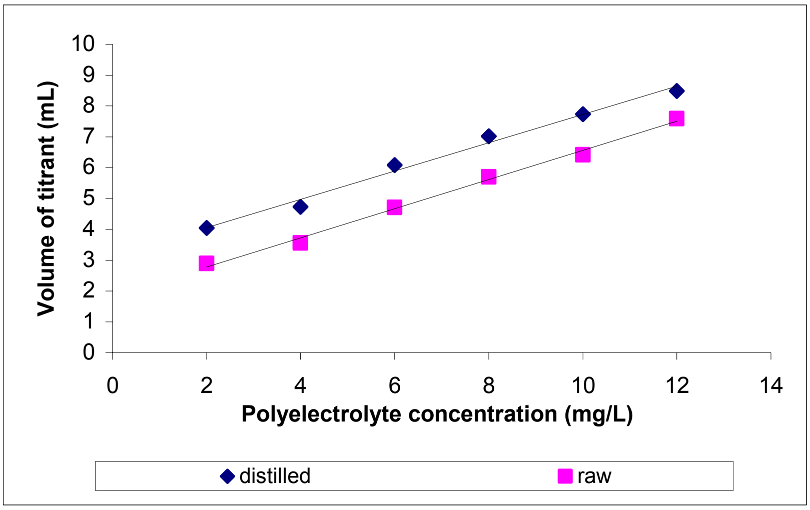

Figure 17: Calibration curves for polydadmac distilled water and polydadmac raw water systems

A comparison of the calibration curves for polydadmac between distilled water and raw water (figure 17) illustrated a slightly reduced quantity for titrant consumption for the raw water system. The response of the raw water calibration curve is still linear and has a comparable gradient to that of the distilled water curve. This shows that the photometric method can be extended to real water systems for polydadmac.

Investigations using epi-dma on a raw water system by the photometric method gave similar results. The statistical analyses are given below in table 19 and table 20:

Table 19: Raw data obtained from six repetitions of the photometric titration of epichlorohydrin-dimethylamine (raw water)

Concentration (mg/L)	Volume of titrant (mL)					
Blank	2.31	2.31	2.31	2.31	2.31	2.31
2	2.730	2.625	2.73	2.730	2.783	2.730
4	3.675	3.675	3.675	3.675	3.675	3.570
6	4.410	4.410	4.200	4.410	4.463	4.410
8	5.355	5.355	5.408	5.460	5.46	5.670
10	6.668	6.668	6.618	6.563	6.300	6.668
12	7.350	7.035	7.350	7.350	7.350	7.035

Table 20: Results of statistical analyses of the six repetitions for epi-dma (raw water)

	Concentration (mg/L)					
	2	**4**	**6**	**8**	**10**	**12**
Mean	2.72	3.66	4.38	5.45	6.58	7.43
Median	2.73	3.68	4.41	5.43	6.65	7.35
Standard Deviation	0.05	0.04	0.09	0.12	0.14	0.16
Co-efficient of Variation (%)	1.90	1.17	2.11	2.15	2.18	2.25

Table 20 displays the standard deviations of epi-dma on a raw water system. These are considerably lower compared to the direct titration method. The comparison of the standard deviations on a raw water system for epi-dma to the direct titrimetric is given in Table 21.

Table 21: Standard deviations of the direct titrimetric method reported by Wang *et al.* (1978) and the photometric method for epi-dma with a raw water sample

Concentration of polymer (mg/L)	2	4	6	8	10	12
Direct Titrimetric Method	1.60	1.06	0.82	0.55	0.86	2.33
Photometric Method	0.05	0.04	0.09	0.12	0.14	0.16

The calibration curve is presented (figure 18) with the minimum and maximum values of each standard in the data set. This illustrates that the calibration curve generated from the mean all values in the data set is an accurate representation of six replicate analyses. The calibration curve was once again linear within the concentration range of interest with a correlation coefficient of 0.9892.

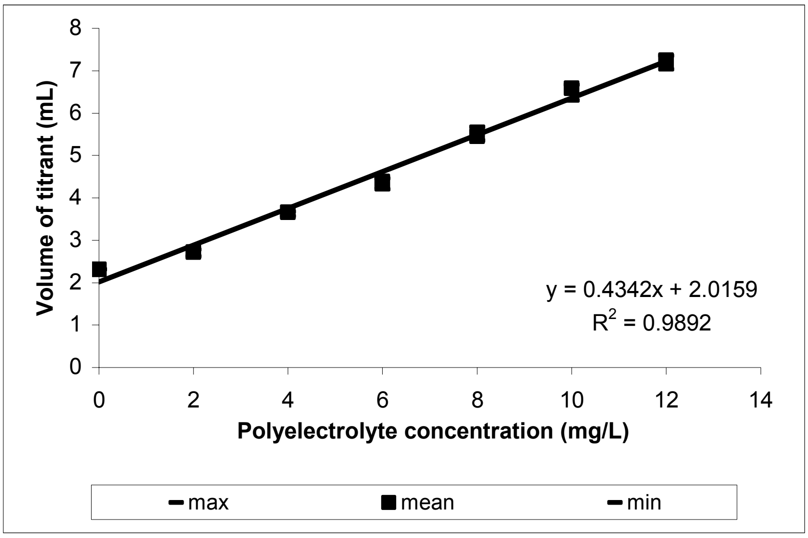

Figure 18: Mean calibration curve for epi-dma using the photometric method

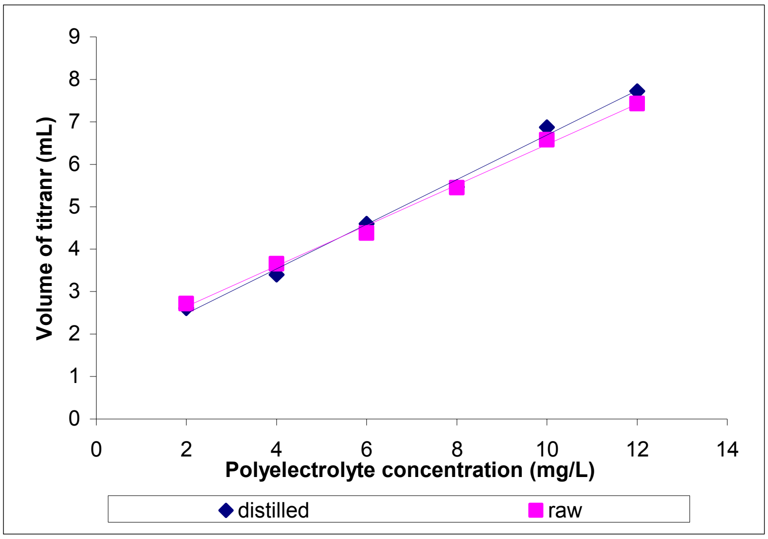

Figure 19: Comparison of calibration curves of polyamine-distilled water system and polyamine raw-water system

A comparison of the calibration curves for epi-dma between distilled water and raw water (figure 19) gave a similar response. The response of the raw water calibration curve is still linear and has a comparable gradient to that of the distilled water curve. This shows that the photometric method can be extended to real water systems for epi-dma determinations.

Numerous advantages of photometric titrations have been identified (Fifield and Kealy, 1990):

- Data near the endpoint are unimportant provided straight line portions can be established and extrapolated. This allows very dilute solutions to be analysed.
- Precision and accuracy are better for a direct absorbance measurement as the titration averages the data relative precision is around 0.5%.
- The substance to be determined need not itself absorb whilst a degree of background absorbance or turbidity can be tolerated.

The photometric method has thus far satisfied the minimum criteria of detecting polymer concentrations with a lower detection limit of 1 mg/L. It can be used successfully for the determination of polymers where no other interfering cationic polyelectrolytes are present. A further experiment at higher concentrations of up to 120 mg/L of the polymer still shows linearity in the calibration curve. Thus, the range of polymer detection may be extended to higher concentrations.

A further investigation of possible interferences from naturally occurring anions on the indicator was conducted. A synthetic sample of the anion with varying concentrations was prepared. The anion was thoroughly stirred with a 0.5 mL of 0.1% solution of toluidine blue and the absorbance measured spectrophotometrically. The anions chosen for the study were: chlorides, nitrates and bicarbonates. Table 20 lists the absorbance value of the indicator with increasing concentration of the anion.

Table 22: Absorbance values of toluidine blue with increasing anion concentration

Concentration (mg/L)	Absorbance (631 nm)			
	Chloride (Cl^-)	Nitrate (NO_3^-)	Bicarbonate (HCO_3^-)	Sulphate (SO_4^{2-})
0	0.60	0.60	0.60	0.60
0.1	0.58	0.62	0.55	0.57
0.2	0.61	0.63	0.56	0.60
0.4	0.59	0.62	0.55	0.61
0.8	0.60	0.63	0.51	0.62
20	0.51	0.56	0.51	0.65
40	0.52	0.52	0.52	0.69
60	0.49	0.51	0.51	0.71
80	0.47	0.51	0.50	0.76
100	0.40	0.49	0.50	0.81

Table 22 indicates that as the concentrations of the anion were increased, the absorbance values of $NaNO_3$, HCO_3^- and NaCl decreased whilst the SO_4^{2-} ion showed an increase in absorbance value. These trends are graphically represented in figure 20.

