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DEVELOPMENT OF CAPILLARY ELECTROPHORESIS METHODS FOR THE SEPARATION OF METAL CATIONS

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Dedication

Dengan nama Allah yang Maha Pengasih lagi Maha Penyayang

To my respected parents,

Haji Zakaria Long

To my beloved wife,

Ruziah Haji Harun

To my lovely sons and daughter,

Muhammad Farhan, Muhammad Fahim , Muhammad Fayyad and Farrah Izzati

To my sisters,

Che Su and Hajjah Rasidah

Thank you for their support, patience and encouragement throughout my research and study at Sheffield Hallam University. May Allah give reward to them for their prayers for me towards success.

ACKNOWLEDGEMENTS

I would like to take this opportunity with deepest condolences and heartfelt sympathies to the family of my former supervisor, Dr Lee Tetler who was killed in a road accident during December, 2000. He gave me a very helpful suggestion, valuable advice and strong support throughout my works. He has providing me a brilliant idea and constructived critisms from the beginning until the end of my reasearch.

It was a great pleasure for me to have a new supervisor, Dr David Crowther for his willingness to take new responsibilities on my thesis from Dr Lee Tetler. Grateful appreciation is also expressed to my second supervisor, Dr Philip Gardiner from Sheffield Hallam University for helping me to design my experimental and providing me a samples for the analysis. The assistance offered by the technical staff in the Analytical Chemistry Lab especially Paul Collins is gratefully acknowledged.

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ABSTRACT

The aim of this investigation is to study the capabilities of Capillary Electrophoresis for the separation of metal cations particularly group I, group II and transition metals in liquid samples. An acac, oxalic acid and acetic acid were employed for the separation of mono and divalent cations via UV/Vis detection. However, the separation of metal cations from group I &II are not possible with the buffer alone without a complexing reagents. In addition, the presence of background electrolyte such as 4-methylbenzylamine and methanol at different concentration in the buffer solution will affect the peak separations. Therefore, the optimisation of the background electrolyte was required to eanable the simultaneous separation of the metal cations. Other factors such as pH, ionic strength and viscosity were also demonstrated for the high performance of the separation of metal cations. An increases the pH buffer, the migration times of all solutes decrease due to an increase in the EOF. As the pH of buffer is increases, the metal cations becomes progressively less protonated and its apparent mobility decreases. An oxalic acid shows poor separation compared to other complexing reagent (K, Na, Ca, Mg and Cd). Good reproducibility for migration times (SD 0.03-0.29, n = 10; $F_{obs} < F_{crit.}$), sensitive detection limits (0.1-0.5 ppm), not satisfactory working ranges (0-1 ppm, $R^2 = < 0.97$), baseline separation, peak efficiencies (52,341-265,047 theoretical plates) were obtained for K, Na, Ca, Mg and Cd. The combination of 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5) was enabled a separation of 14 metals in less than 7 min. However, separation of Fe did not prove possible with this buffer. Good reproducibility for migration times (SD 0.07-0.19, n = 10; $F_{obs} < F_{crit.}$), sensitive detection limits (0.12-0.94 ppm), satisfactory working ranges (1-40 ppm, $R^2 = 0.9841$ to 0.9977), baseline separation, peak efficiencies (204,494-609,667 theoretical plates) were obtained for Na, Ca, Mn, Co, Ni, Zn, Cr, Al, Cu and Pb. A complete separation of 8 metal cations (K, Na, Ca, Mg, Co, Ni, Cd and Pb) was achieved in less than 8.5 min using 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0). Good reproducibility for migration times (SD 0.10-0.23, n = 10; $F_{obs} < F_{crit.}$), sensitive detection limits (0.17-0.76 ppm), not satisfactory working ranges (1-25 ppm, $R^2 < 0.95$), baseline separation, peak efficiencies (178,163-223,749 theoretical plates) were obtained for K, Na, Ca, Mg, Co, Ni, Cd and Pb. The separation of metal cations in mineral water by CE is quite straight forward compared to ion chromatograph (IC). Lactic acid was found as an excellent complexing reagent for the separation metal cations in acid mine discharges by CE-UV/Vis detection. Good sensitivity and satisfactory working ranges (1-20 ppm, $R^2 = 0.9907$ to 0.9928), baseline separation, peak efficiencies (114,719-774,365 theoretical plates) were obtained for K, Na, Ca, Mg, Mn and Fe. A new developed method for the separation of 13 metal cations (K, Ba, Na, Ca, Mg, Mn, Cd, Cr, Fe, Co, Ni, Zn and Cu) was achieved in less than 8.5 min using the buffer conditions of 20 mol/L lactic acid, 15 mmol/L LiOH, 10% MeOH (pH 4.3) by CE with conductivity detection. Good reproducibility for migration times (standard deviation, SD 0.03-0.23, n = 10, $F_{obs} < F_{crit.}$), sensitive detection limits (0.4-2.9 ppm), satisfactory working ranges (1-100 ppm, $R^2 = 0.9905$ to 0.9960 except for Fe), baseline separation, peak efficiencies (311,782-770,735 theoretical plates) were obtained for K, Na, Ca, Mg, Mn and Fe in acid mine discharged. Further development need to be carried out for improving the accuracy and precision of result in quantitative analysis for CE-Conductivity detection.

COMPLETION DATE OF MY WORKS FOR THESIS SUBMISSION

The preliminary work on my thesis started in January 1996. Initial research/investigations on the application of ion chromatography techniques for the separation aid detection of cation were done under the supervision of Prof. M. Cooke. The supervision work then was undertaken by Dr L.Tetler to emphasis on the development of capillary electrophoresis (CE). Further research/investigations were focused on the separation of metals by CE using a UV/Vis detector, a conductivity detector and analysis of acid mine discharges. These experimental works were completed on September, 1998 and the first draft of the thesis was submitted on January 1999, before my returned to Malaysia due to economic recession. After that I went to United Kingdom for three weeks in April, 2000 for further discussion with my supervisor pertaining to my thesis. A second draft of the thesis was submitted before I left the United Kingdom. The corrected thesis on a second draft was received on December 2000. Unfortunately, all my emails to Dr. L. Tetler were not answered until May 2001. I was informed by Dr David Crowther that Dr L.Teler was killed in a road accident. I am grateful that Dr. David is willing to be my supervisor until the completion of my thesis.

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CHAPTER 1

INTRODUCTION

1.1. Introduction

CE refers to a group of techniques where separation of analytes is achieved under the influence of an applied electric field. There are several modes employed in CE. These include capillary zone electrophoresis (CZE), capillary isotachophoresis (CITP), micellar electrokinetic chromatography (MEKC), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF) and capillary electrochromatography (CEC). CZE is used as the separation technique for analysis of small inorganic ions and is also referred to as capillary ion electrophoresis (CIA).

Early pioneers in the use of narrow bore tubes for electrophoresis were Tiselius (1), Konstantinov and Oshurkova (2), and Everaerts (3). In 1967, Hjerten (4) improved the technique by using 3-mm I.D. glass tubes and UV detection. Later, Virtanen (5) reduced the I.D. to between 200-500 μm and employed potentiometric detection but the efficiency of separation was very poor at the low voltages used. An excellent separation was achieved by Mikkers et al. in 1980 (6). This work used Teflon tubing with 200 μm I.D. and a separation was obtained with a plate height of less than 10 μm. Further improvements were made by Jorgenson and Lukacs (7 – 9). They experimented with applied high voltages and smaller I.D. capillaries (10 to 100μm) and achieved plate heights of less than 1 μm. In-line methods were employed for the detection of separated zones. It was demonstrated that capillary electrophoretic

methods were able to generate high resolution separations, based on differences in the electrophoretic mobilities of charge carrying species. The first analysis of metal cations, using electrophoresis, was done by Hjerten (4) for a separation of bismuth and copper in 1967. Since then, several groups of investigators have described the separation of small inorganic ion species in aqueous media by CE (7-38). Some of those analytes, conditions, applications and detection methods used are listed in Table 1.1.

Table 1.1 Examples of typical applications

Analyte	Conditions (running electrolyte, pH, capillary length, length to detector, Inner diameter, voltage)	Detection, concentration range and sample type	Ref
K^+, Ca^{2+}, Na^+ Mg^{2+}, Cu^{2+}	5 mmol /L UV Cat-1, 6.5 mmol/L HIBA, pH 4.4 60 cm (52 cm) x 75 μm, 20kV	Indirect UV at 214 or 185 nm, $1.10^{-5} - 5.10^{-4}$ mol/L (tap water and juice)	(22)
Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Cs^{2+}	5 mmol/L UV Cat-1, 6.5-40 mmol/L HIBA, pH 4.4 60 cm (-) x 75 μm, 20 kV	Indirect UV at 214 nm $4.10^{-5} - 2.10^{-2}$ mol/L	(56)
K^+, Na^+, Ca^{2+}, Mg^{2+}	12 mmol L^{-1} HIBA, 6 mmol/L imidazole, pH 3.95 62 cm (50 cm) x 50 μm, 25 kV	Indirect UV at 214 nm (Tap water)	(55)
K^+, Na^+, Ca^{2+} Mg^{2+}	4 mmol/L tartaric acid, 6 mmol/L imidazole, pH 4.00 62 cm (50 cm) x 50 μm, 25 kV	Indirect UV at 215 nm (Tap water)	(55)
Li^+, Na^+, K^+ Mg^{2+}, Ca^{2+}, Ba^{2+}	5 mmol/L imidazole, 4.0 mmol/L malonic acid, pH 4.0	Indirect UV at 214 nm 4.10^{-7} mol/L	(58)
NH_4^+, K^+, Ca^{2+} Na^+, Mg^{2+}, Li^+	10 mmol/L imidazole, 2.5 mmol/L 18-crown-6, pH 4.5	Indirect UV at 214 nm 4.10^{-7} mol/L	(74)
NH_4^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Li^+, Ba^{2+}	4.0 mmol/L $CuSO_4$, 4.0 mmol/L HCOOH, 4.0 mmol/L 18-crown-6, pH 3.0 50 cm (-) x 50 μm, 20 kV	Indirect UV at 215 nm $3.10^{-5} - 1.10^{-3}$ mol/L (Drinking water)	(71)
K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Li^+	5 mmol/L UV Cat-2, 6.5 mmol/L tropolone, pH 4.4 60 cm (52 cm) x 75 μm, 20 kV	Indirect UV at 185 nm $1.10^{-5} - 1.10^{-4}$ mol/L	(64)

Table 1.1 (*continued*)

Analyte	Conditions (running electrolyte, pH, capillary length, length to detector, Inner diameter, voltage)	Detection, concentration range and sample type	Ref
K^+, Ba^{2+}, Sn^{2+}, Li^+, Ca^{2+}, Mg^{2+}, Na^+, Rb^+, NH_4^+, Cs^+	5 mmol/L benzimidazole, 40 mmol/L 18-crown-6, 0.1% HEC, 0.1% mHEC, tartaric acid, pH 5.2 25 cm (-) x 300 μm, copolymer inside, 75 μA	Indirect UV at 254 nm 10^{-5} – 2.10^{-2} mol/L, (Drinking and rain water, mineral water)	(68)
Na^+, K^+, Mg^{2+}, Ca^{2+}	5 mmol/L UV Cat-1, 6.5 mmol/L HIBA, 6.2 mmol/L 18-crown-6, 25% CH_3OH, 60 cm (52 cm) x 75 μm, 20 kV	Indirect UV at 185 nm or 254 nm, 4.10^{-4} – 3.10^{-3} mol/L (Seawater and formation water)	(188)
Cs^+, NH_4^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Li^+	4 mmol/L METOL, 2 mmol L^{-1} 18-crown-6, 80 cm (65 cm) x 75 μm, 30 kV	Indirect UV at 220 nm 1.10^{-4} mol/L	(73)
Rb^+, K^+, Na^+, Li^+	20 mmol/L MES, HIS, pH 6 60 cm (-) x 75 Indirect UV at 185 nm 1.10^{-5} – 1.10^{-4} μm, 15 kV	On-column conductivity 2.10^{-5} mol/L, (Human serum)	(38)
Mn^{2+}, Cu^{2+}, Al^{3+}, Cd^{2+}, Fe^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}	10 mmol/L $Na_2B_4O_7$, 0.1 mmol/L HQS, pH 9 42 cm (35 cm) x 75 μm, 15 kV	UV at 254 nm 6.10^{-5} – 1.10^{-4} mol/L	(83)
Cr^{3+}, Ca^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Fe^{2+}, Zn^{2+}, Fe^{3+}, Cd^{2+}	10 mmol/L $(NH_4)_3PO_4$, 75 mmol L^{-1} SDS, 0.1 mmol/L PAR, pH 8 50 cm (42 cm) x 75 μm, 15 kV	UV at 254 nm 6.10^{-5} – 2.10^{-4} mol/L	(37)
Co^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}	10 mmol/L phosphate, 7.5 mmol/L SDS 10^{-4} mmol /L PAR, pH 8, 50 cm (-) x 75 μm, 50 cm (-) x 75 μm, 15 kV	UV at 254 nm 7.10^{-5} – 5.10^{-4} mol/L	(37)
Pd^{2+}, Pt^{4+}, Au^{3+}	0.1 mol/L HCl, 0.4 mol/L NaCl 70 cm (50 cm) x 50 μm, - 7 kV	Indirect UV at 220 nm 4.10^{-8} – 1.10^{-7} mol/L	(89)
Fe^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, Cr^{3+}, Hg^{2+}, Pd^{2+}, Ag^+, Co^{2+}	20 mmol/L Na_2HPO_4, KH_2PO_4, 2 mmol/L NaCN, pH 9.4, pH 4.4 60 cm (-) x 75 μm, 20 kV	UV at 214 nm 4.10^{-7} – 5.10^{-6} mol/L	(36)

Table 1.1 (*continued*)

Analyte	Conditions (running electrolyte, pH, capillary length, length to detector, Inner diameter and voltage)	Detection, concentration range and sample type	Ref
Fe^{3+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Cu^{2+}	10 mmol/L $Na_2B_4O_7$, 1 mmol/L CDTA, pH 9.0, 50 cm (42 cm) x 75 μm, 15 kV	UV at 215 nm $6.10^{-4} - 5.10^{-3}$ mol/L	(98)
Cr^{6+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Co^{3+}	10 mmol/L HCOOH, 1 mmol/L CDTA, NaOH, pH 3.8 50 cm (42 cm) x 75 μm, 20 kV	UV at 214 nm $3.10^{-3} - 1$ mol/L	(98)
Cr^{3+}, Fe^{3+}, Cu^{2+}, Pb^{2+}	0.1 mmol/L acetate buffer, o.1 mm TTAB, pH 5.5 80 cm (60 cm) x 50 μm, - 30 kV	UV at 225 nm $6.10^{-6} - 3.10^{-5}$ mol/L	(96)
Al^{3+}, Bi^{3+}, Cr^{3+}, Cu^{2+}, Pd^{2+}, Ag^{+}, Tl^{+}, Sb^{3+}, Sn^{4+}, U^{5+}, V^{4+}, Zr^{4+}	20 mm $Na_2B_4O_7$, 1 mmol/L CDTA, 5% ethylene glycol, pH 9.0 50 cm (42.5 cm) x 75 μm, 12.5 kV	UV at 214 nm 1.10^{-6} mmol/L	(85)
K^{+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^{+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Li^{+}, Zn^{2+}, Cu^{2+}	6.5 mmol/L HIBA, 5 mmol/L UV Cat-1, acetic acid, pH 4.4 52 cm (-) x 75 μm, 20 kV	Indirect UV at 215 nm $3.10^{-6} - 3.10^{-5}$ mol/L	(23)
K^{+}, Na^{+}, Pb^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}	2 mmol/L phthalic acid, mmol/L UV Cat-1, 20% CH_3OH, pH 3.3 60 cm (-) x 75 μm, 15 kV	Indirect UV at 214 nm	(29)
K^{+}, Na^{+}, Li^{+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Mn^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}	2.5 mmol/L tartaric acid, 6 mmol/L p-toluidine, 20% CH_3OH, pH 4.8 60 cm (-) x 75 μm, 30 kV	Indirect UV at 214 nm $3.10^{-6} - 2.10^{-5}$ mol/L	(29)
K^{+}, Ba^{2+}, Sr^{2+}, Na^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^{+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, lanthanoids	15 mmol/L lactic acid, 8 mmol/L UV Cat-1, 5% CH_3OH, pH 4.25 60 cm (52 cm) x 50 μm, 25 kV	Indirect UV at 214 nm $1.10^{-5} - 8.10^{-5}$ mol/L	(29)
K^{+},Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^{+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Li^{+}, Ni^{2+}, Zn^{2+}, Cu^{2+}	12 mmol/L HIBA, 6 mmol/L imidazole, pH 3.95 62 cm (50 cm) x 50 μm, 25 kV	Indirect UV at 214 nm Prediction, optimization 1.10^{-4} mmol/L	(55)