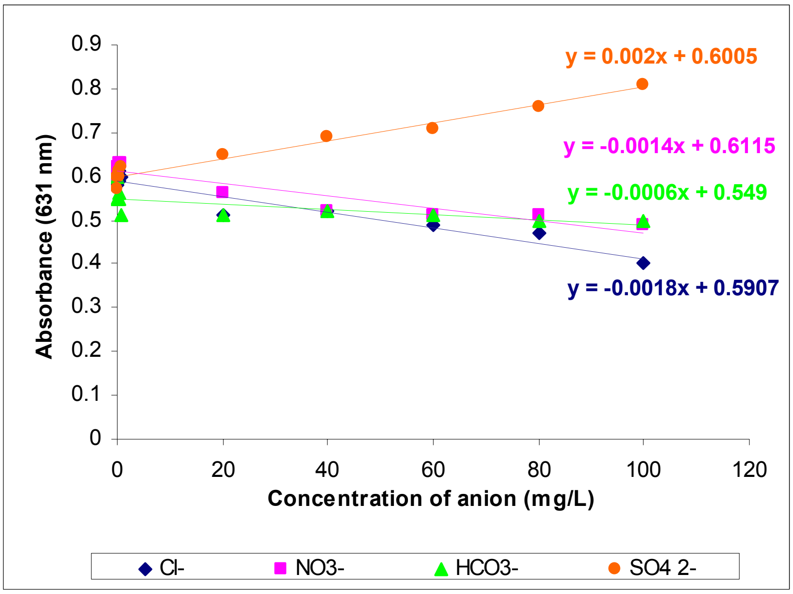

Figure 20: Illustration of the effect of increasing anion concentration on the absorbance of toluidine blue

The decrease in absorbance values for $NaNO_3$, HCO_3^- and NaCl were minor as seen by the gradients of the graph for each anion i.e. -0.0014, -0.0006 and -0.0018 for $NaNO_3$, HCO_3^- and NaCl respectively. HCO_3^- showed the least amount of change in sensitivity implying that the smallest amount of dye was consumed. Increasing concentrations of SO_4^{2-} ions caused the absorbance value to increase and can be seen by a change in the gradient of 0.002. This was due to the occurrence of a yellowish colour imparted on the solution by the presence of SO_4^{2-} ions caused which caused an overlap on the absorbance maximum of the toluidine blue dye thereby increasing the absorbance.

Although the presence of these anions interferes with the absorbance of the dye, the sensitivity is not severely affected when $NaNO_3$, HCO_3^- and NaCl ions are present and will still allow the determination of polyelectrolytes to continue with some error. The interference caused by the presence of SO_4^{2-} presented a larger error on the determination of these polyelectrolytes. Prior treatment of the sample is required to eliminate this error.

3.2. PONCEAU S Dye Method

This spectrophotometric method is simple to perform and displays good sensitivity to the determination of both polyelectrolytes under examination. The polycation-dye complex that forms is red in colour and can be seen clearly at the interface between the aqueous and organic phases. A noticeable change in the light absorbance of the dye in the aqueous phase at 520 nm is observed before and after complexation with the polyelectrolyte (figure 21).

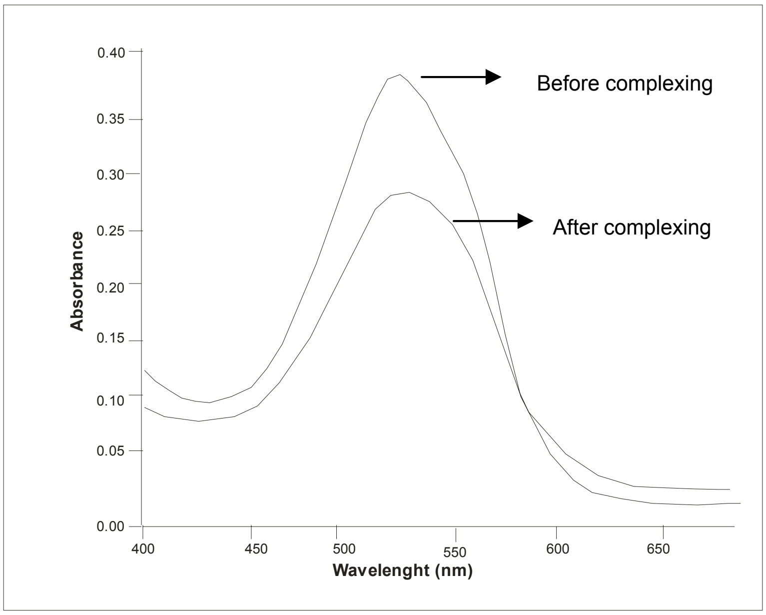

Figure 21: Absorbance maximum of the ponceau s dye before and after complexation with the polyelectrolyte

Preliminary studies of ponceau S dye stability and pH were conducted, as these are essential aspects that need to be considered in order to obtain accurate results in spectrophotometric determinations. For the measurement of the dye stability, a stock solution of the dye (200 mg/L) was made from where 2.5 mL of dye was added to a volume of distilled water equivalent to the sample volume. The absorbance of this solution was then measured over a period of time. This observation indicated that the ponceau S dye is relatively stable for up to 120 hrs (table 23). The gradient of the best line through the points (figure 22) is negligible, implying that the dye is relatively stable and can be used for up to 5 days.

Table 23: Absorbance measurements of ponceau S dye measured over a period of time to determine dye stability

Time (hrs)	Absorbance (520 nm)
0	2.20
2	2.38
4	2.40
6	2.39
24	2.21
48	2.20
120	2.28

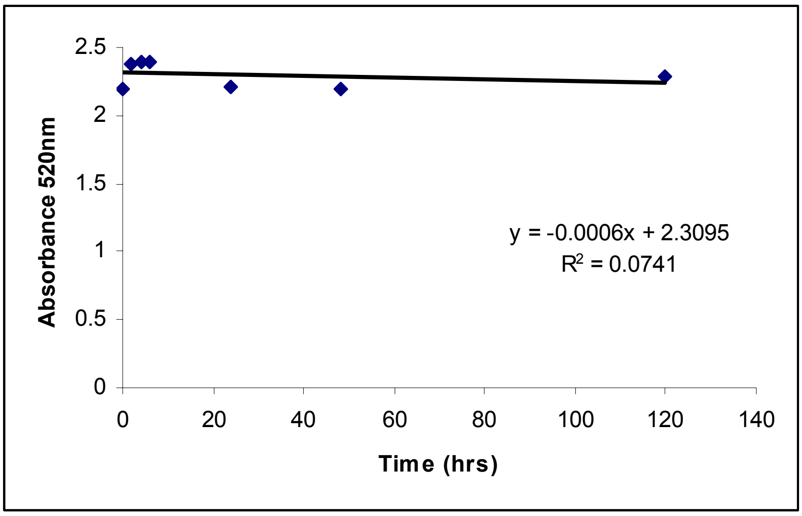

Figure 22: Absorbance measurements of ponceau S dye measured over a period of time

Variation of pH (in increments of 1 from pH 2 – pH 11) showed inconsistent values in absorbance measurements (table 24) for a sample containing 1 mg/L of polyelectrolyte. Higher absorbance values were observed at more acidic pH values (figure 23).

Table 24: Absorbance values of ponceau S dye with increasing pH

pH	Absorbance 520 nm
2	2.11
3	1.65
4	1.52
5	1.51
6	1.46
7	1.43
8	1.31
9	1.29
10	1.21
11	1.09

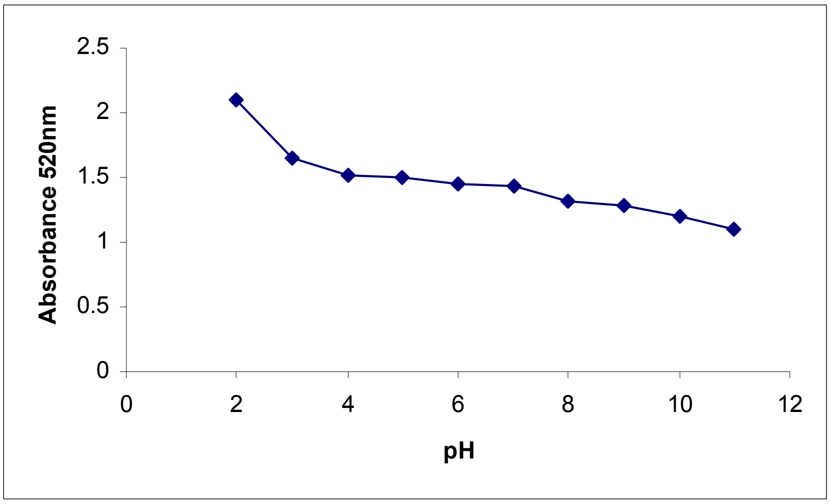

Figure 23: Graph showing effect of pH on absorbance measurements of ponceau S dye

It was further observed that as pH became more alkaline, absorbance values decreased and the colour of the solution began to change indicating a shift in the absorbance maximum of the dye.

Experiments in this study were performed in an acidic solution in keeping with previous authors' (Fielding *et al.*, 1999) findings. They established that a change in pH had a negative effect on the slope of the calibration curve causing the gradient to decrease as the solution became more alkaline.

The determination of polydadmac and polyamine investigated here was conducted in the same manner described by Fielding and colleagues (1999) to verify whether the prescribed method displayed the same results. The concentration range of interest remained the same and the only difference being that a 50 mm cuvette was used for polydadmac absorbance measurements instead of a 40 mm cuvette.

The previous authors' (Fielding *et al.*, 1999) used a 40mm cuvette as they observed an improvement in the sensitivity of the calibration curve when using a cuvette with a larger path length whilst taking absorbance measurements. A repetition of this by comparing the use of a 10 mm cuvette (figure 21) and a 50 mm cuvette for the determination of polydadmac was conducted.

A gradient of -0.0149 for a 10 mm cuvette compared to a gradient of -0.0581 for a 50 mm cuvette verified that the sensitivity was better when using a cuvette with a larger path length. A correlation co-efficient of 0.9777 (figure 24) was obtained for the cuvette with a larger path length compared to 0.9217 for the smaller cuvette suggesting improved calibration curves with the former cuvette.

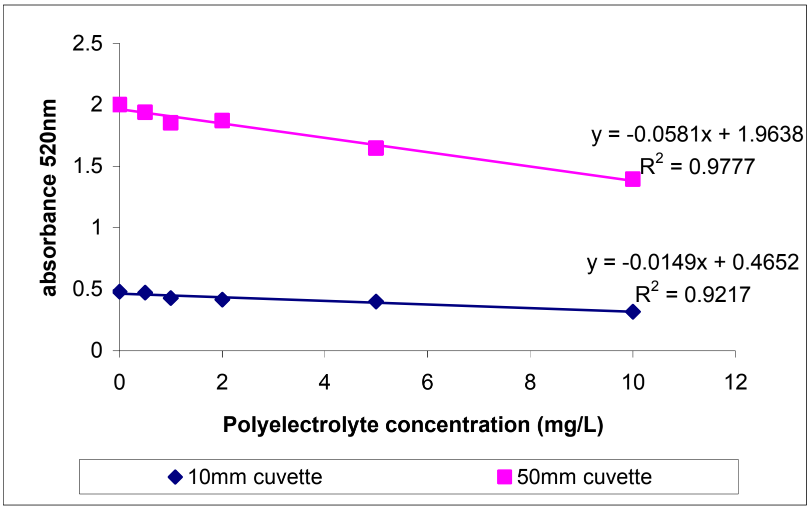

Figure 24: Comparison of calibration curves for polydadmac when using cuvettes of different size

Six repetitions of the spectrophotometric determination were conducted on a distilled water sample from where various statistical analyses were then applied to assess the performance of the method. The raw data and statistical analyses for the spectrophotometric method are given in Tables 25 and 26 respectively for polydadmac.