Table 1.1 (*continued*)

Analyte	Conditions (running electrolyte, pH, capillary length, length to detector, Inner diameter and voltage)	Detection, concentration range and sample type	Ref
Li^+, K^+, Ca^{2+}, Cr^{3+}, Zn^{2+}, Al^{3+}, Cu^{2+}	5.2 mmol/L ephedrine, 4.7 mmol/L HIBA, pH 2.8 50 cm (-) x 50 μm, 15 kV	Indirect UV at 204 nm 3.10^{-6} mol/L	(61)
Rb^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^+	10 mmol/L lactic acid, 8 mmol/L UV Cat-1, 15% CH_3OH, pH 4.9, 60 cm (52.5 cm) x 50 μm, 20 kV	Indirect UV at 214 nm 1.10^{-4} mol/L	(193)
K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+}, Li^+	6.5 mmol/L HIBA, 5 mmol/L UV Cat-1, pH 4.4, 60 cm (52 cm) x 75 μm, 20 kV	UV at 185 nm $3.10^{-6} - 3.10^{-5}$ mol/L	(64)
NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Li^+	10 mmol/L imidazole, 5 mmol/L lactic acid, 0.5 mmol/L 18-crown-6, pH 4.5 57 cm (50 cm) x 75 μm, 20 kV	Indirect UV at 214 nm optimization $1.10^{-6} - 2.10^{-5}$ mol/L	(66)
K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Li^+, lanthanoids	6 mmol/L DBA, 4.2 mmol/L HIBA, acetic acid, pH 5.0, 0.2 mmol/L TX-100 60 cm (-) x 75 μm, 30 kV	Indirect UV at 214 nm $1.10^{-5} - 4.10^{-4}$ mol/L	(28)
Ca^{2+}, Na^+, Fe^{2+}, Zn^{2+}	6.5 mmol/L HIBA, 5 mmol L^{-1} UV Cat-1, pH 4.4 60 cm (-) x 75 μm, 20 kV	Indirect UV at 185 nm $8.10^{-6} - 1.10^{-4}$ mol/L	(60)
NH_4^+, K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}	6.5 mmol/L acetate HIBA, 5 mmol/L imidazole, 0.53 mmol/L 18-crown-6, 20% CH_3OH, pH 4.5 60 cm (-) x 75 μm, 20 kV	Indirect UV at 214 nm $3.10^{-5} - 9.10^{-5}$ mol/L tea infusion	(62)
Cs^+, Rb^+, NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Sr^{2+}, Cd^{2+}, Ba^{2+}, Li^+	5 mmol/L DHBP, 6 mmol/L glycine, 2 mmol/L 18-crown-6, 2% CH_3OH, pH 6.52 57 cm (50 cm) x 75 μm, 25 kV	Indirect UV at 280 nm 7.10^{-6} mol/L – 6.10^{-5} mol/L (Atmospheric aerosol)	(76)
Fe^{2+}, Pd^{2+}, Co^{2+}, Pt^{2+}, Fe^{3+}, Cr^{3+}, Au^+, Ag^+	5 mmol/L phosphate/triethanolamine, 0.8 mmol/L HMB, pH 8.5 60 cm (-) x 75 μm, 20 kV	UV at 214 nm	(86)
NH_4^+, K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}	5 mmol/L imidazole, 6.5 mmol/L HIBA, 20% CH_3OH, 0.53 mmol/L 18-crown-6, pH 4.5 60 cm (-) x 75 μm, 20 kV	Indirect UV at 214 nm $3.10^{-5} - 9.10^{-5}$ mol/L	(78)

1.2. Background theory of Capillary Electrophoresis [CE]

The basic concept, underlying the separation of ionic species in CE, is based on the differential order of migration achieved by the combination of electroosmotic flow (bulk flow resulting from the interaction between the negatively charged inner capillary wall and buffer cations followed by the application of an electric field) and electrophoretic mobilities (the movement of ions toward the electrode of opposite polarity).

1.2.1. Electroosmotic Flow [EOF]

This process is also referred to as the electroendoosmotic flow. The inner surface of a fused silica capillary is negatively charged owing to the ionisation of surface silanol groups (Si-OH) to silanoate ($Si\text{-}O^-$) when an appropriate buffer is introduced into the capillary. The degree of ionisation of silanol groups relies on the pH of the buffer, the pK_a of the silanol group is about 3.0. Under this conditions the surface silanol groups are fully dissociated. The positively charged ions from the buffer solution will be attracted to the negatively charged silanoate groups to form an inner layer. This layer is tightly held by the inner wall and is immobile (i.e. fixed layer). A second, diffuse layer of cations (i.e. mobile layer) is less tightly held and will migrate towards the cathode under the influence of an applied electrical field. The ions in this diffuse layer exchange continuously with those in the rest of the solution, and are indeed indistinguishable from them. In this relation, the boundary occurring between these two layers is called the plane of shear. An electric imbalance exists at this boundary. This will affect the potential difference called the zeta potential, ζ across the layers. Meanwhile, the magnitude of EOF is proportional to the zeta potential which is

proportional to the thickness of the double layer. The zeta potential is given by the following equation:

$$\zeta = \frac{4\pi\delta e}{\varepsilon} \qquad (1.1)$$

where δ is the thickness of the diffuse double layer, e is the charge per unit surface area, and ε is the dielectric constant of the buffer.

When an electric field is applied across the capillary, the mobile layer will migrate toward the cathode. Since most of cations are solvated, they drag the buffer solution with them toward the cathode. The simple process of the electroosmotic flow can be represented schematically as in Figure 1.1.

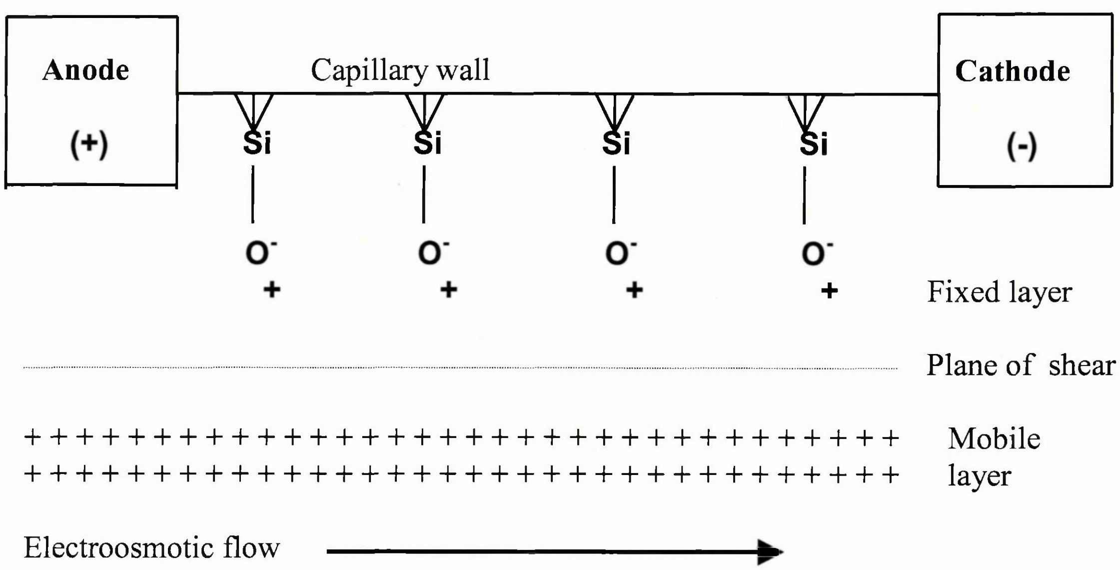


Fig. 1.1. Process of Electroosmotic flow

In reversal of the EOF, the positively charged hydrophilic ends of the quaternary amines attach to the capillary wall through ionic interaction with silanoate groups on the capillary wall. The hydrophobic, hydrocarbon ends of the attached quaternary amines associate with the hydrocarbon ends of the quaternary amines in solution through hydrophobic interaction. The hydrophilic, positively charged ends of the associated end then attract anions from buffer solutions i.e. a negatively charged double layer. As a result, negative charges in buffer solution will be attracted to the positive electrode upon application of a voltage and thus will reverse a flow in the direction of the anode. As the detector is sited at the cathodic end in CE instrument the electrode polarities are also reversed such that analytes continue to pass the detector. The order of elution is anions, neutral, then cations. This process can be represented in the schematic diagram Fig. 1.2.

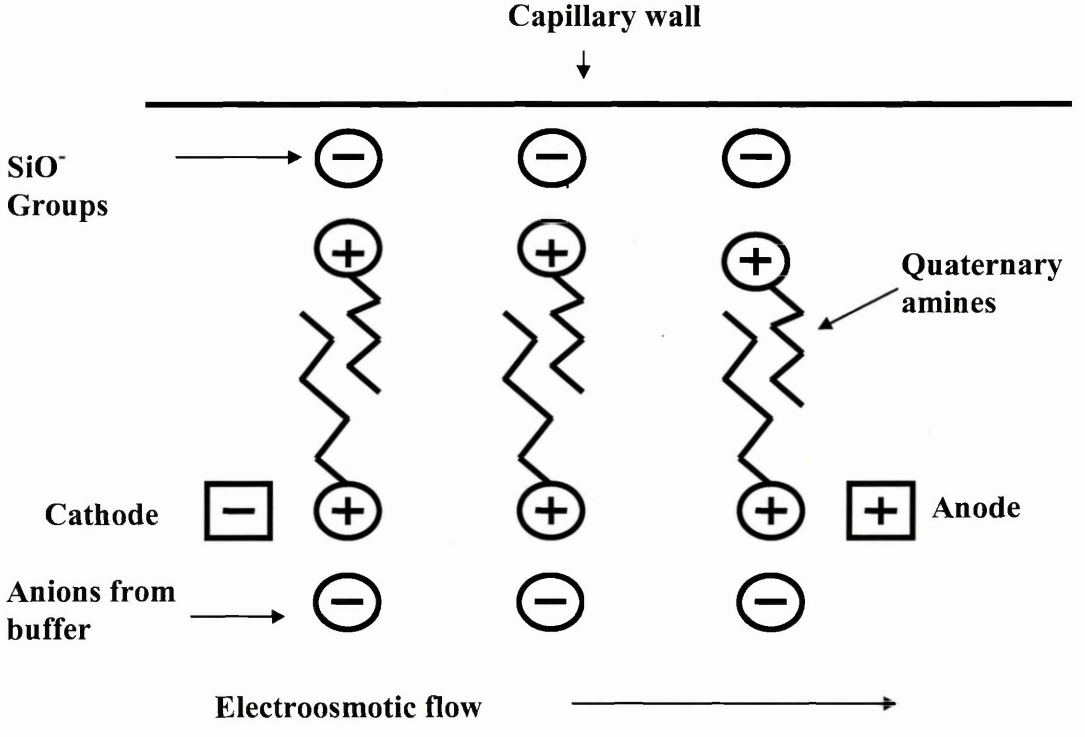


Fig. 1.2. Schematic diagram of the inside of the capillary wall representing reversal of electroosmotic flow.

The magnitude of the EOF is expressed in terms of velocity or mobility as shows by the following equation:

$$\nu_{eof} = \frac{\varepsilon \zeta E}{\eta} \qquad (1.2)$$

where ν_{eof} is the velocity of EOF, ε is the dielectric constant of the buffer, ζ is the zeta potential, E is the applied electric field in volts/cm and η is the viscosity of the buffer .

$$\mu_{EOF} = \frac{\varepsilon \zeta}{4\pi\eta} \qquad (1.3)$$

where μ_{EOF} is the electroosmotic mobility, ε is the dielectric constant of the buffer, ζ is the zeta potential and η is the viscosity of the buffer .

The zeta potential is essentially determined by the surface charge on the capillary wall. Since this charge is strongly pH dependent, the magnitude of the EOF varies with pH. At higher pH, where the silanol groups are predominantly deprotonated, the EOF is significantly greater than at lower pH where they become protonated. Depending on the specific conditions, the EOF can vary by more than an order of magnitude between pH 2 and 12. The zeta potential is also dependent on the ionic strength of the buffer,

as described by double-layer theory. Increased ionic strength results in double-layer compression, decreased zeta potential, and reduced EOF.

The electroosmotic flow in CE is a flat profile flow compared to a laminar or parabolic flow in HPLC. Therefore in CE, a uniform distribution of the buffer solution is maintained at a maximum driving force along the capillary and the flow reduction near the capillary wall can be minimised. Due to this, solutes elute as narrow bands giving narrow peaks of high efficiency. A shear force produced by an external pump will affect a laminar or parabolic flow profile in HPLC. Therefore the solutes at the centre of the column will move faster than those nearer the wall. This uneven flow profile results in relatively broad peaks. The flow profiles of CE and HPLC are represented in Fig. 1.3.

a) Flat profile flow in CE b) Laminar profile flow in HPLC

Fig. 1.3. The profile flow between EOF and laminar flow

The magnitude of the EOF in CE is reliant on certain variable factors and any alteration of these parameters will affect the EOF. Those factors involved are discussed below.

1.2.1.1. The voltage

The voltage applied across the capillary will affects the speed of migration of analytes, the higher the voltage the shorter the migration time. The higher voltage also gives higher efficiencies. However, as the voltage increases so does the current and this leads to Joule heating within the capillary. Unless this heat can be efficiently dissipated, degassing and eventual boiling of the buffer will occur thus disrupting the electrophoretic process. An Ohm's Law plot may be used to optimise the voltage applied during the analysis. The current versus applied voltage is plotted to obtain an optimum voltage as represented by the following equation:

$$V = IR \qquad (1.4)$$

where V is applied voltage, R is the resistance and I is the current.

The graph obtained should be linear and a maximum voltage can be determined when a deviation from linearity and an increase in slope is observed. This nonlinearity occurs due to excessive heat and decrease in resistance thus causing an increase in current. However, the maximum voltage also relies on the buffer composition, pH and concentration. The capillary length and inner diameter also will influence the maximum voltage that may be used and the shorter the capillary length the lower the maximum voltage. As an example when the length of the capillary is reduced, the resistance is

also decreased and this causes an increase in current flow. The maximum voltage can also be determined with the reduction of inner diameter of the capillary. With a small inner diameter, the resistance increases and hence leads to a decrease in the current flow.

1.2.1.2. pH buffer

In CE the magnitude of electroosmotic flow is dependent on the pH of the buffer and is directly proportional to the zeta potential. The zeta potential is also directly proportional to the surface charge produced at the capillary wall (see Eq. 1.1). As explained earlier, the ionisation of silanol groups at the capillary wall is more pronounced above the pK_a value of the silanol group. As the buffer pH is increased, a greater number of silanol groups at the capillary wall will be dissociated. As a result, a larger zeta potential is generated and this will increase the electroosmotic velocity. In contrast, less silanol groups will be ionised if the pH buffer is below the pK_a value of silanol groups. Most of the silanol groups are fully protonated at low pH (~2) and hence no electroosmotic flow is produced.

1.2.1.3. Ionic strength/concentration

With control of the capillary temperature, the increasing of ionic strength or concentration of the buffer will reduce the electroosmotic flow. This is because the zeta potential is determined by the thickness of the diffuse double layer, charge per unit surface area and the dielectric constant of the buffer (see Eq. 1.1). As an example when the buffer concentration is increased the dielectric constant also will increase. This will lower the strength of the zeta potential. The viscosity of the buffer increases

at higher concentrations and will influence the velocity of EOF. As can be seen from Eq. 1.2, an increase in buffer concentration or ionic strength will affect the reduction of both in zeta potential and the electroosmotic flow.

However, if the capillary temperature is uncontrolled, the increasing of buffer concentration or ionic strength may cause an increase in electroosmotic flow. At high concentration, the current flow and the temperature are increased meanwhile the viscosity is reduced. As a result the velocity of EOF may move faster under the uncontrolled temperature conditions.

Usually, the concentration (or ionic strength) that may be employed is between 10 mmol/L to 100 mmol/L in order to obtain a good result with reasonable analysis times. The application of low concentration should be avoided because it may cause broadened and asymmetric peaks.

1.2.1.4. Temperature

An increase in temperature will reduce the viscosity of the buffer solution and hence will increase the electroosmotic flow. As an example, a temperature increase of 1^0C from 20 to 21^0C will reduce the viscosity of water by 2.4 %. This will increase the magnitude of EOF. Meanwhile, an increase in temperature will also decrease the dielectric constant and hence lead to a decrease in the magnitude of the electroosmotic flow (see Eq. 1.2). The decrease in dielectric constant for water is only about $0.5\%/^0$C which is a smaller change compared to the reduction in viscosity. Due to this, the EOF moves faster at higher temperature.

1.2.1.5. Organic solvent

The addition of organic solvents to the buffer solution will change the characteristics of the viscosity, dielectric constant and zeta potential and will thus affect on the EOF. In general, the amount of organic solvent added to the buffer solution also will determine the affect on the EOF. When a constant electric current is applied to the capillary tube, the voltage will gradually increase with the increasing concentration of organic solvent due to the reduction in ionic strength of the medium being employed.

According to Reijenga et al. (39) the zeta potential (ζ) induced between the inner wall of a fused silica tube and an aqueous methanol solution gave a declining response versus methanol concentration. As mentioned earlier (see Sec. 1.2.1.4), the effect of changing viscosity is greater than changing the dielectric constant, therefore the ratio of dielectric constant (ε) to viscosity (η) is slightly decreased with the increasing organic solvent concentration (see Eq. 1.2). The mobility of the EOF, expressed as $\varepsilon\eta^{-1}\zeta$, decreased as the concentration of organic solvent increased. Therefore, the migration times will be longer and resolution better.

Several organic solvents with different viscosities and dielectric constants may be employed to optimise the electroosmotic flow. In general, methanol was shown to decreases the electroosmotic flow of the buffer solution when compared to acetonitrile. In several literature reports (40,41), the addition of methanol plays a very effective role, improving the separation of analytes with the addition of up to 30% (v/v). With increasing percentage of methanol, the mobilities of all the cations decreased almost linearly and elution of the cations was greatly delayed. However, overuse of methanol can produce a negative effect on the separation efficiency owing to broadening of the

peak shape. It was reported (42) that the addition of large amounts of organic solvents can cause electrical breakdown and have no advantages except for the improvement of the solubility of hydrophobic compounds. When the percentage of methanol reached the 50% level, the resolution clearly deteriorated. Therefore, 30%(v/v) methanol added to the buffer solution was proposed to give a very effective improvement in obtaining the separation of analytes.

In contrast, acetonitrile is less effective when compared with methanol in modifying the viscosity of the running buffer. With an increase in acetonitrile concentration, the coefficient of EOF(μ_{eo}), value was increased at almost a constant rate. Tsuda et al. (43), stated that the μ_{eo} value for methanol was lower than that of water, whereas the value for acetonitrile was higher than water. As a result a slight increase of zeta potential with increase of acetonitrile concentration may be predicted because the $\varepsilon\eta^{-1}$ value is increased. The level of acetonitrile concentration to take affect on the viscosity of the running buffer and the electroosmotic flow still remains unclear. These result are in agreement with those recently reported by Van Orman et al. (44).

1.2.1.6. Modification of the inner capillary wall

The electroosmotic flow can be reduced, eliminated or reversed by altering the chemistry of the inner capillary wall. Two approaches may be employed, permanent modification with covalent bonded or physically adhered phases or dynamic deactivation using running buffer additives.

Decreasing the EOF is achieved by shielding the silanols on the capillary wall via a chemical bonding process. The uses of some materials may reduce the EOF owing to

increases in the viscosity of the buffer. The thicker the film, the more viscous, and therefore, the slower the EOF. The introduction of some particular chemicals i.e. trimethylchlorosilane will block or inhibit the ionisation process of silanol groups at the capillary wall and hence the EOF will be suppressed or eliminated. Reversal of the electroosmotic flow may be required if analysis of anions is undertaken . This reversal method will reduce the migration times and change the flow direction from cathode to anode. Some quaternary amines, as an alkyl ammonium salts e.g. cetyltrimethylammonium bromide (CTAB), tetradecyltrimethylammonium bromide (TTAB) and others may be added to the buffer solution in order to reverse the direction of the electroosmotic flow (see Fig. 1.2).

1.2.2. Electrophoretic Mobility

1.2.2.1. Introduction

Under the influence of an electric field, an electrically charged solute will migrate to the electrode of opposite charge with an electrophoretic velocity, v_{EP} , in cm/s, as given by the following equation:

$$v_{EP} = \mu_{EP} E \qquad (1.5)$$

where μ_{EP} is the electrophoretic mobility and E is the applied electric field.

Meanwhile the electrophoretic mobility of a charged species is expressed as follows:

$$\mu_{ep} = \frac{q}{6\pi\eta r} \qquad (1.6)$$

where q is the analyte charge, η is the buffer viscosity and r the analyte radius.

The speed of migration to the respective electrode is dependent on the value of the applied electric field. High electrophoretic mobility can be achieved if the ratio of charge to size (q/r) as indicated in equation (1.6) is large. A small analyte which is highly charged will migrate faster, whereas a large molecule similarly charged will migrate at slower rate. Neutral molecules have an electrophoretic mobility of zero because the q is zero. The electrophoretic velocity is relies on both mobility and electric field, whereas electrophoretic mobility is relies only of solute and buffer properties and is independent of the applied electric field.

The velocity of a solute is influenced both by its electroosmotic mobility and the EOF. In this relation, the observed electrophoretic velocity, V_{OBS} is given by the following equation:

$$V_{OBS} = V_{EP} + V_{EOF} \qquad (1.7)$$

where V_{EP} is the electrophoretic velocity

V_{EOF} is the velocity of EOF

Under normal conditions, the EOF will migrate from the source vial toward the detector which is situated at the cathodic end. At this point the observed velocity of anions is less than the EOF as they attempt to migrate in the opposite direction to the EOF, that is V_{OBS} (anions) < V_{EOF}. Meanwhile, the observed velocity of cations is greater than the

electroosmotic velocity, that is V_{OBS} (cations) > V_{EOF} as they migrate in the same direction as the EOF. Neutral solutes will move through the capillary column under the influence of the EOF as their observed velocities are the same as the EOF, that is, $V_{OBS,}$ (neutral) = V_{EOF} . Neutral solutes will therefore move toward the detector as the same rate as the electroosmotic flow but faster than anionic solutes because their electrophoretic mobilities are zero. Usually the use of a neutral compound i.e. thiourea will allow the experimental measurement of the EOF.

1.2.2.2. Factors affecting in electrophoretic mobilities

The electrophoretic mobility of solute ions is influenced by the charge on the solute, solute size and the viscosity of the buffer (see Eq. 1.6). Any modification or changes in these parameters will affect both the electrophoretic mobility and electroosmotic flow. As an example the change of pH will change the degree of ionisation of the solute, and hence, its charge, while also changing the electroosmotic flow. Those factors that will change the viscosity such as a change in temperature or organic solvent, will also affecting both electrophoretic mobility and electroosmotic flow.

1.2.2.2.1. Solute charge

The charge of an ionised solute may be influenced by the pH of the buffer. As an example, the mobility of weakly anionic solute increases as the pH is raised due to the negatively charge exists predominantly at higher pH. Meanwhile at a low pH, most of the negative charges are neutralised and leads to a decrease in mobility. Wainright (11) shows that the mobilities of negatively charged anti-inflammatory compounds increase as the pH is increases. Observed migration times became shorter at higher pH and longer at lower pH. In this investigation a capillary was coated with a linear

polyacrylamide to eliminate the electroosmotic flow, so that the observed changes in migration times were due only to changes in solute mobilities. In contrast the mobility of cationic solutes is increased at lower pH.

1.2.2.2.2. Solute size

Some modification to the degree of hydration of solute ions may improve the separation of analytes. This effect can be seen when an organic solvent is added to the buffer solution e.g. methanol. With the presence of methanol in the buffer solution, Harold et al. (12) was able to obtain a better separation of analyte components. In their experiments, they separated iodide from chloride and azide from perchlorate anions in the presence of methanol but these separations were not possible without methanol in the buffer solution. It clearly shows that methanol may influence a change in the degree of hydration of iodide relative to chloride. In this investigation they demonstrated that the enthalpy of hydration for iodide is less than chloride, so that the water of hydration is easier to displace from iodide than chloride.

1.2.3. Effect of the analytical parameters on the electrophoretic separation

1.2.3.1. Introduction

Among the variable factors of an electrophoretic separation in CE are time, efficiency, selectivity and resolution. These factors are reliant on the electrophoretic parameters include voltage, electrophoretic mobilities, electroosmotic flow and capillary length.

1.2.3.2. Migration time

The time solute ions take to migrate from inlet to the detector through the effective capillary length, l, is called the migration time, t_m, and is given by the following

equation:

$$t_m = \frac{l}{V_{OBS}} \qquad (1.8)$$

where l is the effective capillary length and V_{OBS} is the observed electrophoretic velocity.

The migration time also can be expressed as below:

$$t_m = \frac{lL}{(\mu_{EP} + \mu_{EOF})V} \qquad (1.9)$$

where l is the effective capillary length, L is total capillary length, μ_{EP} is the electrophoretic mobility, μ_{EOF} is the electroosmotic flow mobility and V is the voltage.

Eq. (1.9), shows that higher voltages, shorter capillaries, and high electroosmotic flows give shorter migration times. This will increase the efficiency but in some cases problems may arise because as the electroosmotic flow increases the resolution becomes poorer. At high electroosmotic flows the analyte components may not have sufficient time for the zones to be separated, even though they are very narrow. In order to attain the best balance of resolution and separation time, a compromise must be made between electroosmotic flow and separation time. In general, if the heat can be effectively dissipated, the best way to reduce the migration time is using a high voltage or a short capillary length instead of increasing the electroosmotic flow.

1.2.3.3. Efficiency

The efficiency is expressed as a number of theoretical plates, N, which is calculated using migration times and peak widths at the base of the peak by the following equation:

$$N = 16(t_m / w)^2 . \qquad (1.10)$$

where t_m is the migration time and w is the baseline peak width

If the peak width is measured at half the peak height, $w_{1/2}$, the following equation is used to determine the efficiency:

$$N = 5.54(t_m / w)^2 . \qquad (1.11)$$

where t_m is the migration time and w is the baseline peak width

It can be seen from both of the above equations that the narrower a peak and the longer its migration time, the higher its efficiency. If a solute zone did not diffuse as it migrated through the capillary, it would exit with the same zone width that it had when it was injected. In CE, the zones do not spread very much because they move through the capillary as a plug flow (flat profile flow). This is different from HPLC where solutes will move through the column under the influence of a laminar flow which causes spreading of the zones leading to broader peaks (see Sec. 1.2.1).

The efficiency can be related to the HETP (height equivalent to a theoretical plate), H, as follow:

$$H = (l/N) \qquad (1.12)$$

where l is effective capillary length and N is number of theoretical plates

The efficiency can also be related to HETP using the van Deemter equation as given below:

$$H = A + \frac{B}{u} + C \cdot u \qquad (1.13)$$

where A is independent of the flow velocity and characterises peak dispersion caused the Eddy diffusion, B is longitudinal diffusion, C is comprises the resistance to mass-transfer between mobile and stationary phases and u is the overall migration rate of the mobile phase

Jorgenson and Luckacs (45) were the first to point out that because of the plug flow profile in CE the only contribution to the plate height was that given by the B term of the Van Deemter equation, i.e., the term which provided for axial diffusion. With no packing there was no contribution from the A term (eddy diffusion), and with no retention at the walls of the tube and no variation in flow velocity across the tube there were no contributions from C term (resistance to mass transfer). Accordingly, we can write the plate height H as follows:

$$\mathrm{H} = \frac{2D_{\mathrm{m}}}{u} \qquad (1.14)$$

where u is the overall migration rate of the analyte and D_m is diffusion coefficient of the solute

According to Giddings (46), the number of theoretical plates is also given by the following equation:

$$N = \frac{(\mu_{EP} + \mu_{EOF})V}{2D} \quad (1.15)$$

where μ_{EP} is the electrophoretic mobility, μ_{EOF} is the electroosmotic mobility, V is voltage and D is diffusion coefficient of the solute

Based on Eq. (1.15), higher efficiency can be achieved when the electroosmotic flow and voltage are increased. In CE the efficiency is independent of the capillary length (if Joule heat is dissipated) compared to chromatography where the efficiency is proportional to the column length. A short capillary may provide a good separation with shorter migration time with no reduction in efficiency. However, the use of a short capillary will reduce the electrical resistance and hence will lead to an increase in Joule heating. Dissipation of heat is more difficult due to smaller surface area when compared with a longer capillary. With no temperature control, the efficiency for a long capillary is increased to certain limits before it remains constant. In this situation Joule heating is effectively dissipated and convective diffusion can be minimised. Even with the control of temperature, the capillary length can be optimised using an Ohm's law plot at a given voltage.

Efficiency is inversely proportional to the solute's diffusion coefficient, and since small solutes have higher diffusion coefficients than larger solutes, of the same charge,

they yield peaks with lower efficiencies. Solutes with high electrophoretic mobilities in the same direction as the electroosmotic flow give peaks with high efficiencies. Thus, small, highly charged cations will give higher efficiencies than larger, monovalent anions.

1.2.3.4. Selectivity

The selectivity is measured based on the distance between the apices of adjacent peaks in an electropherogram when they pass through the detector. Selectivity ,α, is given by the following equation:

$$\alpha = \frac{\mu_1 \cdot \text{constant}}{\mu_2} \qquad (1.16)$$

where μ_1 and μ_2 are the electrophoretic mobilities of the solutes.

Eq. (1.16), shows the selectivity relies on differences in electrophoretic mobilities of solute ions. This also indicates that the most effective way to change the selectivity of the solute ions is by changing the pH of the buffer. Any alteration of the buffer pH will also change the EOF. For instance, adequate resolution may be obtained at a low pH, but if the higher pH is used to alter a solute charge, this will increased the EOF so that solutes may elute before resolution is achieved. Therefore it would be necessary either to increase the effective capillary length or reduce the EOF. In some cases changing of pH may affect the elution order of the analyte components.