Table 25: Raw data obtained from six repetitions of polydadmac, ponceau S and distilled water

Concentration (mg/L)	Absorbance (520 nm)					
0	2.34	1.76	1.95	1.99	2.01	1.95
0.5	2.21	1.75	1.87	1.96	1.95	1.90
1	2.07	1.73	1.79	1.87	1.85	1.81
2	1.99	1.76	1.83	1.91	1.89	1.85
5	1.94	1.63	1.55	1.63	1.59	1.55
10	1.44	1.55	1.40	1.41	1.31	1.27

Table 26: Results of statistical analyses of the six repetitions for polydadmac, ponceau s and distilled water

	Concentration (mg/L)					
	0	**0.5**	**1**	**2**	**5**	**10**
Mean	2.00	1.94	1.85	1.87	1.65	1.40
Median	1.97	1.92	1.83	1.87	1.61	1.40
Standard Deviation	0.19	0.15	0.12	0.08	0.15	0.09
Co-efficient of Variation (%)	9.462	7.90	6.39	4.18	8.99	7.10

The standard deviation values are small with co-efficient of variation values of less than 10%. This indicates that the method is reproducible provided the experimental parameters are kept constant.

The calibration curve is presented (figure 25) with the minimum and maximum values of each standard in the data set. The calibration curve was once again linear within the concentration range of interest with a correlation coefficient of 0.9783. For instances where large box plot spreads are obtained, more replicates are recommended in order to attain accurate results.

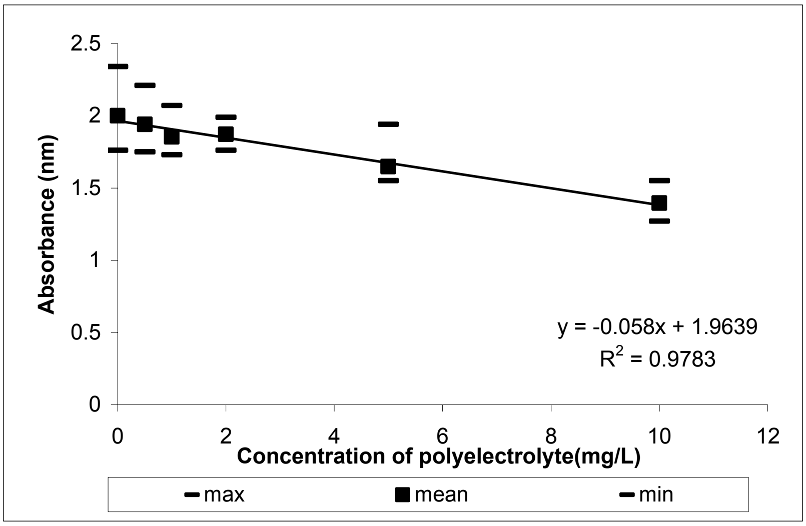

Figure 25: Calibration curve for polydadmac using the ponceau S dye method

The interval estimate calculations for the mean at a 95% confidence level for each concentration is specified below (Table 27) i.e. the true population parameter in approximately 95% of the cases may be found within these intervals.

Table 27: Interval estimates for population mean at 95% confidence interval for polydadmac

Concentration (mg/L)	Interval Estimate (Absorbance $\lambda_{520\ nm}$)
0	1.85 - 2.15
0.5	1.82 - 2.06
1	1.76 - 1.95
2	1.81 - 1.93
5	1.53 - 1.77
10	1.32 - 1.47

Epi-dma experiments showed a similar trend in the results. The raw data and statistical analyses for the spectrophotometric method using distilled water are given in Tables 28 and table 29 respectively for epi-dma.

Table 28: Raw data obtained from six repetitions of epi-dma and ponceau S

Concentration (mg/L)	Absorbance (520 nm)					
0	0.48	0.44	0.47	0.41	0.47	0.47
0.5	0.49	0.44	0.46	0.41	0.46	0.44
1	0.47	0.43	0.44	0.39	0.45	0.44
2	0.43	0.37	0.37	0.34	0.37	0.35
5	0.24	0.17	0.17	0.27	0.21	0.26
10	0.41	0.06	0.05	0.07	0.12	0.14

Table 29: Results of statistical analyses of the six repetitions for epi-dma, ponceau s and distilled water

	Concentration (mg/L)					
	0	**0.5**	**1**	**2**	**5**	**10**
Mean	0.46	0.45	0.44	0.37	0.22	0.09
Median	0.47	0.45	0.44	0.37	0.23	0.09
Standard Deviation	0.03	0.02	0.03	0.03	0.04	0.04
Co-efficient of Variation (%)	5.8	5.45	5.83	7.95	19.45	40.37

The standard deviation values were higher than those of polydadmac and the co-efficient of variation values increased to more than 10% at epi-dma concentration levels of 5 mg/L an above. Although the method is reproducible, the higher co-efficient of variation values are attributed to the fact that at higher concentrations of epi-dma, more of the dye has complexed leaving virtually no more dye in solution to complex with the polyelectrolyte.

The calibration curve is presented (figure 26) with the minimum and maximum values of each standard in the data set. The calibration curve was once again linear within the concentration range of interest with a correlation coefficient of 0.9715. For instances where large box plot spreads are obtained, more replicates are recommended in order to attain accurate results.

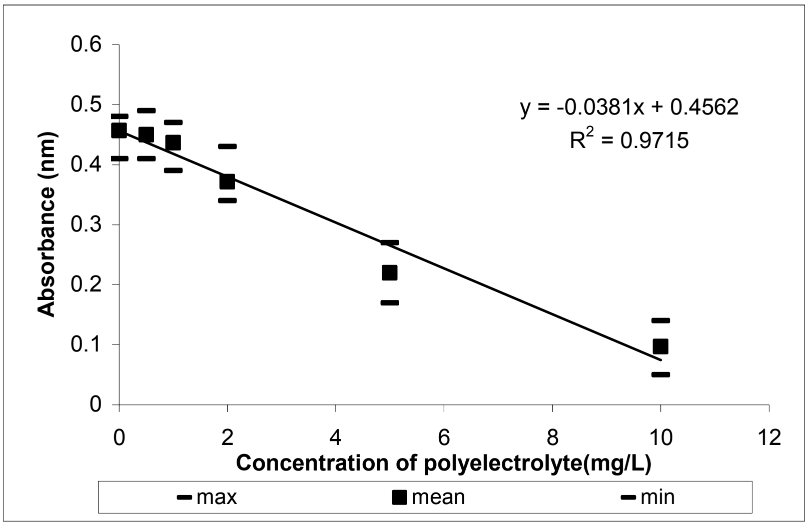

Figure 26: Mean calibration curve for epi-dma using the ponceau S dye method

The interval estimate calculations for the mean at a 95% confidence level for each concentration is specified below (Table 30) i.e. the true population parameter in approximately 95% of the cases may be found within these intervals.

Table 30: Interval estimates for population mean at 95% confidence interval for epi-dma

Concentration (mg/L)	Interval Estimate (Absorbance $\lambda_{520\ nm}$)
0	0.44 - 0.48
0.5	0.43 - 0.47
1	0.42 - 0.46
2	0.35 - 0.40
5	0.18 - 0.25
10	0.06 - 0.12

It was observed that the polyelectrolyte-dye complex (colloid) was bigger and formed more easily for epi-dma than for polydadmac. This may be due to the reduced steric interactions between the epi-dma and the dye molecule when considering the structure of the monomer units and the type of polymer chain for each polyelectrolyte.

A comparison of the gradient from the calibration curves of epi-dma (figure 26) highlights the sensitivity of the method compared to that of polydadmac (figure 25). A gradient of -0.0388 for epi-dma compared to a gradient of -0.0581 for polydadmac implies that the method is more sensitive to the determination of epi-dma.

The disadvantage of this method is that once all of the dye has complexed with the polyelectrolyte, one can no longer continue with the experiment. Determinations are restricted to the concentration of the dye in solution, so if the amount of polyelectrolyte in solution is higher than that of the dye, one cannot continue with the experiment without increasing the concentration of the dye.

Since ponceau S is an anionic dye, there is possibility that it may complex with any naturally occurring cationic species found in raw water. Studies were then undertaken to determine whether naturally occurring metal ions found in raw water sources e.g. calcium (Ca^{2+}), magnesium (Mg^{2+}), iron [II] (Fe^{2+}) and manganese [II] (Mn^{2+}), had an effect on the determination of polyelectrolytes using the ponceau S dye method. These metal ions were chosen as they are frequently found in high concentrations in some areas.

Specific sites were identified that are known to contain high concentrations of these metal ions. Raw water was then sampled and used in the determination of the polyelectrolytes using the standard addition method. Shongweni Dam (KwaZulu-Natal) and the Vaal Dam (Gauteng) were selected as sites from different regions containing high calcium and magnesium levels and Inanda Dam (25 m below surface depth) in KwaZulu-Natal was selected as the site that was known to contain high levels of iron and manganese. Table 31 below lists the values of the selected metal ions present in the different raw water sources by ICP analysis.

Table 31: Values for selected metal ions from different water samples

Water Source	Calcium (mg/L)	Magnesium (mg/L)	Iron (II) (mg/L)	Manganese (II) (mg/L)
Shongweni Dam	12.8	8.69	-	-
Vaal Dam	16.7	7.81	-	-
Inanda Dam	-	-	0.38	0.56

Table 32 and table 33 below, lists the absorbance values for poydadmac and polyamine determinations respectively, using the different raw water samples. Absorbance values for distilled water samples are also listed in order to draw a comparison on the effect of the metal ions during polyelectrolyte determination.