1.2.3.5. Resolution

Resolution is defined as how far apart adjacent peaks are. There should therefore be a baseline separation between the peaks and no coelution or overlap of the tail of one peak with the leading edge of the next peak. Chromatographic peaks approximate to a Gaussian distribution so it is difficult to determine the minimum separation, in terms of retention times, achieving baseline resolution. Ideally, adjacent peaks (*A* and *B*) should be separated by 6σ ($3\sigma_A + 3\sigma_B$), that is, there would be <0.3% overlap since ± 3σ includes 99.7% of a Gaussian distribution. In practice, resolution (R_S) is assessed by comparing the difference in retention time for A and B with the half widths of the peaks since 95.5% of a Gaussian distribution is contained within ± 2 σ, therefore for a 2.3% overlap separation should be at least $2\sigma_A + 2\sigma_B$ or $4\sigma_B$, peak width of the broader peak (Fig. 1.4). Resolution, R_S, can be measured by the following equation:

$$R_S = \frac{t_{RB} - t_{RA}}{2\sigma_B + 2\sigma_A} \approx \frac{\Delta t}{4\sigma_B} \qquad (1.17)$$

$$However, 2\sigma = \frac{1}{2} w_b = w_h, therefore$$

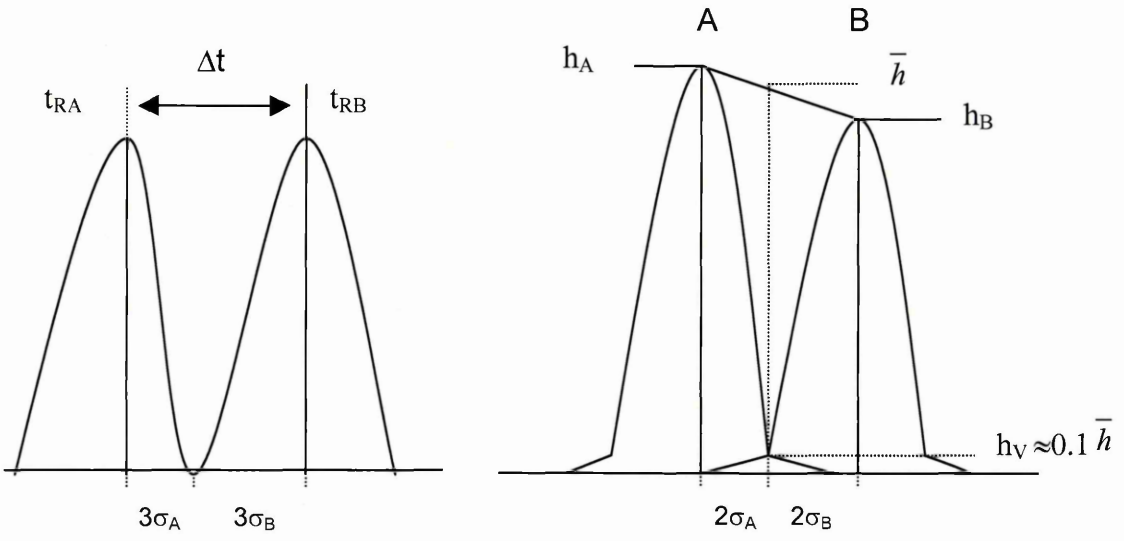
$$R_S = \frac{\Delta t}{\frac{1}{2}(w_{bB} + w_{bA})} = \frac{\Delta t}{(w_{bB} + w_{bA})} \approx \frac{2\Delta t}{2w_{bB}} = \frac{\Delta t}{w_{bB}} \qquad (1.18)$$

also

$$R_S = \frac{\Delta t}{w_{hB} + w_{hA}} \approx \frac{\Delta t}{2w_{hB}} \qquad (1.19)$$

where t is the migration time, w is baseline peak width (in time), and σ is temporal the standard deviation subscripts A and B refer to the two solutes.

Ideally, R_S should be between 1.2 and 1.5, corresponding to peak overlaps of approximately 1% (± 2.5σ) and 0.2% (± 3σ) (Fig. 1.4). R_S = 1.0 corresponds to a peak overlap of ~2.3% (± 2σ). R_S values of more than 1.5 or greater indicate too much separation which can lead to long analysis times and band broadening of the eluting peaks.



(Resolution, $R_S \approx 1.5$) (Resolution, $R_S \approx 1.0$)

Fig. 1.4 Schematic electropherogram of resolution equation

Resolution between two adjacent peaks can also be expressed as

$$R = \frac{1}{4}(N)^{\frac{1}{2}}\left(\frac{\Delta\nu}{\nu_{AVE}}\right) \qquad (1.20)$$

where $\Delta\nu$ is the velocity difference between two solutes and Δ_{AVE} is their average velocity. The term $\Delta\nu/\Delta_{AVE}$ is the relative velocity difference and is given by

$$\frac{\Delta\nu}{\nu_{AVE}} = \frac{(\mu_2 - \mu_1)}{(\mu_{AVE} - \mu_{EOF})} \qquad (1.21)$$

where $\Delta\nu$ is the average electrophoretic mobility of the solutes. Substituting Eqs. (1.15) and (1.21) into Eq. (1.20) gives

Jorgenson and Lukacs (46) derived an equation below to calculate the resolution, R, from the electrophoretic parameters :

$$R = \frac{1}{4}\left[(\mu_{EP} + \mu_{EOF})\frac{V}{2D}\right]^{\frac{1}{2}}[(\mu_2 - \mu_1)/(\mu_{AVE} + \mu_{EOF})] \qquad (1.22)$$

$$R = 0.177(\mu_2 - \mu_1)\{V/[(\mu_{AVE} + \mu_{EOF})D]\}^{1/2} \qquad (1.23)$$

where μ_{AVE} is average of the electrophoretic mobility, D is diffusion coefficient, μ_{EOF} is the electroosmotic mobility, μ_1 and μ_2 are the electrophoretic mobilities of the adjacent solutes and V is the applied voltage.

Eq. (1.23), shows that resolution is proportional to the square root of the voltage. Increasing the voltage will improve the resolution but not as much the efficiency which is directly proportional to the voltage. However, if a high voltage (as determined by a linear Ohm's law plot) is used it will give the fastest separation and hence will increase efficiencies and the resolution. Usually, the efficiency increases at a high electroosmotic flow. Differences in the electrophoretic mobilities of the solutes, lead to resolution and the greater the difference the better the resolution. In

CE, differences in electrophoretic mobilities may be obtained by the alteration of buffer pH or by employing an organic solvent in the buffer solution.

1.3. Overview of CE instruments

1.3.1. The main system of CE

The main components of a CE system are a sample vial, buffer containing source and destination vials, a capillary, a detector, a high-voltage power supply, and a data output and handling device such as an integrator or computer (Fig. 1.4). The ends of the fused silica capillary column dip into the vials each containing the two electrolyte buffer solutions. Platinum electrodes are placed in the electrolyte vials across which a high voltage (up to 30 kV) is applied. A small volume of sample solution is introduced at the anodic end of the capillary and the sample components subsequently migrate through the column under the force of the applied voltage. The separated analytes are detected on-column by one of a variety of methods e.g. UV/Vis, conductivity. The detector is usually situated at the end of the cathodic capillary i.e. prior to the outlet vial. Detected signals are displayed as peaks on an electropherogram.

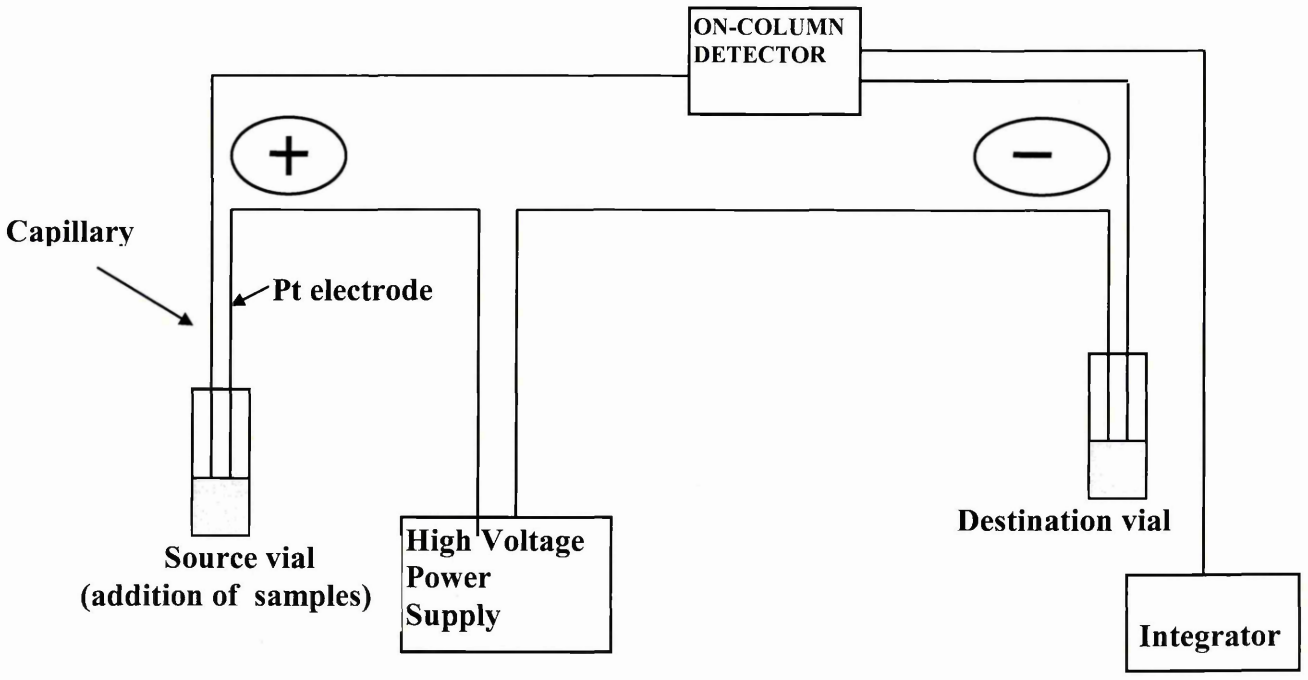


Fig. 1.4. Schematic of capillary zone electrophoresis system

1.3.2. Sample loading

Most systems allow the user to inject the sample in a number of ways, depending on the type of CE being performed. These are either electrokinetic (electromigration) or hydrodynamic injections (hydrostatic).

1.3.2.1. Electrokinetic injection

This offers the simplest method of loading a sample onto the column. A high voltage is usually applied for a short period while the capillary inlet is immersed in the sample solution. Sample ions migrate into the capillary according to their electrophoretic mobilities and electroosmotic flow.

The quantity injected, Q_{inj} can be represented by the following equation:

$$Q_{inj} = \frac{V \pi c t r^2 (\mu_{EP} + \mu_{EOF})}{L} \qquad (1.19)$$

where V is the voltage, c is the sample concentration, t is the time duration the voltage is applied, r is the capillary radius, μ_{EP} is the electrophoretic mobility of the solute, μ_{EOF} is the electroosmotic mobility and L is the total capillary length.

In electrokinetic injection, sampling bias is a very common problem especially when the samples are dissolved in different solvent or buffer composition. The problem may arise when repetitive injections are performed from the same sample vials. This will deplete a sample especially when a small volume of a very dilute solution is used.

1.3.2.2. Hydrodynamic injection

In hydrodynamic injection, sample ions are loaded into the capillary by creating a pressure differential while one end of the capillary is immersed in the sample solution. Various forms of displacement are available: A sample can be injected by applying pressure with an inert gas to the sample vial (pressure injection), by applying a vacuum to the capillary outlet (vacuum injection), or by changing the relative heights of the sample and outlet buffer vials (gravity injection).

In pressure injections, the volume of sample injected, V_i can be represented by the following equation:

$$V_i = \frac{\Delta P r^4 \pi t}{8 \eta L} \qquad (1.20)$$

where ΔP is the pressure across the capillary, r is the capillary inner diameter, t is the time the pressure is applied, η is the viscosity of the sample solution, and L is the total capillary length.

The length of sample plug, L_p can be calculated by the following equation:

$$L_p = \frac{V_i}{\pi r^2} = \frac{\Delta P r^2 t}{8 \eta L} \qquad (1.21)$$

where r is the capillary radius, V_i is the volume of sample injected, ΔP is the pressure across the capillary, t is the time the pressure is applied, η is the viscosity of the sample solution and L is the total capillary length.

Gravity injections is given by the following equation:

$$\Delta P = \rho g \Delta H \qquad (1.22)$$

where ΔP is the pressure across the capillary, ρ is the density of the buffer in the capillary, g is the gravitional constant and ΔH is the difference in heights of the liquid in the sample and the destination vials

If the liquid level in the source and destination vials is uneven during the injection, it may cause the siphoning of sample or buffer. This may affect a slight variation in injection volume and will lead to nonreproducibility in peak area and peak height. The injection time also must be as short as possible to avoid any siphoning effects. To ensure good reproducibility, the pressure(or vacuum) applied should be constant because the injection volume is reliant on pressure as shown in equation (1.20). Any changes in temperature also will affect the reproducibility because the injection volume is dependent on the viscosity of sample solution. This effect can be observed when the sample is taken from a refrigerator and the repetitive injections are made at room temperatures. As a result the volume of sample injected will increase as the sample warms, until the temperature becomes stable.

1.3.3. Capillary for UV/Vis detection

Various type of capillaries have been used including Teflon (48), Pyrex (48,49), silica (49) and PTFE (50,51). Of these, fused silica capillaries are most commonly used for applications in CE. The outer layer of this capillary is coated with a polyimide to make it stronger because the silica itself is easily broken. In order to create an optically transparent window for detection via UV/Vis, a small section of this polyimide coat must be removed. This is achieved by applying heat to a small area of the outer layer of

the capillary (This step is discussed further in Chapter 3 for the preparation of the capillary). The simple diagram of fused silica capillary can be shown in Fig. 1.5.

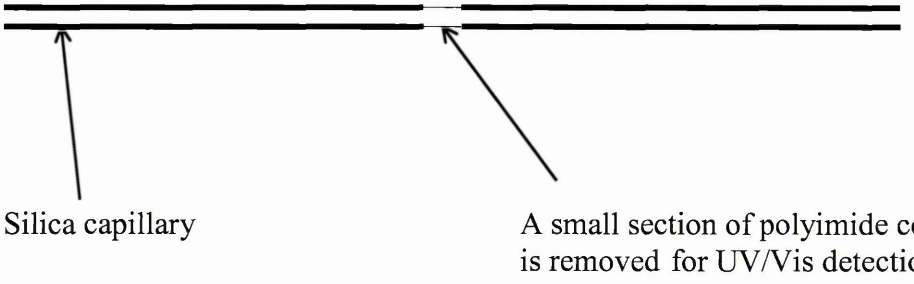


Fig. 1.5. The diagram of fused silica capillary with a small window

The bore of the capillary ranges from 10 to 100 μm but the most widely used are typically 50μm or 75μm I.D. The larger the inner diameter of the capillary, the more heat is generated because the temperature increases is proportion to the square of the inner radius of the capillary. The large inner diameter of the capillary will be of more benefit for UV/Vis detection because the optical path length is longer but may result poorer peak separations. However, the smaller the inner diameter the better the effective heat dissipation therefore it is most usual to employ inner diameters of between 50 to 100μm I.D. The effective capillary length also will affect the migration times and peak spacing, these increasing with increasing capillary length. The migration times are proportional to capillary length and resolution is proportional to $(l/L)^{1/2}$ where l is the effective capillary length and L is the total capillary length.

1.3.4 End-capillary for conductivity cell detection

Conductivity detection in CE has recently become available in a commercial CE instrument. The new conductivity cell is based on an end-capillary concept. The conductivity sensor and the detection end of the fused-silica capillary are permanently encapsulated in two individually modified coupling connectors (ConTip and ConCap that employed for conductivity cell detection were manufactured by Orion research, Boston, MA, U.S.A). This open-architecture cell permits interchangeablility of sensors and capillaries, while maintaining a precisely defined detection volume between those two components when inserted into the detector cell block. The detector's performance is evaluated for sensitivity, linearity, and reproducibility using low-mobility electrolytes. Electropherograms comprising a variety of ionic class separations including inorganic and organic anions, organic surfactants, alkali metals, alkaline earths, transition metals, and organic amines are shown along with separations of actual samples. The conductivity cell of the detector is a function of the ConTip sensor and the ConCap connected together. The ConTip sensor is unique in that both electrodes are incorporated on the same surface of the sensor. Figure 1.6 shows a close view of the ConTip detection surface.

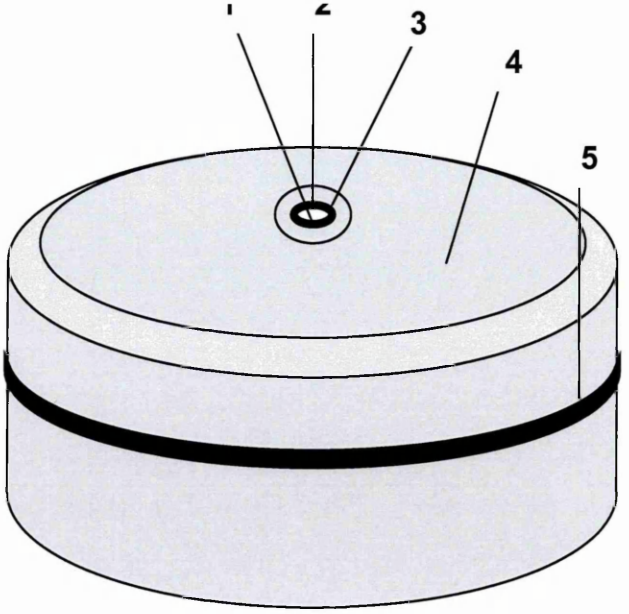


Fig. 1.6 Perspective view of the sensor half of the detector cell (ConTip). From schematic diagram; 1= Pt center electrode, 2=polyimide insulation, 3= electrode spacer, 4=S.S. second electrode and 5= O-ring.