Table 32: Comparison of absorbance values for polydadmac determination using a distilled water sample and different sources of raw water samples

Concentration of polymer (mg/L)	Absorbance 520 nm			
	Distilled Water	Shongweni Dam	Vaal Dam	Inanda Dam
0	2.00	2.15	1.47	2.04
1	1.85	2.16	1.11	1.20
2	1.87	2.11	1.04	1.53
5	1.65	1.93	1.13	1.58
10	1.40	1.55	0.94	1.18

Table 33: Comparison of absorbance values for epi-dma determination using a distilled water sample and different sources of raw water

Concentration of polymer (mg/L)	Absorbance 520 nm			
	Distilled Water	Shongweni Dam	Vaal Dam	Inanda Dam
0	0.46	0.39	0.30	0.16
1	0.44	0.36	0.19	0.30
2	0.37	0.31	0.19	0.31
5	0.22	0.19	0.17	0.24
10	0.09	0.08	0.08	0.17

The calibration curves for polydadmac and epi-dma generated from the absorbance values above are illustrated below in figure 27 and figure 28 respectively.

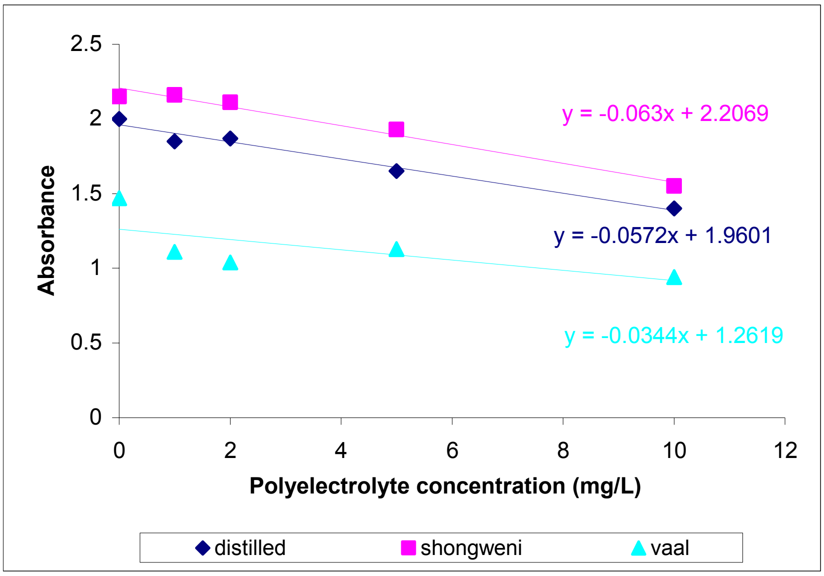

Figure 27: Comparison of calibration curves for polydadmac using distilled water and different sources of raw water that contain Ca^{2+} and Mg^{2+} ions

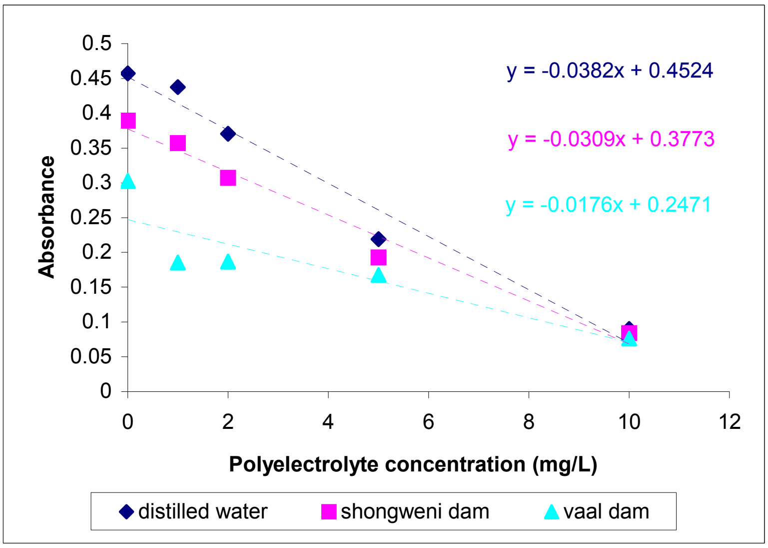

Figure 28: Comparison of calibration curves for epi-dma using distilled water and different sources of raw water that contain Ca^{2+} and Mg^{2+} ions

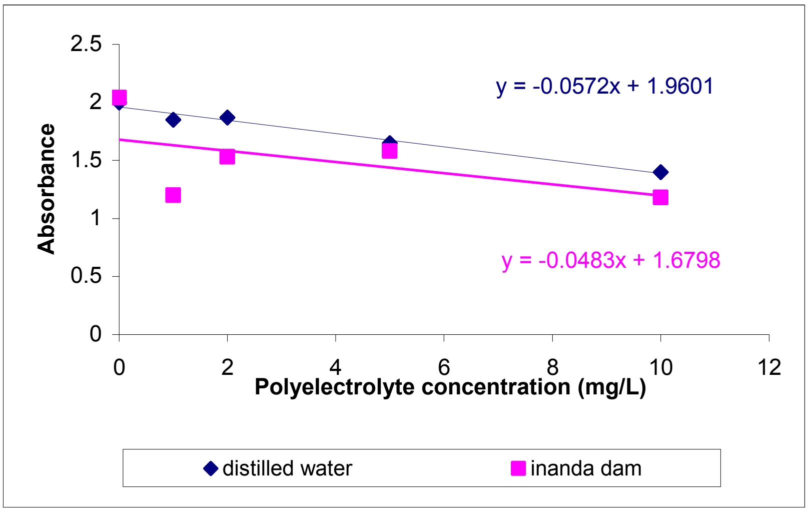

Figure 29: Comparison of calibration curves for polydadmac using distilled water and raw water (Inanda dam) that contain Fe^{2+} and Mn^{2+} ions

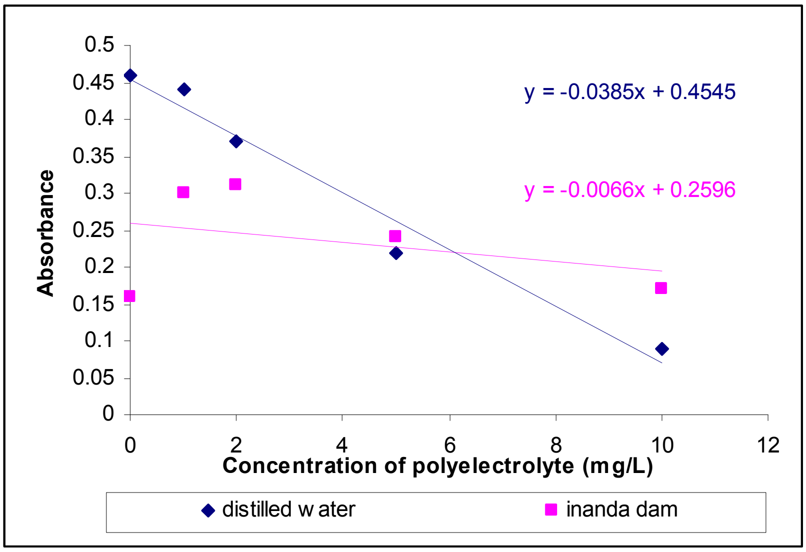

Figure 30: Comparison of calibration curves for epi-dma using distilled water and raw water (Inanda dam) that contain Fe^{2+} and Mn^{2+} ions

An inconsistency in the absorbance values were noted when an assessment of distilled water and the different raw water samples was made. The calibration curves of Vaal Dam water showed a considerable decrease in absorbance and a noticeable difference in the sensitivity when the gradients of the calibration curves were compared i.e. -0.0572 to -0.0344 (figure 27) and from –0.0382 to –0.0176 (figure 28).

Shongweni Dam samples displayed absorbance values that were slightly higher than the distilled water samples for polydadmac. The sensitivity of the calibration curve was not significantly affected for polydadmac although a decrease in sensitivity was noted for epi-dma shown by a decrease in the gradient of the calibration curve from –0.0382 to –0.0309 (figure 28).

Inanda Dam samples containing high concentrations of iron and manganese also gave inconsistent results. Polydadmac determinations gave comparable results for the sensitivity of the curve and the correlation co-efficient was severely affected (figure 29). For polyamine determinations, the calibration curve gave a change in gradient from -0.0385 to -0.0066 (figure 30). This shows that the presence of these metal ions have an effect on the determination of polydadmac and epi-dma.

It was observed that a larger and darker colloid formed at the interface for the raw water samples compared to the polyelectrolyte determination using distilled water samples. The inconsistency in absorbance values suggests that the presence of metal ions may have an effect on the determination of polyelectrolyte.

Polyelectrolyte determinations were then carried out at in the presence of the selected metal ions i.e. calcium (Ca^{2+}), magnesium (Mg^{2+}), iron [II] (Fe^{2+}) and manganese [II] (Mn^{2+}), in accordance with the Umgeni Water's standards for potable water. The standards were used, as these values are the maximum allowable limit at which the metals ions may be present in potable water and is a good indication of whether they are interferents in polyelectrolyte determination. The maximum allowable levels of the metal ions in potable water are given in table 34.

Table 34: Maximum allowable levels of the selected metal ions according to the Umgeni Water standards

Metal Ion	Umgeni Water Standard (mg/L)
Calcium	150
Magnesium	70
Iron (II)	0.2
Manganese (II)	0.1

The absorbance values and corresponding calibration curves for polydadmac determination in the presence of calcium and magnesium follow in table 35 and figure 31 respectively.

Table 35: Absorbance values during polydadmac determination when high concentrations of Ca^{2+} and Mg^{2+} are present in solution

Concentration Polyelectrolyte (mg/L)	Absorbance (520 nm)		
	Distilled Water	Ca^{2+}	Magnesium
0	2.00	0.13	1.45
0.5	1.94	0.07	1.49
1	1.86	0.06	1.45
2	1.87	0.05	1.43
5	1.65	0.05	1.47
10	1.40	0.04	1.29

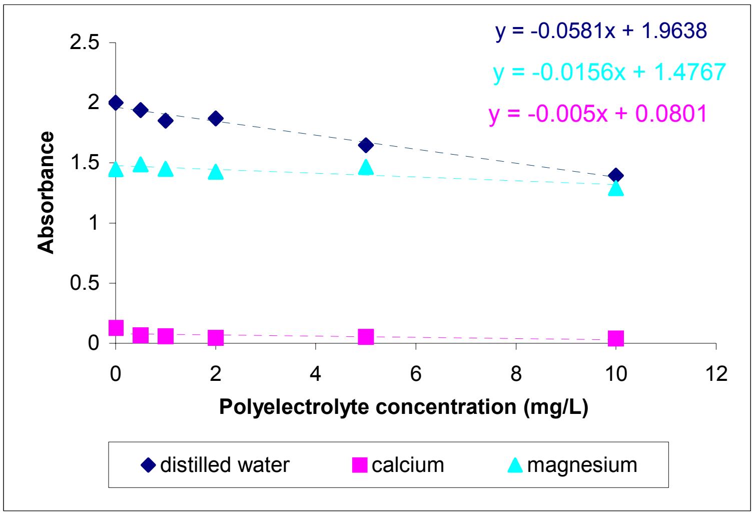

Figure 31: Illustration on the effects of Ca^{2+} and Mg^{2+} on polydadmac determination

Figure 31 above, undoubtedly shows that the presence of calcium and magnesium interfere in the determination of polydadmac.