The ConCap fused silica capillary is designed to be used inconjunction with conductivity detection. The ConCap sensor is placed at the end of the capillary and is protected with a blue plastic cover which is removed prior to installation. The ConCap portion of the detector is based on the same basis sensor geometry as the ConTip, but houses the fuse silica capillary (Fig. 1.7) .

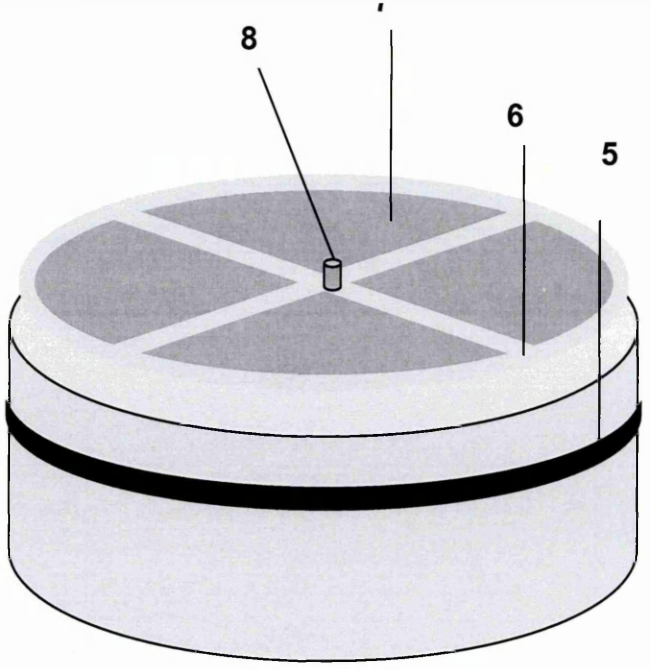


Fig. 1.7 Perspective view of the sensor half of the detector cell (ConCap). From schematic diagram; 5= O-ring, 6=Cross-channel groove, 7= Contact surface with CinTip and 8=Fused silica capillary.

The contact surface has two grooves cut into it. These grooves permit rapid flushing with electrolyte for replenishment of electrolyte, removal of capillary conditioning solutions and or bubbles. The cross channel configuration also eliminates the need for a special radial orientation with respect to the fluidic connections. Key for efficient separations is the spacing of the capillary from the ConTip sensor. This spacing defines the detection cell volume and the efficiency of the separation. The closer the capillary tip is spaced from the ConTip sensor the smaller the cell volume and the higher the cell constant. Higher cell constants translates to lower net conductivity readings and lower detection responses. The inset spacing of the capillary tip in ConCap I from ConTip I sensor is 24.1 ± 1.2 microns. This spacing was experimentally determined as the optimum distance for minimal bandspreading of peaks for efficient separations.

Therefore, the ConTip and the ConCap (Fig 1.8) must be properly connected such that they are in firm contact with each other. If the ConTip and ConCap are not in firm contact with each other then the conductivity reading will read higher than usual due to the increased cell volume and decreased cell constant. The result will be loss of efficiency due increased fronting and tailing.

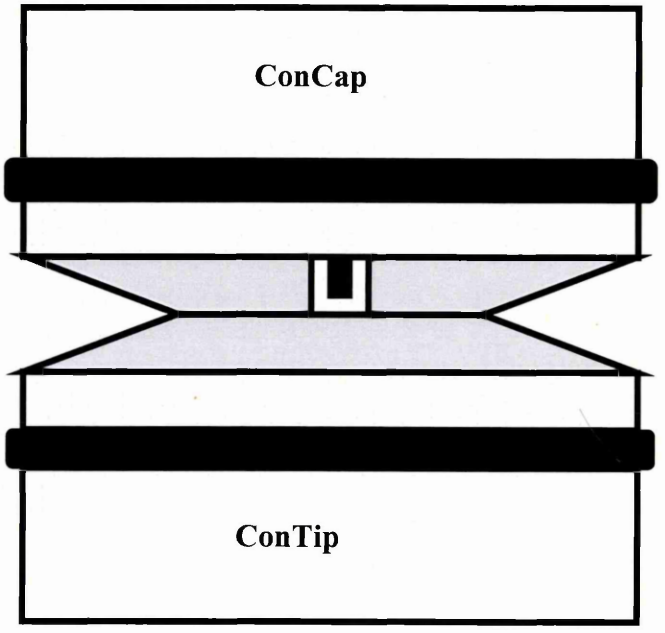


Fig. 1.8 Sequential close-ups of the detector cell.

1.3.5. Detectors

A variety detectors may be employed including UV/Vis, fluorescence, laser-induced fluorescence, mass spectrometry, conductivity, amperometric, radiometric and refractive index. The most commonly used is UV/Vis absorbance and can be employed in a wide range of applications. The conductivity and UV/Vis detectors are discussed further in section 1.4.

1.3.6. Power supply

The power supply is used to provide an electric field across the capillary. It must be able to operate in either constant voltage, constant current, or constant power mode. The power supply should also include features to enable the polarity to be reversed. Applied voltages up to 30 kV, currents up to 300μm, and power up to 6W are generally used. For the normal application of CE, the constant voltage mode of operation is widely used. The ability of power supply to provide a constant voltage is very important to ensure the migration times are constant throughout the run.

1.3.7. CE instruments

Commercial instruments became available in the mid-1980s and a selection of these are listed in Table 1.2 (52). They offer different options and features to suite with a wide variety of applications. Most of the systems are compatible with stripchart recorders, integrators, or PC-based data acquisition systems.

Product	Manufacturers
Prince CE 300 Series	Prince Technologies.
P/ACE 5000 Series	Beckman, USA.
HP 3D	Hewlett Packard, USA.
SpectraPhoresis Series	Thermo Separation Products, USA.

Table 1.2. Summary of representative products

1.4. Detection methods in CE

There are many types of detector employed in CE including UV/Vis (direct and indirect), refractometry, electrochemical (conductivity, amperometry and potentiometry) and mass spectrometry. Emphasis will be on the use of direct and indirect UV/Vis and conductivity detection methods as applied to metal cations. Other detection methods for the separation of metals will be discussed in Chapter 2.

1.4.1. UV/Vis detection

1.4.1.1. Introduction

The detection method is based on a drop in the absorbance of UV or light as it passes through the detector. This process can be represented as in Fig. 1.9. The intensity of light, I_{In} from the original light source will be reduced after passing through the capillary window. The sample will absorb some of the light which causes reduction in intensity, I_{Out} and will be recorded by a photodetector, usually a photodiode.

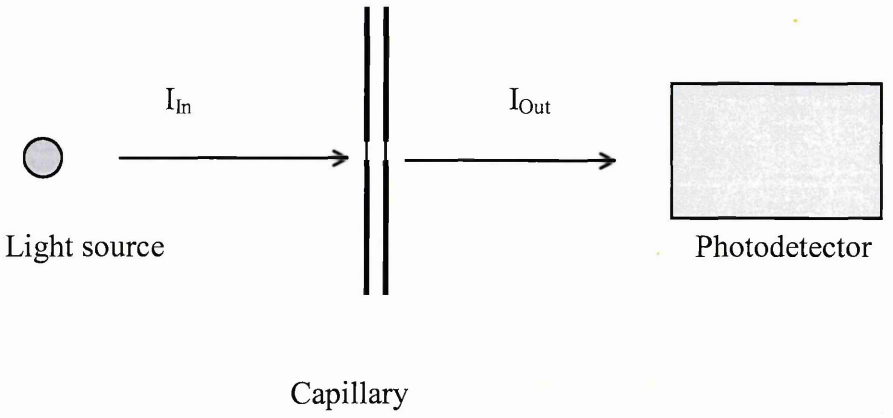


Fig. 1.9. Schematic diagram of the light path in UV/Vis detection.

Transmittance, T, is given by the following equation:

$$T = \frac{I_{out}}{I_{in}} \qquad (1.23)$$

where I_{out} is the intensity of light after passing through the detector and I_{in} is the original light source

UV/Vis absorbance relies on Beer's law where absorbance, A, is represented by the following equation

$$A = log\ 1/T = abc \qquad (1.24)$$

where T is transmittance, a is absorptivity, b is path length in sample compartment in cm (the capillary I.D.) and c is solute concentration.

When the unit of concentration is molarity, *a* is referred to as molar absorptivity and is abbreviated with the symbol, ε, and has units of $L.mol^{-1}.cm^{-1}$. Absorptivity depends on the chromophore of the solute, the wavelength of incident light, the pH and composition of running buffer. The photodetector measures light intensities, and the electronic detector converts this to absorbance using Eq. 1.23 and 1.24. Based on the above equation (Beer's law), absorbance is directly proportional to path length, which is equal to the capillary's inner diameter. Therefore, the concentration sensitivity of UV/Vis detection is relatively poor.

1.4.1.2. Direct UV/Vis detection

Direct method detection is not so sensitive for determination of metal cations. The reason is that many of these metal compounds lack chromophores at useful wavelengths or have such low molar absorptivities as to preclude adequate sensitivity with absorption detection. In CE the interactions of the cations with the surfaces of the fused-silica capillaries can result in band broadening, which reduces both detectability and resolution of analytes. However, direct detection techniques have been developed and are reviewed in several journals (40-47). They employ a chromogenic reagent which is used to complex with the metal cation. As a result, a stable complex with high molar absorptivity in the visible and/or UV regions is formed for a wide variety of metal cations. Very sensitive detection can be obtained for various type of samples. The review of direct detection UV/Vis is discussed further in Chapter 2.

For direct UV/Vis there are some advantages for the analysis of metal cations. First, certain matrix interferences can be identified and eliminated during the

precomplexation stage. Second, the selectivity can be enhanced by differences in the formation of metal complexes.

1.4.1.3. Indirect UV/Vis detection

This method is usually employed for those sample ions that do not absorb enough light to be detected by UV/Vis. In order to create a high background signal for detection, a chromophoric reagent which is a light-absorbing compounds is usually used. The employing of a background UV-absorber overcomes the lack of a chromophore for many inorganic compounds particularly for metal cations. Many the aromatic bases have been found to be useful chromophores e.g. Imidazole, benzylamine, ephedrine and pyridine. A decrease in absorption is measured.

Indirect UV/Vis techniques offer several advantages for the investigation of analytes by CE. Indirect detection is universal, and can be used for compounds which lack chromophores. The applications of this method are discussed further in Chapter 2.

1.4.2. Conductivity detection

1.4.2.1. Introduction

The basic principle of conductivity detection is based on a change in electrical conductivity of a solution when positive or negative ions enter an electric field. The conductance can be defined by electrical properties such as ratio of current to voltage between two points within a liquid. A conductivity detector has two electrodes in the cell, and a high-frequency alternating potential is applied to the electrodes. When the buffer or ionic solutes flow between two electrodes and reach the cell, it will decrease the electric resistance of the solution, thus increasing the electrical conductance. The

conductance can be detected if the cell has electrodes of 1 cm^2 surface area that are 1 cm apart. A conductivity detector measures conductance, which is then corrected by the cell constant to give conductivity. Since conductivity values will vary as the distance between the points vary, a standard measure of conductivity called the specific conductivity is used for analytical purposes. The unit of conductivity are siemens (S).

Conductivity of an ion in a dilute solution, κ in S/cm, is proportional to concentration as given by the following equation:

$$\kappa = \Sigma_i \lambda_i^0 C_i/1000 \qquad (1.25)$$

where C_i is the concentration of ions in equivalents/L. Equivalents equals the number of moles of the ion times the charge on the ion. λ_i^0 is the ionic limiting equivalent conductivity, in $S.cm^2$/equivalent, and is specific for a given ion.

In general, the background noise from a conductivity detector is proportional to the background signal. Conductivity is proportional to concentration as mentioned in Eq. (1.25), so the more concentrated the run buffer, the higher the background noise, which makes it difficult to detect trace level of solute ions. In some applications, suppressed conductivity may employed to overcome this problem.

1.4.2.2. Direct conductivity detection

Direct detection is used when the background electrolyte conductivity is low when compared to sample ions. This methods is more suitable for the analysis of small inorganic and organic ions. The applications of this method are discussed in Chapter 2.

1.4.2.3. Indirect conductivity detection

Indirect detection is used when the conductivity of sample ions is low and a high conductivity buffer is used. Usually the separation will appeared as negative peaks and must be inverted by the detector. This method is suitable for sensitive detection of larger ions with low ionic mobilities. The applications of this method are discussed in Chapter 2.

1.5. Aims and objectives of this work

The aim of this project was to undertake analysis of metal cations using CE techniques. In particular, group IA, group IIA and those transition metals associated with industrial pollution. Development and the subsequent evaluation of methods employing CE was the prime objective of the research.

Initial experiments were limited to a number of mono- and di-valent metal cations:- sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca). A larger group including transition metals was used for verifying the separation capability of the techniques. Optimisation of the background electrolyte was required to enable separation of the metal cations. In addition, the presence of a complexing reagent was often necessary to obtain full separation and the type and concentration of such species was investigated. The effect of other factors such as pH, ionic strength and viscosity was also studied .

Evaluation of detection methods i.e. indirect UV/Vis and conductivity was undertaken. Several type of samples were investigated including river water and mineral water to make sure the techniques of CE can be applied for the real samples. The optimised methods were employed to determine the metal content of acid mine discharges.

CHAPTER 2

SEPARATION OF METALS BY CAPILLARY ELECTROPHORESIS

2.1. Introduction

Separation in CE relies on the differential migration of charged analytes, the migration rates being influenced by their charge density. In the application of CE to the analysis of metal cations, particularly for the transition metals and lanthanide series, problems arise in obtaining adequate separation. This is because of the nearly identical charge to hydrated ionic radii ratio for many of their ions which leads to similar ionic mobilities. Much of the literature reviewed in this chapter will focus on the methods of partial and full complexation in order to enhance the separation of metal cations.

2.2. The optimisation of the separation of metal cations

In CE, free uncomplexed metal ions will migrate based on the differences in the electrophoretic mobilities of their hydrated ions. These differences are only sufficient for separation of those metals from group I and group II. The migration differences of these metals in a carrier electrolyte strictly depend on their charge-to-size parameters. For cations with a similar charge, the hydrated radius increases proportionally to the charge density of the metal ion. Usually smaller ions are more strongly hydrated when compared to larger ions and this has the affect of lowering their electrophoretic mobility (μ_{EP}). The separation of such metal cations is often not possible based only on migration behaviour without any modification or alteration of chemical equilibrium

of buffer composition. There are many ways to enhance the separation of metal cations in CE:

2.2.1. Partial complexation with organic acids

The mobilities of inorganic cations can be selectively moderated by complexation within the capillary, i.e. by the formation of metal complexes of differing stability and (therefore providing effective) differing charge to mass ratios. Free metal cations are expected to migrate toward the negative electrode in the same direction as the electroosmotic flow.

When the cations migrate in the same direction as the electroosmotic flow (i.e. toward the detector) this is termed co-electroosmotic migration. Co-electroosmotic migration mode, when used for the separation of alkali and alkaline earth metals, will provide rapid and efficient separation based on differences in electrophoretic mobilities between cation analytes.

The transition metal ions and the lanthanides series have similar electrophoretic mobilities, and they are difficult to separate relying only on migration behaviour. In this case partial complexation can be introduced as an additional separation mechanism. Jackson and Haddad (53) stated that the separation, in CE, exists because of different electrophoretic mobilities of analytes in the electrolyte buffer under the influence of electric field. If the electrophoretic mobilities of analytes are similar, a reagent which partially complexes with sample ions must be added to the buffer to increase differences in effective mobilities and thus enable resolution of analytes.

Introduction of a weak complexing reagent into the carrier electrolyte will convert a fraction of metal ions into the complex species which has a bigger size compared to the uncomplexed metal ion. This complexed metal ion will move slower than the free

cation, which migrated at the rate proportional to its ionic mobility. Generally reducing the characteristic of charge density will influence the differences in mobility of ion complexes for cation analytes. The separation may also depend on the concentration and pH of reagent.