The presence of calcium has a larger negative effect on the sensitivity of the calibration curve with a gradient of –0.005 than for magnesium that has a gradient of –0.0156. Polyamine determinations yielded similar results as seen in table 36 and figure 32 below.

Table 36: Absorbance values during epi-dma determination when high concentrations of Ca^{2+} and Mg^{2+} are present in solution

Concentration Polyelectrolyte (mg/L)	Absorbance (520 nm)		
	Distilled Water	Ca^{2+}	Mg^{2+}
0	0.46	0.052	0.32
0.5	0.45	0.05	0.28
1	0.44	0.06	0.27
2	0.37	0.07	0.23
5	0.22	0.081	0.17
10	0.09	0.066	0.08

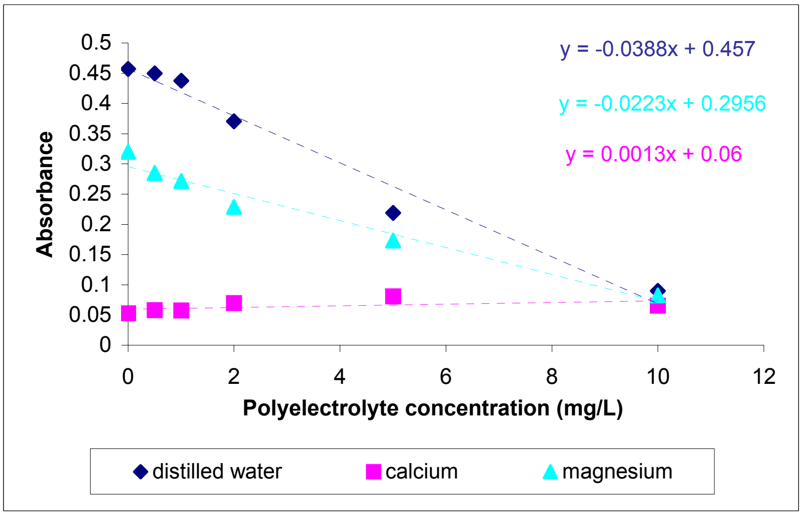

Figure 32: Illustration on the effects of Ca^{2+} and Mg^{2+} on epi-dma determination

The absorbance values and corresponding calibration curves for polydadmac determination in the presence of iron (II) and manganese (II) are given below in table 37 and figure 33 below respectively.

Table 37: Absorbance values during polydadmac determination when Fe^{2+} and Mn^{2+} ions are present in solution

Concentration Polyelectrolyte (mg/L)	Absorbance (520 nm)		
	Distilled Water	Fe^{2+}	Mn^{2+}
0	2.00	2.36	2.27
0.5	1.94	2.49	2.48
1	1.85	2.46	2.53
2	1.87	2.31	2.33
5	1.65	2.21	2.14
10	1.40	1.78	2.05

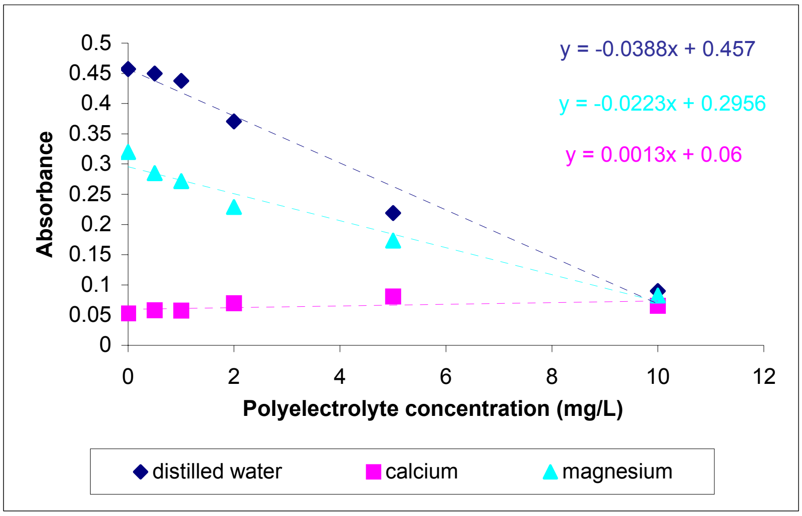

Figure 33: Illustration on the effects of Fe^{2+} and Mn^{2+} on polydadmac determination

Absorbance values for polydadmac determination in the presence of iron (II) and manganese (II) were slightly higher than for distilled water. The sensitivity of the calibration curve was influenced for both solutions containing metal ions, although the determination containing manganese had a more prominent effect on the calibration curve with a gradient of –0.0396 (figure 33).

Polyamine determinations showed the presence of these metal ions did affect the determination of polyelectrolyte. Results are given below in table 38 and figure 34 below.

Table 38: Absorbance values during epi-dma determination when Fe^{2+} and Mn^{2+} are present in solution

Concentration Polyelectrolyte (mg/L)	Absorbance (520 nm)		
	Distilled Water	Fe^{2+}	Mn^{2+}
0	0.46	0.35	0.34
0.5	0.45	0.41	0.34
1	0.44	0.41	0.38
2	0.37	0.40	0.44
5	0.22	0.33	0.39
10	0.09	0.23	0.34

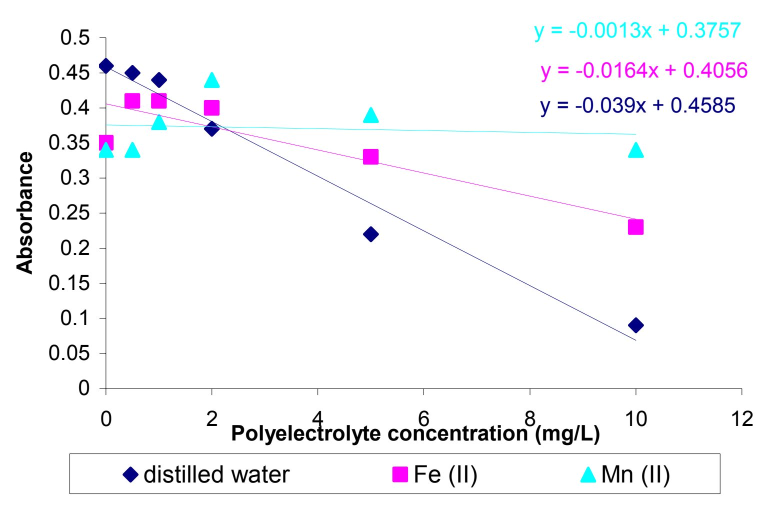

Figure 34: Illustration on the effects of Fe^{2+} and Mn^{2+} on epi-dma determination

Epi-dma determination appeared to be significantly affected by the presence of Mn^{2+}. The sensitivity of the gradient was reduced from -0.039 for distilled water to -0.0013 for the sample containing manganese (figure 31). The effect of iron on the determination was also noted with a decrease in sensitivity of the gradient from -0.039 for distilled water to -0.0164 (figure 34).

An analysis of the threshold limit of the metal ions was investigated i.e. the concentration at which the metal ion causes a gross error in the determination of both polyelectrolytes. A fixed concentration of the polyelectrolyte was used as a blank with increasing amounts of the metal ion being added to this solution. Absorbance values were then measured and are listed in tables 39 and table 40 for polydadmac and epi-dma respectively.

Table 39: Absorbance values on of 2 mg/L polydadmac solution with increasing metal ion concentration

Concentration of metal ion (mg/L)	Absorbance			
	Ca^{2+}	Mg^{2+}	Fe^{2+}	Mn^{2+}
0	1.87	1.87	1.87	1.87
0.1	1.63	1.63	2.23	2.42
0.2	1.31	1.53	2.30	2.48
0.4	1.47	1.70	2.19	2.36
0.8	1.25	1.55	2.18	2.32
20	0.27	1.17	1.18	1.39
40	0.15	1.06	1.28	1.36
60	0.10	0.99	1.20	1.19
80	0.15	0.94	1.06	1.11
100	0.08	0.72	1.00	1.09

Table 40: Absorbance values on of 2 mg/L epi-dma solution with increasing metal ion concentration

Concentration of metal ion (mg/L)	Absorbance			
	Ca^{2+}	Mg^{2+}	Fe^{2+}	Mn^{2+}
0	0.37	0.37	0.37	0.37
0.1	0.45	0.35	0.43	0.49
0.2	0.39	0.44	0.44	0.49
0.4	0.36	0.41	0.40	0.42
0.8	0.30	0.35	0.40	0.41
20	0.08	0.32	0.31	0.29
40	0.06	0.31	0.25	0.26
60	0.05	0.26	0.25	0.26
80	0.05	0.22	0.27	0.28
100	0.05	0.22	0.29	0.25

Tables 39 and table 40 indicate that as the concentration of the metal ion increases, the absorbance value decreases indicating that the presence of these ions interfere with the determination. Below 20 mg/L, the determination can be carried out with some error but for concentrations of 20 mg/L and over, the presence of these ions creates a gross error. These interferences can be removed by prior treatment of the sample.

This exploration is conclusive that the presence of metal ions e.g. calcium, magnesium, iron (II) and manganese (II) have a negative effect on the determination of polyelectrolytes The metal ions also form a complex with the dye and in turn, consumes the dye and leading to false positive results for polyelectrolyte determination. The reduced steric interactions between the metal ions and the dye make it easier to form a complex compared to the polyelectrolyte molecules. The dye appears to be more selective to complex formation with the metal ion.

3.3. The Tannic Acid Method

This method was developed for the determination of flocculent in real wastewater (Hanasaki *et al.*, 1985). It has been used successfully for the determination of both cationic and non-ionic flocculants e.g. polyacrylamide cation, ester polyaminocarbonate cation and polyacrylamide nonion.

Hanasaki and co-workers (1985) established that the optimum transmittance values of the tannic acid solution with polyelectrolyte measured with a spectrophotometer are found at 554 nm and 830 nm with the former wavelength being more sensitive. The calibration curves demonstrated good linearity and non-ionic flocculants were able to be determined at low concentrations due to the sensitivity of the curve.

Hanasaki and co-workers (1985) investigated the effects of temperature, salt concentration and pH on the determination of polyelectrolyte. Temperature and salt concentration did not have an effect of the determination while a variation in pH did. Transmittance values were high with acidic pH and decreased with a change to alkaline pH. Hanasaki *et a*l. (1985) established that the colloidal particles in tannic acid solution are large at acidic pH and small at alkaline pH. The alkaline pH influenced oxidation of tannic acid resulting in a darkening of the color of the solution. pH determinations below 7 were recommended when using this method.

The tannic acid method described by Hanasaki *et al.* (1985) did not prove to be a successful method for the determination of polydadmac and epi-dma.

A scan of transmittance across the ultraviolet and visible regions of the electromagnetic spectrum by a single beam spectrophotometer did not produce any response from the samples under evaluation. Peaks were not observed at both 554 nm and 830 nm as suggested by Hanasaki *et al.* (1985). This trend was observed for both polyelectrolytes under investigation in this study.

Various experimental parameters were investigated in order to attain a response for polydadmac and epi-dma determinations using this method. These are outlined in the appendix.

3.4. High Pressure liquid chromatography (HPLC)

HPLC is an automated technique that offers many advantages over other methods like spectroscopic and flow injection analyses. The most significant advantage of HPLC is that complex mixtures can be simultaneously analysed for a number of components over a wide concentration range and with relatively simple calibration. HPLC produces results within minutes with sensitivities ranging from nanogram (10 x $^{-9}$) to femtogram (10 x $^{-15}$) and with a reproducibility of ± 1%.

3.4.1. Normal Phase HPLC

The concentration of polyelectrolyte sample used in the following HPLC experiments were considerably lower than those used in the other methods described thus far. This is possible because the low sensitivity ranges i.e. nanogram (10 x $^{-9}$) to femtogram (10 x $^{-15}$) make lower detection limits possible. Following the HMSO (1996) procedure as a guideline, the chromatogram below (figure 35) was obtained for a 20 µg/L sample of polydadmac.

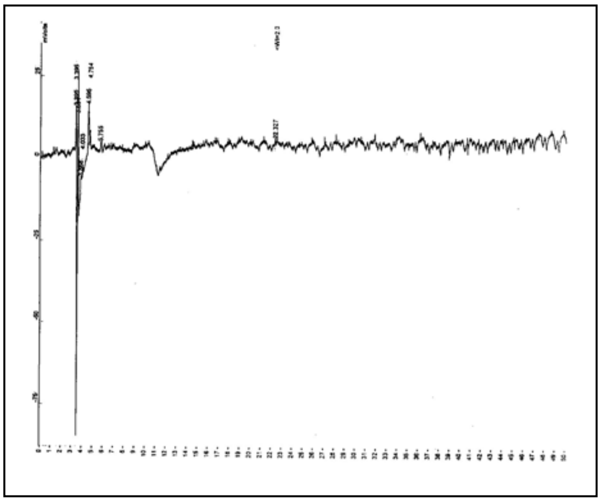

Figure 35 – Chromatogram of a 20µg/L sample of polydadmac deteced by a RI detector

After running the sample on the column for 50 minutes the hplc machine response for the sample detected a total number of 8 peaks that were rejected. The peak numbers and their retention times are tabulated below (table 41).

Table 41: Detected peaks and their corresponding retention times for polydadmac as given by the hplc machine

Peak Number	Retention Time (minutes)
1	3.395
2	3.591
3	3.868
4	4.033
5	4.595
6	4.754
7	5.755
8	22.327

The lack of response prompted further investigation of the following:

- Running the mobile phase only on the column and observing the chromatogram for irregularities
- Increasing the injection volume thereby increasing the concentration of the polymer
- Changing the polarity of the mobile phase by slowing increasing the amount of methanol thereby making it more polar
- Change of flow rate
- Change of detector response

The variables examined did not provide any single component peaks that could be used for the determination of polydadmac. The determination of epi-dma gave a similar outcome indicating that the method is not suitable for the determination of polydadmac and epi-dma.

3.4.2. Size exclusion chromatography

Size exclusion studies demonstrated to be a successful tool for identifying polydadmac and epi-dma. Detection at lower concentrations described earlier did not produce any peaks. The concentration of polyelectrolyte was then increased in increments of 100mg/L until a response by the detector was achieved. The first response was observed with a 700mg/L sample of polydadmac as demonstrated with a flow rate of 1.0 mL/min (figure 38). The peak obtained (figure 36) was close to the baseline and not well resolved, prompting the concentration of polyelectrolyte to be further increased until well resolved single component peak was obtained.

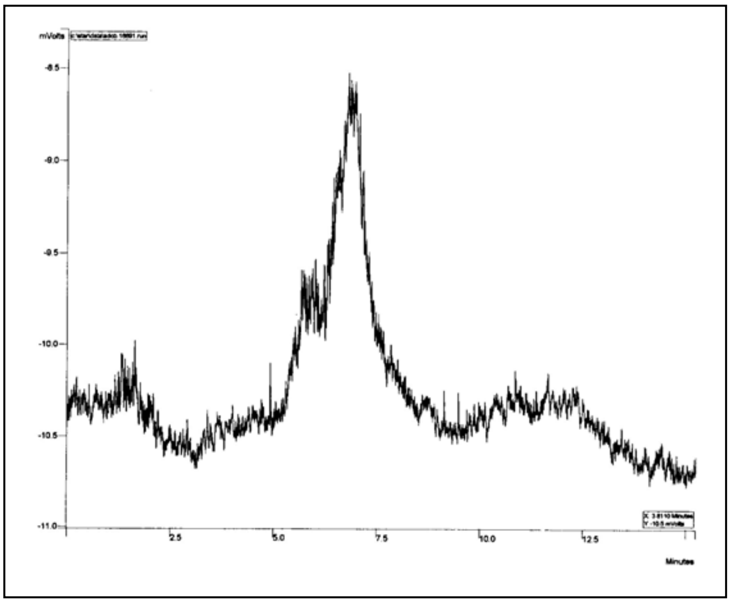

Figure 36 – Chromatogram of 700mg/L polydadmac solution detected by ELS detector

The limit of detection identified for polydadmac and epi-dma were 4300 mg/L and 3130 mg/L respectively. These concentrations produced single component peaks that were well resolved. The retention time observed was 15.39 minutes for polydadmac with a peak height of (56.2 + 8.39) mV (Figure 37) at a flow rate of 0.6 mL/min.

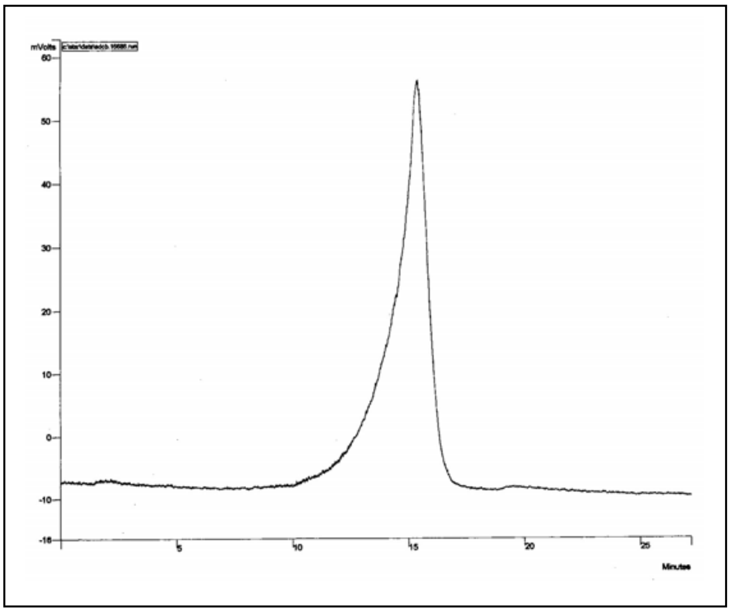

Figure 37 –Chromatogram of 4300 mg/L polydadmac solution detected by ELS detector

Epi-dma produced a retention time of 14.87 with a peak height of (84.4 + 8.91) mV (Figure 38) at the same flow rate.

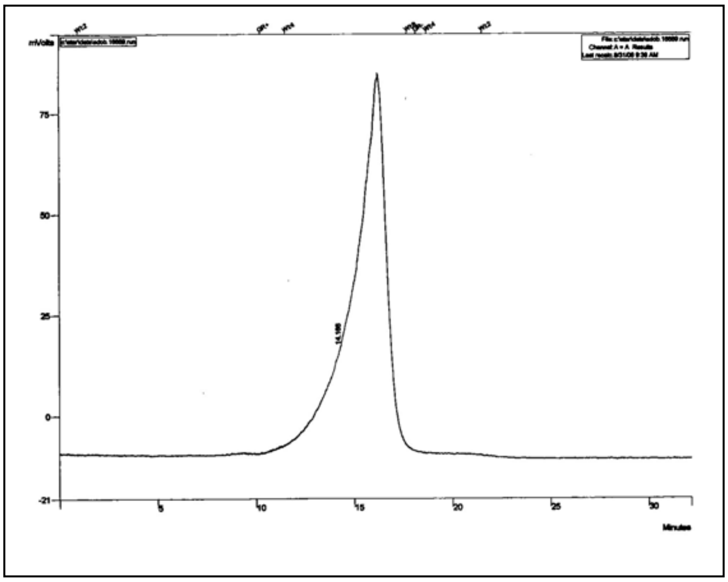

Figure 38 – Chromatogram of 3130 mg/L epi-dma solution detected by ELS detector

Based on the column specifications and molecular weight of the polyelectrolytes, this method illustrated good separation and quantification. No interferences were observed on the chromatogram even for 15 minutes after the polyelectrolyte was separated. It is also a useful technique to use when more than one polyelectrolyte product is found in a solution.

3.5. Summary of results

In order to prove that all the methods investigated in this study are acceptable for their intended purpose, method validation studies were conducted. During the validation process, each method was analysed using certain criteria. A summary of the study is tabulated below (Table 42).