Complex-formation can be represented by the equilibrium equation as follows:

$$M^{n+} + mL^{-} \Leftrightarrow M_m^{(n-m)+} \qquad (2.1)$$

where L^- is a monovalent, monodentate ligand and M is metal ion.

At present most of the successful separations of metal cations have involved the use of a weak organic acid as the complexing reagent. Those complexing reagents used have either been monoprotic acids e.g. acetic acid, α-hydroxy isobutyric acid (HIBA), glycolic acid and lactic acid), diprotic acids (e.g. oxalic acid, malonic acid, succinic acid, malic acid and tartaric acid) or triprotic acids (e.g. citric acid). This technique was first employed by Foret et al. (54) and then by Jandik and others (22-24).

Among those complexing reagents employed to date, HIBA (22-24,26,28-30,54-64) and lactic acid (29,30,58-62,65,66) or citric acid (23,58) followed by tartaric (29,58,65,68) and oxalic acids (58,67) appear to offer the most satisfactory separations. Most of these complexing reagents enable a complete separation to be obtained in a very short time and with high selectivity. The fastest separation so far reported was of

27 metal ions (including alkali, alkaline earths, transition and lanthanide metals) in only 6 min. This was achieved by employing 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine and 5% methanol at pH 4.25 (65). In a later development, Chen and Cassidy (28) obtained a good separation of 26 metal ions in 10 minutes employing 4.2 mmol/L HIBA, 6 mmol/L *N,N*-dimethylbenzylamine, 0.2 mmol/L Triton X-100 at pH 5.0. Most of the metals separated under this investigation included alkali, alkaline earth, transition metals and lanthanides. Other complexing reagents used have been phthalic acid (29,58) or acetic acid (26,58,67,70) in the separation of alkali and alkaline earth and transition metals.

Incomplete separation due to lack of complexing ability can be improved by employing two ligands which have different abilities for complexation e.g. HIBA and acetate (69). A combination which consisted of 1 mmol/L oxalic and 100 mmol/L acetic acid as the complexing reagent was employed in the separation of alkali, alkaline-earth and transition metals (67). These two complexing reagents have different abilities to form a stable complex with polyvalent cation metals. In this experiment, employing indirect conductivity detection, a separation of 16 metals in only 7 minutes was obtained. Alkali and alkaline earth metals may be more efficiently separated using succinic acid (57,58) or malonic acid (58) while glycolic acid gave a higher selectivity for a mixture of alkali, alkaline-earth and transition metals (57,58).

2.2.2. Complexation with macrocyclic ligands

The separation of alkali and alkaline-earth metal cations may be improved by using a suitable macrocyclic polyether. Crown ethers are an example of such compounds and when present in the buffer will complex with the metal. The structure of 18-crown-6 is

shown in Fig. 2.1. Metal-crown ether complexes rely on the inclusion of the metal cation into the crown ether cavity to form the stable complex. This complex formation can be specific e.g. 18-crown-6 will complex with potassium, barium and strontium in the presence of ammonium. Separation is based on the degree of complexation and therefore the differences in the mobility of the complexed species. As the ligands are neutral pH changes of the buffer have no effect. Concentration of the crown ether in the buffer is kept low (between 2 and 5 mmol/L) to ensure differences in the effective mobilities of the metal-crown ether complexes.

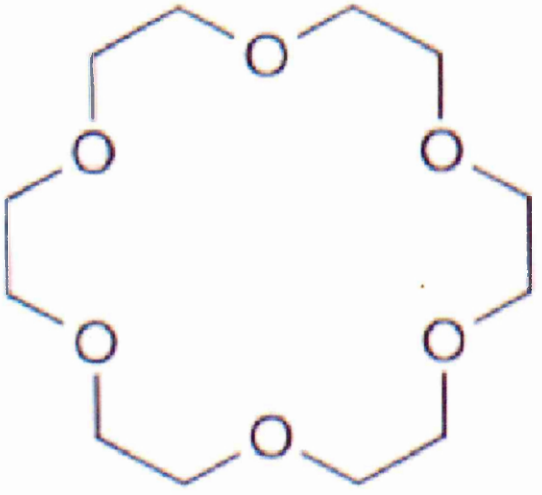


Fig. 2.1. Structure of 18-crown-6

The introduction of a macrocyclic polyether e.g. 18-crown-6 was proposed by Bachmann et al. (35). This was employed to obtain better separations for metals with similar ionic mobilities. Addition of 18-crown-6 into the buffer changes the electrophoretic mobilities especially for divalent and monovalent metal ions e.g. the separation of potassium and barium from ammonium may be achieved. Riviello et al.

(71) employed a buffer composition of 4.0 mmol/L copper (II) sulphate, 4 mmol/L formic acid and 4.0 mmol/L 18-crown-6 at pH 3 to separate alkali and alkaline earth metals. These conditions enabled the separation of potassium from ammonium and calcium from strontium.

The application of metal-crown complexation is widely used and has been reviewed (66,72-74). For most of them the crown ether is used to separate ammonium from potassium. Other macrocyclic compounds may be used in CE separation of metal cations include cryptands, e.g., cryptand-22 (75). This macrocyclic ligand is used become of its remarkable cation complexing ability.

2.2.3. Solvation

The separation of free metal cations, whose mobilities are similar can be improved by employing an organic modifier added to the carrier electrolyte. The introduction of an organic solvent into the buffer composition will affect the charge density of metal cations and also the overall viscosity of the aqueous mixture. These effects may cause an increase in the migration time and thus improve the resolution of the separation.

Solvation effects will result in different behaviour especially for divalent and monovalent metal cations. Increasing the amount of organic modifier decreases the mobilities of divalent metal cations compared to monovalent cations. This effect is due to an increase in the degree hydration of divalent metal cations compared to monovalent metal cations with the presence of an organic modifier. Examples of this are the addition of acetone and ethylene glycol. Much of the literature reports that higher concentrations of organic solvents, usually methanol (30,62,65,76-78) or acetonitrile, in the electrolyte buffer affords an improvement in the separation and resolution of analyte compounds. Polyethylene glycols (PEG) are able to offer some

advantages for increasing the selectivity in metal cation separations by CE. This compound will enhance the differences in effective mobility due to the formation of metal-PEG complexes. This type of complexation is not as specific when compared to crown ethers. The application of polyethylene glycol was reported by Stathakis and Cassidy (79). They showed that polyethylene glycol enhanced differences in the effective mobilities. This depended on the nature of the metal ions with the polyvalent metal ions forming stronger complexes when compared to smaller monovalent cations.

2.2.4. Ion pairing

This technique contributes minor changes in metal selectivity for non-complexed metal ions. In principle, the method is based on the interaction of a positively charged ions with a negative ion-pairing reagent. An example of this can be seen from the use of sodium dodecyl sulphate (SDS) to delay migration of a group of alkaline-earth and transition metals. This method was employed by Buchberger et al. to separate strontium from barium (80). The non-ionic surfactant, Triton X-100 strongly reduces the electroosmotic flow velocity and can be used for modifying the resolution of partially complexed metal ions.

2.2.5. Full complexation

An alternative technique in CE for the separation of metal ions is the complete transformation into stable complexes prior to separation taking place. The complexes formed may have different mobilities and, depending on the reagent, a different charge state. Several migration modes can be selected depending on the type of metal ion complex, i.e. cationic metal complexes, neutral metal complexes or anionic metal complexes. In order to form a stable, strong metal complex, a suitable chelating reagent must be chosen. The chosen reagent should contain at least one ionisable

group, e.g. sulphate, carboxylate or a quarternary ammonium group, which does not participate in the complexation.

An early application using preformed metal complexes was published by Yotsuyanagi et al. in 1989 (81). They showed the separation of transition metal ions in the form of 4-(2-pyridylazo)resorcinol (PAR) complexes using micellar CE. Two years later, Motimizu and coauthors separated a mixture of alkaline earth and transition metals complexed with 1,2-cyclohexanediaminetetracetic acid (CDTA) in free solution CE (82). Other reports (83-85) mention the use of CDTA as the complexing reagent in the separation of metal cations. The employing of monodentate inorganic ligands e.g. cyanide (36,86) or chloride (87-90) has been applied in several investigations (86,91) but they are less useful due to difficulties in controlling the stability of the complexation conditions. In certain cases, MEKC may be employed in conjunction with complexing ligands especially for neutral metal chelates. These applications are based on the partition of neutral solutes between the aqueous and micellar phases (92,93). The utilisation of preformed metal complexes can be discussed according to the charge of the metal complex:

2.2.5.1. Anionic metal complex

For negatively charged complexes, the electrophoretic mobility of the analyte is directed towards the positive electrode against the electroosmotic flow (EOF). Only the highly mobile complexes with a large charge and/or small size can be successfully detected. In certain cases where the size of organic ligand is large, the migrating anionic metal complexes will move slowly toward the anode. These metals complexes may not be detected or have long migration times (to the cathode) because the electrophoretic mobilities of the solutes are unable to overcome the EOF. As an

example, metal cyanides (36,94) exceed the speed of the EOF and may be detected at reasonable migration times. Where the size of organic ligands is large, the migration velocity of the ionic complexes will be insufficient to overcome the EOF. This will lead to long migration times. Reversal of polarity and/or reduction of the EOF by treatment of the capillary wall with a cationic surfactant may overcome this problem. In 1992, Timerbaev et al. (15) employed tetraalkylammonium salts during a pre-complexation before the separation of anionic metal complexes in the CE column. This will reverse the electroosmotic flow and therefore enable detection of anionic metal complexes. In a later development Timerbaev et al. (83) then reversed the polarity and the electroosmotic flow to enable the fast separation of anionic metal complexes.

Timerbaev et al. (15) employed anionic metal complexes of 8-hydroxyquinoline-5-sulfonic acid (HQS) to obtain a separation of Zn (II) in a sample of tap water in 1992. The buffer used consisted of 10 mmol/L Borate and 0.1 mmol/L HQS. Later Regan et al. (95) used anionic metal complexes with PAR to separate Fe(II) from Zn(II) in pond water and in vitamin tablets. They employed 10 mmol/L TAPS [N-tris(hydroxymethyl)methyl-3-aminopropane sulfonic acid] and 0.1 mmol/L PAR. In 1995, Baraj et al. (96) were able to separate Cr(III) in waste water involving an anionic metal complex with EDTA. The buffer employed consisted of 100 mmol/L sodium acetate and 0.1 mmol/L TTAB (tetradecyltrimethyl ammonium bromide). In 1996, Semenove et al. (97) and Timerbaev et al. (98) employed anionic metal complexes with CDTA to obtain the separation of Cr(III), Cr(VI), Cu(II), Mn(II) and Zn(II) in a chromium plating solution, rinse water from a plating bath and pharmaceutical samples. For the plating bath solution they employed 10 mmol/L formate buffer and 1 mmol/L

CDTA. Meanwhile for pharmaceutical samples Timerbaev et al. used 10 mmol/L Borate buffer and 1 mmol/L CDTA.

The separation of anionic metal complexes can also be obtained by employing the micellar electrokinetic chromatography (MEKC) technique. This method relies on two mechanisms, micellar solubilisation and electrokinetic migration under MEKC conditions. Usually a micellar solution i.e. sodium dodecyl sulphate (SDS) is employed with an anionic metal complex of 4-(2-pyrydylazo)resorcinol (PAR) to obtain a separation of Cr(III), Co(II), Cu(II), Pb(II), Ni(II), Fe(II), Zn(II), Fe(III) and Cd(II) (37). Meanwhile in another development Arsenazo III [disodium salt of 2,7-bis(2-arsono-phenylazo)-1,8-dihydroxynapthalenedisulphonic acid] was used as a chelating reagent by Timerbaev et al. (37) to obtain the separation of Ce(III), La(III), U(VI), Cu(II), Pb(II), Co(II) and Fe(II).

2.2.5.2. Cationic metal complex

In positively charged complexes, the electrophoretic mobility of the cationic metal complex is directed towards the cathodic electrode and migrates in the same direction as the electroosmotic flow (EOF). Therefore the separation of cationic metal complexes relies on coelectroosmotic migration, this offers an advantage in relation to analysis time. These separations are restricted to simple mixtures because of a narrow migration range and insufficient differences in mobilities. In certain cases the separation of cationic metal complexes may be poor due to short migration times. This is because the cationic metal complexes may require a long migration times to achieve a resolution owing to small differences in electrophoretic mobilities. In order to overcome this problem, a cation surfactant was employed to reverse the EOF and the

polarity was reversed. This enabled the obtaining of a better separation although the migration times are longer (99).

The separation of cationic metal complexes can also be obtained by employing the micellar electrokinetic chromatography (MEKC) technique. In this method the reversal of EOF was used and the polarity was reversed. Timerbaev et al. (99) employed cationic alkyltrimethyl ammonium surfactants in the electrophoretic solution to improve the resolution of positively charged metal chelates of 2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone). The gain in resolution observed with increasing the content of surfactant was considered by the authors as a result of co-operative affects of changes in the phase ratio (i.e. ratio of the volume of micellar phase to that of the bulk aqueous phase) and electroosmotic mobility (due to increasing viscosity and ionic strength). Due to this effect, the migration range of cationic metal complexes can be enlarged.

2.2.5.3. Neutral metal complex

MEKC may be applied to both electrically charged and to neutral metal complexes (92,93). The resolution of neutral metal complexes relies on the partition between the bulk aqueous and micellar phases. In 1996, Xu et al. (100) employed a neutral metal complex to enable the separation of Fe(III) in water (rain, tap and lake). They used thioglycolic acid as reduction reagent to convert Fe(III) to Fe(II). The buffer solution consisted of 50 mmol/L acetate and 1,10-phenanthroline was employed as a complexing reagent to obtain a separation of metal complexes.

2.3. Detection in CE

2.3.1. Introduction

In this section the emphasis will be on the application of detection methods used for metal separations. Primarily UV/Vis and conductivity detection have been employed in the separation of metal cations and will be discussed in detail. Other detection methods are briefly reviewed.

2.3.2. UV/Vis detection

2.3.2.1. Direct UV/Vis detection

This method was not employed much in the preliminary investigations of metal separation. The problem being due to a lack of sensitivity for direct detection by UV/Vis. Recently, researchers have employed chromogenic reagents to form pre-column complexes, either ionic or neutral, to enable direct UV/Vis detection. As a result, the sensitivity was increased and the method can be applied to a wide variety of samples. In 1991, Saitoh et al. (34) used a water-soluble porphine as a precapillary derivatising reagent to enable direct detection by UV/Vis. In this investigation, the separation of zinc at a concentration of about 8×10^{-5} mmol/L was obtained. The employing of cationic metal complexes formed with 8-hydroxyquinoline-5-sulfonic acid (HQS^-) to enable a separation of metal cations by CZE with direct UV/Vis detection has also been reported (15,83). In another development 4-(-pyridylazo)resorcinol (37,81,101), Arsenazo III (37), and several aminopolycarboxylic acids (81,102), have been employed for direct absorbance detection of alkaline earth, transition, post-transition, and lanthanide metal ions. Since the performance of the separation relies on the stability of metal chelates, the separation of metals is obtained without the addition of a free ligand to the carrier electrolyte. This ensures a lower

background signal and will increase the detection sensitivity for analytes. In some cases the complexing reagent was introduced directly into the electrolyte to create the absorbing species without preliminary complexation (complexing reagent was employed in electrolyte buffer). For example, visible absorbance detection of calcium with o-cresolphthalein complexone containing electrolyte (103), has been noted.

The main disadvantage is that the stability of metal complexes are usually not consistent before or during the process of the electrophoretic separation. This may cause difficulties in optimising the separation and detection conditions of the metal complexes. A second disadvantage is that multiple complexes may exist in solution leading to the appearance of multiple peaks, making it very difficult to distinguish the sample peaks. A third disadvantage is that the analytes are chemically altered and collection for further studies is almost impossible. A fourth disadvantage is that derivatisation is both time consuming and inefficient.

2.3.2.2. Indirect UV/Vis detection

Indirect UV/Vis absorbance detection is used to overcome the lack of a chromophore and is widely used in the separation of metal cations. The method is easy to perform and can be classified as a universal detection method.