Table 42: Summary of method validation

CRITERIA FOR METHOD VALIDATION	METHOD				
	KPVS	**PONCEAU S**	**TANNIC ACID**	**NORMAL PHASE HPLC**	**SEC**
Analytical Technique	Titration	Spectrophotometric	Spectrophotometric	HPLC/RI Detector	SEC/ESD detector
Preparation Time	15 min	30 min	-	15 min	15 min
Sample Size	40 mL	50 mL	15 mL	20 µL	20 µL
Limit of Detection	1 mg/L	1 mg/L	-	-	4300 mg/L polydadmac 3130 mg/L epi-dma
Reproducibility	± polydadmac 12% ± epi-dma 11%	± polydadmac 13% ± epi-dma 3%	-	-	-
Linearity in calibration curve	Linear	Linear	-	-	-
Specificity	Non-specific (unblended coagulants)	Non-specific (unblended coagulants)	-	Specific (blended coagulants)	Specific (blended coagulants)
Interferences	Other cationic polyelectrolytes and Anionic content of raw water matrix (Cl^-, NO_3^-, SO_4^{2-} and HCO_3^-)	Other cationic polyelectrolytes and Cationic content f raw water matrix eg. Metal ions (Ca^{2+}, Mg^{2+} Mn^{2+} and Fe^{2+})	-	Interferences from water matrix can interfere. Sample pretreatment by ultrafiltration or SPE	None. Separation is based on sample size. Larger molecules elute first. Small molecules in water matrix will take longer to elute
Stability	Buffer to be prepared fresh daily Other reagents are stable	Dye stable up to a week. H_2SO_4 solution to be prepared daily	Tannic acid susceptible to oxidation. Must be prepared daily	Reagents to be prepared daily	Reagents to be prepared daily
Robustness	Affected by change in pH	Affected by change in pH	Affected by change in pH and ionic strength of solution	Affected by change in pH, temperature and mobile phase composition	Affected by change in pH and tempearture
Level of Operation	Operator level	Operator level	-	Skilled level in lab	Skilled level in lab

4. Conclusion

The presence of unreacted monomer residues (contaminants) from the production of polyelectrolytes is cause for concern especially since certain contaminants in polyelectrolytes could have an adverse effect on the health of water consumers (WHO, 1993; Letterman and Pero, 1990; Holtzhausen, 2005). Polyelectrolytes and their contaminants may also react with treatment chemicals added from other processes like ozonation and chlorination to form undesirable by-products.

This concern is further highlighted by the fact that low concentrations of polyelectrolyte remains in the water after the filtration stage and that continued exposure to low concentrations of contaminants such as epichlorohydrin is important as it is an animal carcinogen. The inadequacy of techniques available for measuring residual organic polyelectrolytes in potable water makes the need to quantify them more critical.

The quantification and identification of polydadmac and epi-dma was accomplished by the KPVS, ponceau S and SEC studies. The tannic acid method and normal phase HPLC were unsuccessful at quantifying both polyelectrolytes.

4.1. KPVS Method

- Can be used when only one polyelectrolyte is present solution
- Use of a buffer is required to maintain the pH to obtain accurate results
- At increasing anion (Cl^-, NO_3^-, HCO_3^- and SO_4^{2-}) concentrations the determination is subject to a small error that needs to be compensated for
- Anion interference can be eliminated by pre-treatment. This can be done by passing the sample to be analysed through a de-ioniser
- Colloidal build-up in walls of the flow cell and through piping can be eliminated by coating the walls of the cuvette and piping with hexamethyldisilioxsane (HMDS) by passing the liquid through the system before the determination commences
- More dilute solutions can be analysed and a degree of background absorbance or turbidity can also be tolerated
- Continuous monitoring of the analyte in a production process is also possible

4.2. Ponceau S Dye Method

- Can be used when only one polyelectrolyte is present solution
- The determination is more sensitive to epi-dma determinations than for polydadmac determinations as observed by the reproducibility measurements
- At cation (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}) concentrations up to 20 mg/L the determination is subject to a small error that needs to be compensated for

- At cation (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}) concentrations more than 20 mg/L the determination is subject to gross error that will lead to major inaccuracies in the determination
- Cation interference from these metal ions can be eliminated by pre-treatment. This is done by adding EDTA to the sample to chelate with the metal ions and “mask” them

4.3. Size exclusion chromatography

- This technique has been successfully used to identify the presence of these polymers in a solution and must be done in a lab with trained personnel
- The determination is possible at detection limits of 4300 mg/L for polydadmac and 3130 mg/L for epi-dma
- It is a useful tool to measure polyelectrolyte concentration when an accidental spill or an overdose occurs
- Can be used to monitor polyelectrolyte behaviour throughout a treatment process. Will give an indication of whether some reaction occurs with other process like ozonation and disinfection to create by-products.
- The method is not subject to major interferences from the sample matrix compared to other chromatographic techniques because the determination is based on size exclusion (the larger molecule will elute first). If there is interference from the water matrix this can be eliminated by ultrafiltration of by solid phase extraction

The KPVS method is more resilient to interferences compared to the ponceau S dye method but these interferences may easily be eliminated by prior treatment of the sample. The methods are easy to understand and simple to perform making them operator friendly. They do not require sophisticated equipment and can be performed at a treatment works on a daily basis.

SEC studies demonstrated to be an excellent tool at identifying the polyelectrolytes and can be valuable in quantifying these polyelectrolytes in the event of an overdose or an accidental spill. This is advantageous as the range of detection using the KPVS and ponceau S dye methods limits the quantification of these polyelectrolytes.

The validation process (Table 42), verifies that KPVS and ponceau S dye methods performed better than the other methods even though they were susceptible to interferences from the raw water matrix.

5. Recommendations

The following recommendations are listed for the methods that were successful in the determination of polydadmac and epi-dma.

5.1. KPVS Method

- The method can be used for routine monitoring of polyelectrolyte samples on a treatment works.
- The method is capable of determining residual polyelectrolyte concentrations between 0 mg/L to 12 mg/L.
- The method can be used for raw water samples containing the following anions i.e. Cl^-, NO_3^-, HCO_3^- with some error in the calibration curve due to their interference. Pre-treatment of raw water samples is recommended to eliminate this error.
- Raw water samples containing high concentrations of SO_4^{2-} also require pre-treatment as their presence obstructs the absorbance maximum of the toluidine blue dye thus interfering with the determination and resulting in an error in the calibration curve.

5.2. Ponceau S Dye Method

- The method can be used for routine monitoring of polyelectrolyte samples on a treatment works.
- The method is capable of determining residual polyelectrolyte concentrations between 0 mg/L to 10 mg/L.
- For higher concentrations of polyelectrolyte (i.e. more than 10 mg/L), more dye is required for the determination to be successful.
- For large box plot spreads, more replicates are recommended in order to attain accurate results.
- The method can be used for raw water samples containing the following cations i.e. Ca^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+} with some error. For cation concentrations exceeding 20 mg/L, the determination is subject to gross error.

5.3. Size exclusion chromatography

- SEC using an ELS detector is an excellent technique for identifying polydadmac and epi-dma samples.
- The method can be used to quantify these polyelectrolytes in the event of an overdose or accidental spill of the polyelectrolyte sample on a treatment works.
- The determination is possible at detection limits of 4300 mg/L for polydadmac and 3130 mg/L for epi-dma. At concentrations below these, well resolved peaks are not observed.

5.4. Future research

Future research for polyelectrolyte determination in drinking water includes:

- Further automation of the KPVS method to allow for continuous monitoring of polyelectrolytes in a production process
- The use of Nuclear Magnetic Resonance (NMR) spectroscopy for quantifying polymers. This has been successfully accomplished for other quaternary ammonium groups with terminal methyls (Chang *et al.*, 2002).

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APPENDIX

1. Tannic Acid Method Optimisation

The inability to detect peaks at 554 nm and 830 nm and the lack of response from the spectrophotometer prompted the following investigations:

1.1. Optimisation of polyelectrolyte volumes for standards

Hanasaki and co-workers (1985) did not specify the volume of polyelectrolyte required to perform the determination. A 12 mg/L solution of polyelectrolyte was prepared from where 5, 10, 15 and 25 mL of polyelectrolyte were used in each determination. A volume of 15ml was chosen as the amount of analyte to add to the test sample.

1.2. Comparison of the use of NaCl and $NaNO_3$ as a suspensoid

A suspensoid is a colloidal solution in which the disperse particles are solid and remain sharply demarcated from the fluid in which they are suspended (Stedman Medical Dictionary, 2004). Hanasaki and co-workers (1985) used $NaNO_3$ or $NaClO_3$ as “suspensoids” in order to keep the colloidal particles of the polyelectrolyte and dye suspended in solution. They established that the use of either salt made no difference to the calibration curve. A comparison of inorganic salts i.e. $NaNO_3$ and NaCl from previous authors (Attia and Rubio, 1975) was carried out to determine if a change in inorganic salt would produce a response in the determination of either polydadmac or epi-dma. The use of either salt, $NaNO_3$ and NaCl, failed to produce any peaks. A comparison of the scans across the electromagnetic spectrum for 0.5 M NaCl and 0.5 M $NaNO_3$ are illustrated in figures 39 and figure 40 respectively.