An organic UV-absorber is added into the buffer solution. Aryl derivatives of aliphatic amines, which form cations when protonated are most commonly used, in particular 4-methylbenzylamine (22,29,30,23,56,57,59,60,64). The employing of 4-methylbenzylamine, with an organic acid as the complexing reagent enables the separation of most alkali, alkaline earth and transition metals. Imidazole can also be

used as a UV-absorber to enable the separation of alkali, alkaline earth and transition metals (57,58,62,63,70,104). Other UV-absorbers employed for indirect UV/Vis detection include benzyltrimethylammonium (28), ephedrine (57,61), 4-methyl-2-benzylamine (59), p-toluidine (29), benzylamine (26), polyethylene glycol (79), 4-methylaminophenol sulphate (known as metol) (105) and benzimidazole (68). In order to optimise the separation and detection efficiencies, it is important to choose a suitable combination of complexing reagent and UV-absorber. Some adjustments in the concentration of a UV-absorber reagents are very important to obtain optimal separation conditions. Concentrations of between 1 - 10 mmol/L of UV-absorber have been used in the separation of metal cations.

Inorganic UV-absorbers such as copper (II) salts can also be used in the electrolyte system (71,80). This method allowing the separation of alkali metals, alkaline-earth metals and ammonium in less than 5 minutes.

The optimisation of the detection efficiency by modification of both hardware and electrolyte components has been reported in several publications (26,28,64,106). These alterations include pH of electrolyte, concentration of complexing reagent and nature of the surface of the capillary. In this relation, Chen and Cassidy (26,28) employed bonded and unbonded capillaries to separate 26 metal ions, including alkali, alkaline earth, transition and lanthanide metal ions using different pH, complexing reagents and surfactants. Meanwhile in other development, Jandek et al. (64) studied the affects on the detection limits of inorganic metal cations by changing the detection wavelength, the mobility of electrolyte co-ion, the length of detection cell and the mode of injection.

2.3.3. Conductivity detection

The method is used for metal ions in CE but has a limited application. The detectability for metal ions such as transition metal and lanthanide series is not so sensitive and therefore requires further development. Conductivity detection was employed by Virtanen in 1974 (5). Later, Mikkers et al. (107) measured the potential differences between two electrodes but the sensitivity was low and this work was further developed by Jorgenson and Lukacs (7,8,108). In 1986, Bocek et al. (109) used a conductivity cell connected with a capillary. They obtained a level of detection at a concentration of about 2×10^{-2} mmol/L.

2.3.3.1. Direct detection

In earlier applications of conductivity detection for the separation of metal cations, direct detection methods was used whereby the conductance of the analyte components were greater than the conductance of the electrolyte buffer. The first metal separation using conductivity detection was demonstrated by Zare et al. (38). This separation has a limited application for a number of metal ions even in combination with ion exchange membrane suppressors. In these experiments they used MES/HIS to get a separation of Rb, K, Na and Li at concentrations of about 2×10^{-2} mmol/L. This detector required further development but was still unable to give a good separation. In 1991 Zare et al. (25) used potassium acetate as the electrolyte to separate alkali and alkaline earths. The separation of metals other than alkali and alkaline earth was not properly optimised. It has been reported that metal cations e.g. Cs, K, Ca, Na, Mg, Ba and Li can be separated using 30 mmol/L HIS/MES and 2 mmol/L of 8-crown-6 with direct conductivity detection (67).

2.3.3.2. Indirect detection

This method has been rarely used in the separation of metal cations. The method is classified as indirect detection when the conductance of analytes is low compared to the conductance of an electrolyte buffer. The most successful investigation of a wide range of metal cations was carried out by Harber et al. (67) in 1996 to separate alkali, alkaline earth and transition metals. Little has been reported since regarding the separation of metal cations using indirect conductivity detection particularly for transition metals.

2.3.3.3. Common problems in conductivity detection

Several problems arise during an investigation using indirect conductivity detection. First, it is advantageous to use a low ionic strength, poorly ionised buffer system to produce a low conductivity background. If good separation is desired, the concentration of background electrolyte must be high relative to the concentration of the sample. However this condition (high electrolyte concentration) results in a decrease in sensitivity due to elevated background conductivity. Second, background noise from the high voltage prevents high sensitivity detection. Third, a large difference between the mobility of the carrier electrolyte ion and that of the analyte ion leads to excessive peak tailing/fronting in CE. Fourth, cell design is difficult since there must be no significant voltage drop between the cell electrodes; small dimensions of the capillary, high ionic strengths and a high separation electric field are challenges for the use of conductivity detection in CZE.

2.3.4. Sensitivity enhancement and limit of detection in CE

In CE, the concentration sensitivity particularly in the case of absorbance-based detection is generally on the order of 10 to 100 fold less than that of HPLC. In addition, the short optical path length (about 50 μm capillary I.D.) typically results in concentration limits of detection (LODs) of the order of 10^{-6} mol/L. The approximate detection limits for various detectors are given in Table 2.1.

Detection method	**Concentration detection limits (mol/L[a])**
UV/Vis Absorption	10^{-5}-10^{-8}
Fluorescence	10^{-7}-10^{-9}
Laser-induced Fluorescence	10^{-14}-10^{-16}
Amperometry	10^{-10}-10^{-11}
Conductivity	10^{-7}-10^{-8}
Potentiometry	10^{-4}-10^{-7}
Refractometry	10^{-6}-10^{-8}
Mass spectrometry	10^{-8}-10^{-10}

[a]Assume 10 nl injection volume.

Table 2.1. Sensitivity of different detection methods.

These show that most of the detection methods in CE especially absorbance-based detection having a lower concentration sensitivity when compared with other analytical separation instruments. However there are a number of ways to enhance the concentration sensitivity and improve the limit of detection in CE:

2.3.4.1. Capillary geometry and improved detector optical design

Low concentration sensitivity in absorbance-based detectors in CE may be due to a number of factors. First, from Lambert-Beer law (see Equation 1.16) the absorbance is proportional to path length and the path length in CE for the detection window is very short. This path length relies on the internal diameter of the capillary column (usually between 50 - 100 μm). Moring et al. (110) employed a *z-cell* with a 3 mm capillary section of path length and obtained a tenfold increase in sensitivity. In another development Tsuda et al. (111) used *rectangular* capillaries of 1000 μm path length with an increase of about 20-fold in sensitivity. Later Wang et al. (112) used a *multireflection cell* to enable an increase of 40-fold in peak height. The path length also can be increased using a *bubble cell* as demonstrated by Heiger (202) when he obtained a 3-fold increase in peak height.

2.3.4.2. Sample concentration strategies

The sample amounts injected and the capillary volume is very small in CE and therefore the concentration of sample injected is very limited. The sensitivity of the technique can be enhanced by using discontinuous buffer systems and pre-electrophoresis sample concentration schemes.

Under normal mode, the sample injection method is employed on either hydrodynamic injection or electrokinetic injection. Both are discussed in Chapter one. Hydrodynamic injections provide a sample plug representative of analyte composition with an injection volume that depends on the injection time, capillary dimensions, buffer viscosity, and pressure drop across the capillary. However, this method will cause significant band broadening due to the electro-osmotic pressure developed at the

boundary between the water and buffer zones. The pressure difference causes generation of laminar flow and results in zone broadening. Another problem that may arise, the total plug length becomes too large providing a negative effect on the separation efficiency. This typically occurs when the injected plug length of is more than 1% of the total separation length. Meanwhile in electrokinetic injection, the amount of material injected is a function of the electrophoretic mobility of each solute, the electrical conductivity of the sample buffer and the running buffer, and the electroendosmotic flow. These parameters are very difficult to control and may affect the degree of reproducibility for each analyses. The amount of each sample component loaded onto the capillary will vary as a function of the mobility of each sample species.

Stacking methods are based on the field strength differences between the sample zone and the running buffer. This method was initially described by Everaerts et al. in 1979 (107). Stacking is obtained when the conductivity of the sample solution is significantly lower than the running buffer. Burgi and Chien determined that the best results for stacking are attained when the sample is prepared in a buffer that is about ten times less concentrated than the run buffer (113). Upon application of the voltage, a proportionally greater field is developed in the dilute sample plug compared to run buffer due its higher resistivity. Electrophoretic velocity is proportional to the electric field, so the solute ions will migrate faster across the sample zone until they reach the concentration boundary between the sample plug and run buffer. Once the ions reach the run buffer boundary, the field decreases and they migrate slower. This continues until all of the ions in the sample zone reach the boundary and cause the sample ions to become concentrated into a small zone. At this point, the field becomes homogeneous

in the zone and normal electrophoresis begins. Several publications discuss the methodology of sample stacking in different applications (114-117). The stacking method can also be enhanced by reversing the polarity of a system to change the direction of the EOF. This technique was attempted by Chien and Burgi in 1992 to obtain a good detection limit for the separation of anions (115). Meanwhile for cations, which have a positive electrophoretic mobility with respect to the electroosmotic flow, one has to reverse the direction of the EOF, either by coating the inside of the column or by adding an organic modifier to the buffer reservoir. In this respect, McClean et al. (118) introduced large volume sample stacking in CE with hydrodynamically injections of up to 300 s for the determination of cobalt and obtained good sensitivity using a UV/Vis detector. They used an electroosmotic flow modifier, cetyltrimethylammonium bromide (CTAB) to reverse the direction of EOF followed by reversing the polarity of the electrodes. This technique improved the limit of detection of selected basic cationic drugs and metal chelates by up to two orders of magnitude.

When a sample is prepared in diluted buffer and injected electrokinetically, this is called field amplification. This method has been described in which up to 50% of the capillary may be filled with sample, the buffer removed by the EOF, and the sample stacked in a small zone at the head of the capillary. The electric field strength in the sample solution is higher than it is in the run buffer. An amplified field occurrs at the point of injection which is proportional to the ratio of concentrations of buffers at the point of injection. When the voltage is applied the solute ions will migrate faster from high electric field to the lower electric field. The movement of solute ions become slower and concentrated when they reach the lower electric field. The volume of

injection depends on the period of injection. Amplified field injection offers a great advantage when compared to electrokinetic injection because the sample is prepared in a more concentrated buffer (119-121). Sepaniak et al. obtained nearly 3 orders of magnitude increase in sensitivity (10 ppb) in the determination of uranyl cations complexed with Arsenazo III (119). One further consideration in the use of stacking during injection is the generation of heat in the sample zone. When a large volume of dilute buffer is injected into the capillary this causes differences in conductivity to be very high between the sample zone and running buffer. Under typical stacking conditions most of the voltage drop occurs in the stacking zone. The corresponding power generation can result in significantly elevated temperatures. In fact, temperatures may raise by up to 100^0C in the sample zone, even with capillary thermostating, this has been shown by Anders and Soeberg (122).

Another option to enhance sensitivity in sample concentration is by Isotachophoresis (ITP). It is performed by sandwiching a sample between a leading and terminating, or trailing, buffer and by applying an electric field in the constant current mode. This method is unable to obtain the separation of anions and cations in a single run. The method employed can enhance sensitivity by up to 3 orders of magnitude (usually between 100-1000 enhancement). This method was carried out by Stegenius et al. to obtain a sensitivity enhancement of the order of 100 times in the separation of fluorescein isothiocyanate-amino acids by ITP-CE (123). Meanwhile, ITP conditions were employed for the investigation of anions by Haber et al. (67). They obtained the detection limits of 100 ppt using a conductivity detector.

2.3.4.3. Alternative detection modes

Techniques that do not require chemical alteration to improve the sensitivity of detection have been reported in the literature as very sensitive for the detection of metal cations in CE. Among these, an end-column ultramicroelectrode detection system was introduced by Cassidy et al. (124,125) for amperometric detection to provide sensitive determination of metal cations. They obtained the CE separation of metal cations using carbon-fibre, platinum, gold, and mercury films electrodes detection system operated under constant and pulse-voltage conditions. Fourteen metal cations were separated when they used mercury-film ultramicroelectrode with the limit of detection of up to 5 x 10^{-7} mol/L. Potentiometric detection with an ion-selective microelectrode in a post-column position (126) or as an on-column CE detector (127) have been employed to determine alkali and alkaline earth cations. Both techniques were equipped with narrow capillaries (≤10μm I.D.) to ensure a high resolution and small sample amounts. In a later development, on-line coupling of CE and with a mass spectrometer (128) was used to separate metal cations.

2.4. Conclusions

In CE, a common problem that arises is the inability to obtain good separations for those metal cations, particularly transition metals and lanthanide groups, whose electrophoretic mobilities are similar. Methods employed to increase differences in electrophoretic mobilities have included partial and complete complexation of the metal in the presence of complexing reagent and other organic modifiers. In general the particular method employed is only applicable for the separation of a certain group of metals and few of them enable the separation of all metal cations in a single run.

Limits of detection and sensitivity in relation to metal cations analysed by CE were generally poor when compared to other analytical instruments such as ICP, AAS, HPLC and IC. This problem is due to a limited path length for optical detection and this is combined with small sample volumes that was employed in CE.

The use of conductivity detection for the separation of metal cations especially transition metals and the lanthanide series is much less frequent, in contrast to other detectors e.g. UV/Vis.

CHAPTER 3

INSTRUMENTATION AND GENERAL PROCEDURES

3.1. Introduction

This chapter consists of those general procedures and instrumental methods used throughout the investigations. Specific experimental details will be discussed further in chapters 4, 5, 6 and 7.

3.2. Instrumentation and apparatus

3.2.1. CE and data output

The CE system employed was the Crystal CE model 310 (ATI Unicam, Madison, WI, USA). The electropherograms were recorded and integrated by a Hewlett Packard 3395 (Waldbronn, Germany) integrator.

3.2.2. Capillaries

For studies involving indirect UV/Vis detection, a polyimide-coated fused-silica capillary was obtained from Composite Metal Services Ltd, Hallow, UK. The capillary dimensions were 50 μm I.D. and 375 μm O.D. with a total length of approximately 63 cm. A window for detection was made 7.3 cm from the outlet end of the capillary. This window was prepared by applying heat for a few seconds to burn off the polyimide coat to allow UV detection through the capillary wall. A Polyimide-coated

ConCap I fused-silica capillary (65 cm x 50 μm I.D. x 375 μm O.D.) from ATI Unicam (Madison, WI, USA) was used with the Conductivity detector.

3.2.3. Detectors

The system was equipped with a Philips 4225 (Handelsweg, TC Emman, The Netherland) UV/Vis detector. For conductivity detection a Crystal 1000 CE detector (ATI Unicam, Madison, WI, USA) was employed.

3.3. Materials and reagents

All the reagents used were of analytical or ACS certified grade. Deionised water was prepared with a Mili-Q system (Milipore, Bedford, MA, USA). Individual standard stock solutions of 1000 ppm were prepared by dissolving metal nitrate salts as follows; K^+ (KNO_3), Na^+($NaNO_3$), Ca^{2+} [$Ca(NO_3)_2.4H_2O$], Mg^{2+} [$Mg(NO_3)_2.4H_2O$], Mn^{2+}[$Mn(NO_3)_2.4H_2O$], Co^{2+}[$Co(NO_3)_2.6H_2O$], Ni^{2+}[$Ni(NO_3)_2.6H_2O$], Cu^{2+}[$Cu(NO)_2.3H_2O$], Pb^{2+}[$Pb(NO_3)_2$], Cd^{2+}[$Cd(NO_3)_2.4H_2O$], Zn^{2+}[$Zn(NO_3)_2.4H_2O$], Al^{3+}[$Al(NO_3)_2.9H_2O$], Ba^{2+}[$Ba(NO_3)_2$], Cr^{3+}[$Cr(NO_3)_2.9H_2O$] and NH_4^+[NH_4NO_3]. The metal standards were purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Working standard solutions containing different concentrations of the above elements were prepared by mixing the appropriate amounts of the standard stock solutions in deionised water.

Metanol and Acetonitrile of HPLC grade (Sigma-Aldrich, Gillingham, Dorset, UK) were used in the preparation of the buffer compositions throughout the investigation. The percentage of methanol and acetonitrile employed in the buffer composition is based on volume per volume (% vol/vol) instead of mass per volume (% g/ml).