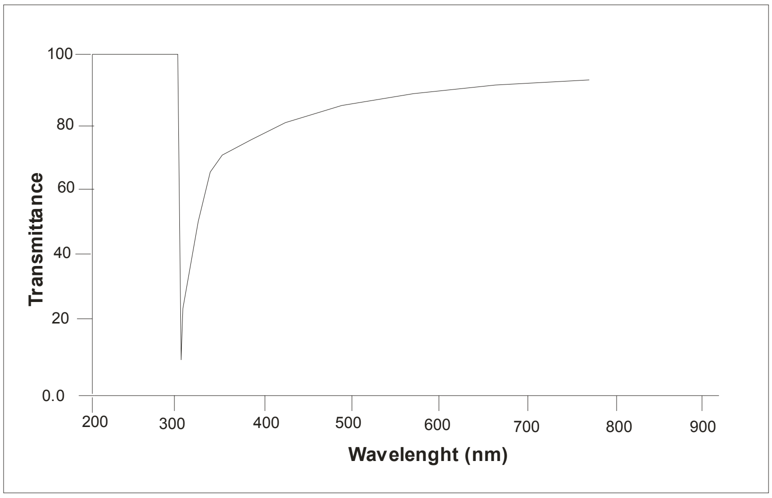

Figure 39: Scan across UV/Visible region of electromagnetic spectrum of tannic acid, polyelectrolytes and 0.5 M NaCl

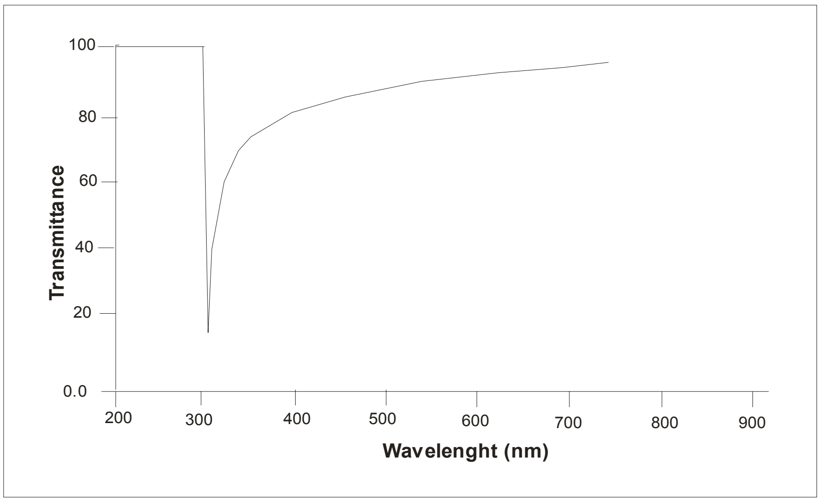

Figure 40: Scan across UV/Visible region of electromagnetic spectrum of tannic acid, polyelectrolytes and 0.5 M $NaNO_3$

1.3. Variation of inorganic salt concentration

Attia and Rubio (1975) carried out their determinations of flocculent at different concentrations of NaCl. Determinations of polyelectrolyte were conducted with different concentrations NaCl at concentrations ranging from 0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M NaCl. Once more, no response was achieved.

1.4. Variation of polymer concentration

Variation of polymer concentration was conducted assuming that the concentration range specified by the previous authors i.e. 2 mg/L-10 mg/L is insufficient when the determination using polydadmac and epi-dma is performed. The following variations of polyelectrolyte concentration were completed:

- 2 - 12 mg/L (increments of 2 mg/L)
- 12 - 48 mg/L (increments of 12 mg/L)
- 50 - 200 mg/L (increments of 50 mg/L)
- 300 - 600 mg/L (increments of 100 mg/L)

In all variations of polyelectrolyte concentration, similar looking scans were obtained. An illustration of the scans for polyelectroyte concentration from 12 – 48 mg/L is given below in figure 41.

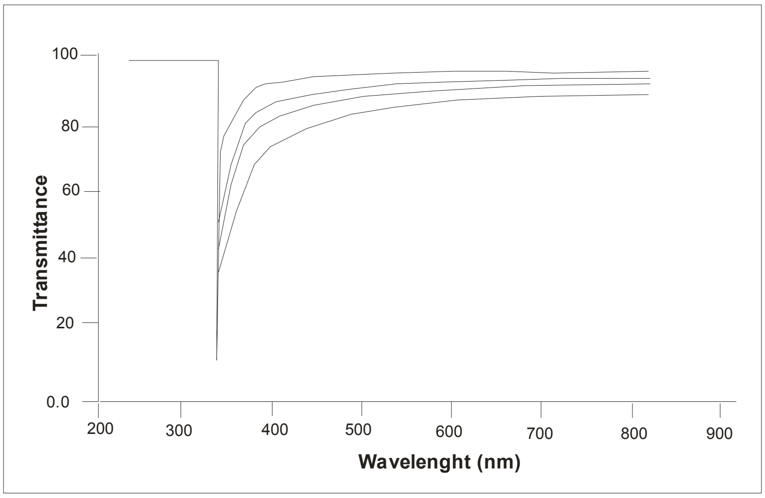

Figure 41: Scan across UV/Visible region of electromagnetic spectrum of tannic acid, and 0.5 M NaCl and polyelectrolyte concentration varying for 12 - 48mg/L

1.5. Variation of pH from 5-11 (increments of 1)

Tannic acid test samples produced a pH of 4. An investigation on the effects of a change pH was conducted. The pH was varied from 5-11 in increments of 1 and the transmittance across the spectrum was monitored. The change in pH did not produce any peaks. At more alkaline pH's, the solution began to discolor to a yellow hue. This confirms the theory of Hanasaki and co-workers (1985) that a darkening of the solution occurs at alkaline pH due to the accelerated oxidation of tannic acid.

1.6. Tannic acid concentration was varied

The polydadmac and epi-dma samples supplied by Zetachem have a solid content of 26.12% and 48.96% respectively. Assuming that the concentration of tannic acid in this experiment was too high for the polyelectrolyte samples under evaluation here, tannic acid solution was varied in concentration to 0.05% and 0.075%. Both solutions were examined with 12 mg/L of polyelectrolyte and 0.5 M NaCl. Once more, no response was observed across the spectrum.

1.7. Reagent concentrations were doubled

Reagent concentrations were doubled i.e. 0.2% tannic acid & 1 M NaCl was used with a 100 mg/L polyelectrolyte when the determination using polydadmac and epi-dma is performed. This was carried out assuming that in this situation, the polyelectrolytes in this study required more reagents (tannic acid and inorganic salt) to produce a response.

1.8. Reagent concentrations were trebled

Reagent concentrations were trebled i.e. 0.3% tannic acid & 1.5 M NaCl was used with a 100 mg/L polyelectrolyte. Reagent concentrations were doubled i.e. 0.2% tannic acid & 1 M NaCl was used with a 100 mg/L polyelectrolyte when the determination using polydadmac and epi-dma is performed. This was carried out assuming that in this situation, the polyelectrolytes in this study required more reagents (tannic acid and inorganic salt) to produce a response.

1.9. Ratio (1:1) of tannic acid to polyelectrolytes is investigated

A 5 mg/L of tannic acid was mixed with a 5 mg/L solution of polyelectrolyte assuming that a 1:1 ratio of tannic acid to polyelectrolyte is required to form ionic bonds and produce a response form the spectrophotometer. The scan across UV/Visible region of electromagnetic spectrum for 1:1 ratio of tannic acid to polyelectrolytes is given below in figure 42.

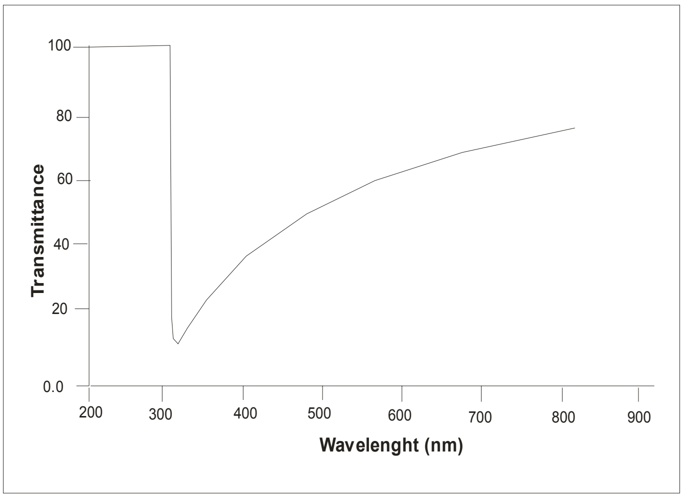

Figure 42: Scan across UV/Visible region of electromagnetic spectrum for 1:1 ratio of tannic acid to polyelectrolyte

1.10. Non-ionic polyacrylamide determined used the tannic acid method

The method was also used for a non-ionic polymer, LT 22 (polyacrylamide non-ion), with similar results being obtained. The scan of LT 22 (figure 43) gave a similar response form the spectrophotometer as with polydadmac and epi-dma.

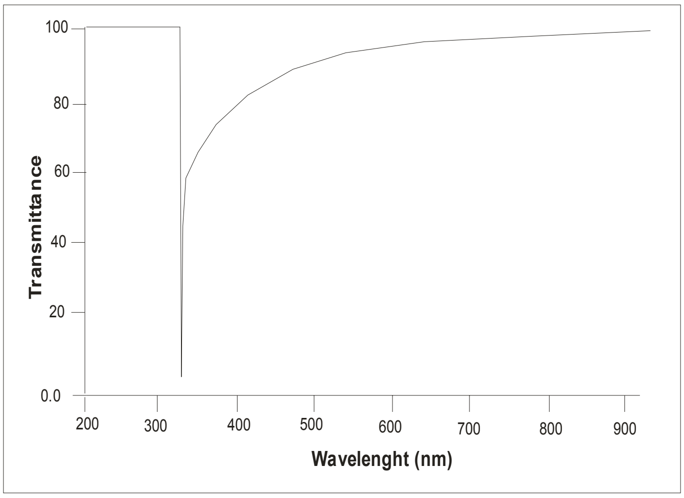

Figure 43: Scan across UV/Visible region of electromagnetic spectrum of non-ionic polyacrylamide LT 22

The investigations listed above still showed broad transmittance readings across the spectrum with no proper peaks from which a calibration curve could be constructed. Based on the variables determined and the response of the spectrophotometer across the UV/Visible spectrum, this method is not suitable for the determination of either polydadmac or epi-dma.

The investigations using tannic acid method showed broad transmittance readings across the spectrum with no proper peaks from which a calibration curve could be constructed. The stability of sample solutions, variations in pH, ionic strength, tannic acid concentration as well as the polelectrolyte concentration did not provide any tangible results.

Tannins are oligomeric compounds that are derived from plant parts i.e. bark, wood, fruit, fruit pods, leaves, roots, and plant galls. They are multiple structure units with free phenolic groups and with molecular weights ranging from 500 to >20,000 (Figure 11). They are soluble in water, with exception of some high molecular weight structures and have the ability to bind proteins and form insoluble or soluble tannin-protein complexes.

The of tannic acid product provided by the manufacturer may have been inconsistent with that of Hanasaki *et al.* (1985) and may have contributed to the inability of the method to quantify polydadmac and polyamine. In addition, the large molecular weights of both tannic acid and the polyelectrolytes may have created steric hinderance thus preventing complexation from occurring.