3.4. General procedures

3.4.1. Conditioning

To maintain a high surface charge density on the inner surface of the capillary and create a strong electroosmotic flow, capillary cleaning procedures are necessary (129). It was sufficient to condition a new capillary with 0.1 mol/L NaOH for about 30 min. This step is repeated and is very important for regeneration of the silica surface by removing any solute or buffer ions from inner wall, especially after three or four days of use. Conditioning of the capillary also ensures it was clean and free from contaminants. The capillary was then rinsed thoroughly with deionised water and equilibrated with the buffer solution to be used for the particular separation. All the cleaning procedures were carried out using on-line pressurisation system incorporated within the CE instrument. In some cases, the investigation of metal cations were carried out between pH 3.5 to pH 4.5. This pH range was used when acetate buffer was employed as a complexing reagent. Difficulties in obtaining a stable baseline may arise when the capillary is flushed with a strongly basic solution (e.g. 1mol/L NaOH). This phenomenon was reported by Lambert and Middleton (130) when the capillary was conditioned at pH 12, followed by use of a running buffer at pH 4-5. They observed significant shifts in migration times (two to three fold) and decreases of electroosmotic mobility due to a very slow equilibration of the silica surface charge. An alternative method of flushing and conditioning the capillary is with weak acid i.e HCl instead of strong basic solution before starting with the actual buffer solution.

The ConCap I capillary requires extra care in order to prolong it's active life. Rinsing the capillary with 0.5 mol/L NaOH followed by a 2 minute rinse with reagent grade water at an applied pressure of 2000 mBar is sufficient to condition the capillary.

3.4.2. Washing

The fused-silica capillary was rinsed with deionised water before and after each experiment. Deionised water was employed at a pressure of 2000 mBar for about 30 minutes to ensure any particles or other substances were removed from the capillary column. These procedures will avoid any cross contamination occurring during analysis.

3.4.3. Buffer replenishment

Refilling of the buffer in the capillary and the reservoirs after a number of repetitive injections has been made is very important. The buffer composition may change after period of time because of the migration of buffer ions and the electrolysis of water. Buffer depletion will result in a drift in the background signal and/or a noisy baseline. For this reason the fresh buffer solutions were used everyday before performing new injections. Several reports in the literature have undertaken the replenishment after each analysis (131-133) in order to increase the reproducibility of migration times, peak height, and peak areas.

3.5. Buffer preparation

All eluents and standards were prepared from reagent grade chemicals and the final solutions were make up with deionised (18MΩ) water. Stock buffer solutions of oxalic acid, acetyl acetone (acac), acetic acid and lactic acid were freshly prepared each month.

3.5.1. For indirect UV/Vis

3.5.1.1. Stock solution of oxalic acid

Oxalic acid (100 mmol/L) was prepared by dissolving 1.8008g of sodium oxalate (MAY & BAKER Ltd, Dagenham, UK) in 200 mL of water in a volumetric flask. This compound dissolved readily in water.

3.5.1.2. Stock solution of acetylacetone (acac)

Acetyl acetone (100 mmol/L) was prepared by dissolving 2.023 g of 2,4-pentanedione (Sigma-Aldrich, Gillingham, Dorset, UK) in 200 mL of water in a volumetric flask. This compound dissolved readily in water.

3.5.1.3. Stock solution of acetic acid

Acetic acid (10 mol/L) was prepared by dissolving 121.3130 g of glacial acetic acid (Fisher Scientific Ltd., Loughborough, UK) in 200 mL of water in a volumetric flask.

3.5.1.4. Stock solution of lactic acid

Lactic acid (100 mmol/L) was prepared by dissolving 0.9008g of lactic acid (Sigma-Aldrich, Gillingham, Dorset, UK) in 100 mL of water in a volumetric flask.

3.5.1.5. Stock solution of 4-methylbenzylamine

4-Methylbenzylamine (100 mmol/L) was obtained from Sigma-Aldrich, Gilingham, Dorset, UK which was used as the UV absorber for indirect UV/Vis required 2.4986g weighed and dissolved in water using a 200 mL volumetric flask. This chemical is very difficult to dissolve in water. In order to obtain a clear solution and complete dissolution, the final solution is immersed in a water bath for 30 minutes then followed

by filtration through a 0.2 micron filter system (Pall Corp., Ann Arbor, MI, USA). This stock solution lasts for 1 month.

3.5.2. For conductivity detection

3.5.2.1. Stock solution of MES and L-Histidine

2-(N-morpholino)ethanesulphonic acid (MES) and L-Histidine were obtained from Sigma Chemical Co., St. Louis, MO, USA. 30 mmol/L MES/Histidine was prepared by weighing 1.1807g of MES and 0.9424g of Histidine and making up to 200 mL with water in a volumetric flask.

3.5.2.2. Stock solution of lactic acid

Lactic acid (100 mmol/L) was prepared as outlined section 3.3.1.4.

3.5.2.3. Stock solution of lithium hydroxide (LiOH)

Lithium hydroxide (100 mmol/L) was prepared by weighing 0.4196g of the salt and making up to 100 mL with water in a volumetric flask.

3.6. Sample handling

3.6.1. Sample loading

Samples are introduced into the capillary column by either hydrodynamic injection or electrokinetic injection. These two methods have already been discussed previously in section 1.2.2. For these investigations most of the samples were injected via the hydrodynamic injection mode.

3.6.2. Sample Preparation

The samples used in this investigation included mineral water and acid mine discharges. The samples did not require any further treatment or digestion procedures because their appearance does not contain any suspended solids that may affects the accuracy of the results. Samples of mineral water and acid mine discharges were acidified to pH 2 with conc. nitric acid and stored in a refrigerator in polyethylene bottles at 4°C prior to analysis. Acid mine discharges were collected from different locations in the Barnsley area and preserved with either acetic acid or nitric acid before use. Most of the samples were filtered through a 0.2 membrane filter before each injection. This step is very important to avoid any blockage of the capillary column or conductivity detector. The samples was then diluted as appropriate.

3.6.3. pH adjustment

The pH meter, (PYE Unicam Ltd., Cambridge, UK) was calibrated every time prior to use with a calibration buffer at pH 4.0 and pH 7.0. This calibration is very important to get a better separation and consistent migration time for metal cations. The pH value was adjusted using the following reagents to obtain the required value.

3.6.3.1. For UV/Vis detection

N,N diethylethanolamine (1mol/L) was used to increase pH and acetic acid or HCl (1mol/L) was used to decrease pH.

3.6.3.2. For conductivity detection

The pH adjustment was affected using 1mol/L N,N diethylethanolamine to increase or 1mol/L acetic acid to decrease.

3.6.4. Degassing and filtration procedure

After the pH adjustment, all buffer solutions were placed in an ultrasonic bath for 30 minutes to allow complete mixing and degassing. This was followed by filtration using a 0.2 micron membrane filter.

CHAPTER 4

APPLICATION OF CE AND PRELIMINARY DETERMINATION OF METALS USING ION CHROMATOGRAPHY

4.1. Introduction

The aim of this work was undertaken to evaluate the capability of CE separations for metal cations in group I and group II present in a real sample e.g. mineral water. In the preliminary stage of this study some of the established techniques using IC have been repeated so as to explore the capability of IC for the separation of group I and group II metals. Limits of detection, linearity, reproducibility and matrix effects were investigated.

Initial experiments involving CE were limited to a number of mono- and di-valent metal cations including sodium, potassium, magnesium, and calcium. Most of the techniques employed for this investigation including acetic acid and lactic acid as a complexing reagents to obtain the separation of metal cations. Development and subsequent evaluation of these methods are discussed in Chapters 5, 6 and 7. Detection of metal cations was carried out by either indirect UV/Vis absorbance or conductimetric methods using CE. In IC only a conductivity detector was available.

4.2. Background theory of Ion Chromatography

Ion chromatography (IC) is a liquid chromatographic technique employed for the separation of inorganic cations and anions. The components are separated via an ion exchange mechanism between the mobile phase and stationary phase. Two general methods have been developed, usually referred to as non-suppressed IC and suppressed IC. In non-suppressed IC ions are separated using a single column (i.e. dependent only on an ion exchange process) placed before the detector whereas in suppressed IC an additional component called the suppressor (second stage of ion exchange process), is situated between the first column and detector. The separation of cations and anions requires separate experiments for both methods. An ion chromatograph is composed of a pump, a injection port, a separation column and a detector. This is the arrangement in non-suppressed IC. For suppressed IC, a membrane suppressor is positioned between the separation column and the detector to reduce the background conductivity of the eluent.

4.2.1. Mechanism of ion exchange

Ion exchange is a process whereby a solution of an electrolyte is brought into contact with an ion exchange resin and the ions on the resin are replaced by ions (ionic species) of similar charge from the analyte solution. Analyte cations and anions are attracted to the stationary phase based on the different polarity of ion exchange sites.

Cation exchangers have negatively charged sites that attract solute cations whereas anion exchangers contain positively charged groups on the stationary phase and attract solute anions. The ion exchange process for anion exchange can be represented by an equilibrium as follows:

$$\text{Res-NR}_3^+E^-_{stat} + A^-_{mob} \Leftrightarrow \text{Res-NR}_3^+A^-_{stat} + E^-_{mob} \quad (1)$$

Where A^- is the sample ion and E^- is the eluent counter ion

The separation of an analyte mixture will be based on the respective affinities of the sample ions and buffer ions to ion exchanger (different equilibrium constant K).

$$K_A = \frac{[A^-]_{stat} \times [E^-]_{mob}}{[A^-]_{mob} \times [E^-]_{stat}} \quad (2)$$

where $[A^-]_{stat,\ mob}$ is the concentration of the sample ion in the mobile phase (mob.) and stationary phase (stat.) and $[E^-]_{stat,\ mob}$ is eluent anion (counter ion) concentration in the mobile phase (mob.) and stationary phase (stat.)

4.2.2. Ion Exchange materials

4.2.2.1. Stationary phase

The functional groups attached to the stationary phase depend on whether the material is an anion exchanger or a cation exchanger. Ion exchange sites may be either strong or weak depending on the nature of the functional group. Ion exchange phases are supported by different types of materials. There are two type of supports normally employed, these are organic polymers and silica-based materials.

Organic polymers – Styrene/divinylbenzene copolymers, polymethacrylate, and polyvinylbenzene resin may be used as substrate materials for ion exchangers. These polymers are stable at extreme pH conditions i.e. 0 and 14.

Silica-based materials ion exchangers are only stable between pH 2 and pH 8. These materials exhibit a high chromatographic efficiency and offer a greater mechanical stability when compared to organic polymers. Swelling and shrinkage problems are not encountered during application, but the range of applicable eluents is limited.

Anion exchange sites – A strong anion exchanger contains quaternary ammonium groups ($-CH_2NR_3^+$) and is active over a wide pH range. Weak anion exchangers usually contain a tertiary amino group ($Res-CH_2NHR_2^+$) which is deprotonated in moderately basic solution and therefore unable to form a stable cation exchange complex. Capacity is defined as the ability to take up exchangeable ions, and is determined by converting the resins to the chloride form then using a neutral nitrate or sulphate solution to displace the chloride, expressing the result on the basis of dry weight.

Cation exchange sites – The functionalities in cation exchangers are acidic (e.g. SO_3H or CO_2H). The sulphonic acid resin ($Re-SO_3H$) is described as a strong acid exchanger because it remains fully ionised and will exchange the protons for other cations even in a strong acidic solutions. Exchanger resins containing carboxyl groups ($Res-CO_2H$), are described as a weak acid exchangers. These are protonated at or below pH 4 and will lose their ionic capacity as the pH goes below the pK_a of the functional group. Ionic capacity is defined as ability to take up exchangeable ions and is determined by converting the resin to

the hydrogen form, then using a neutral solution of a sodium salt to displace H^+ ions, expressing the result on the basis of dry weight.

4.2.2.2. Eluent/counter ion

In IC the eluent used depends on the detection system being employed and whether it is performed with or without chemical suppression. For cation exchange chromatography dilute mineral acids (e.g. hydrochloric acid or nitric acid) are used as eluents for the analysis of alkali metals, ammonium and aliphatic amines. Divalent cations such as alkaline-earth metals are unable to elute with mineral acids by using non suppressed IC because they exhibit higher affinity toward the stationary phase (resin) of a strong acid cation exchanger. If the acid concentration is increased sufficiently to displace them then the background conductance will increase too high a level. This makes it difficult to get a sensitive detection using electrical conductivity. Use of suppressor overcomes this problem, though the life of the suppressor may be shortened by strong acidic. In the micromembrane suppressor the eluent required is a mixture of 2,3-diaminopropionic acid (DAP) and hydrochloric acid (HCl). These eluents provided good separation for alkaline-earth metals and allow adjustment of the elution power via dissociation of the carboxyl group. Chloride is removed by the suppressor, while DAP may exist in the mobile phase as a monovalent cation, divalent cation or mixture of both, and is converted in the suppressor to the zwitterionic form without intrinsic conductance. In non-suppressed IC alkaline-earth metals are separated using a mixture of ethylenediamine and an aliphatic dicarboxylic acid (e.g. tartaric acid or oxalic acid). Weak organic acids are usually used as complexing agents in the analysis of heavy metals and transition metals.

For anion exchange chromatography with chemical suppression, the eluents used are weakly dissociated and have a low background conductivity (e.g. sodium carbonate and bicarbonate). In non-suppressed IC, the eluents used have a low conductivity and are presented in low concentration to prevent the development of a high background conductance (e.g. aromatic acid such as benzoic acid and phthalic acid).

4.2.3. Suppressed Ion Chromatography

The IC suppressor is located between analytical column and cell detector. The IC suppressor relies on chemical suppression to reduce the ionic strength from background eluents by exchanging the counter ion for H^+ (cations) or OH^- (anion), so that high sensitivity conductivity detection can be used. The process taking place for both cations and anions is as follows:

Anion analysis – After the anion exchange process in the analytical column the component analytes (M^+A^-) and eluents (Na^+OH^-) will be eluted to the membrane suppressor, where the counter ions (e.g. Na^+) are exchanged across a perm-selective membrane for H^+, which then combines with OH^- to yield neutral H_2O. Analyte anions are converted to the corresponding acid form ($H+A^-$) and are detected by a conductivity detector. A mineral acid (e.g. H_2SO_4) is required for the suppressor.

Cation analysis – After the cation exchange process in the analytical column the component analytes (M^+A^-) and eluents (H^+Cl^-) are eluted to the membrane suppressor. HCl from eluents are converted to water (H_2O), analyte cations to corresponding base form (M^+OH^-) and detected by a conductivity detector.

4.2.4. Non-suppressed Ion Chromatography

This system does not have a suppressor and the separation of analyte anions and cations is dependent only on an ion exchange system in the analyte column. The non-suppressed method relies on electronic rather than chemical suppression of the eluent conductivity and requires very low capacity of ion exchange media combined with very dilute eluent solutions, which in most instances have low equivalent conductivities.

4.2.5. Detection techniques in IC

There are several methods employed in IC. These include electrochemical methods (conductimetric and amperometric detection), UV/Vis absorbance or fluorescence and refractive index. Only conductivity and UV/Vis absorbance detection will be discussed further in this section.

4.2.5.1. Conductimetric detection

This detection is based on the conductivity of analyte solutions. Conductivity is defined as the ability of an electrolyte solution to transport a current between two electrodes under the influence of an applied electric field. There are two methods to determine inorganic ions, direct and indirect conductimetric.

Direct conductimetric - Eluent ions exhibit a much smaller value of conductance than eluent ions. Normally phthalate or benzoate are used as the counter ion, these exhibit a low equivalent ionic conductance, and thus conductivity increases when a solute ion passes through the detector.

Indirect conductimetric – Eluents exhibit a much higher value of conductance than solute ions. A strong conducting eluent is used and a reduction in conductivity is observed when a weaker conducting solute band enters the detector. Potassium hydroxide is used as an eluent for anion analysis. In cation analysis, dilute nitric acid or nitric acid/ethylene diamine mixtures are used.

4.2.5.2. UV/Vis detection

UV/Vis detection is used either directly or indirectly. Direct UV/Vis detection is applicable where the analyte ions absorb strongly at the appropriate wavelength. Indirect UV is based on the decrease in absorbance at a convenient wavelength as transparent sample ions replace UV absorbing buffer ions. Usually aromatic based compounds such as phthalate, p-hydroxybenzoate and benzoate are used as eluents in analysis of anions. In cation analysis, copper sulphate is used to determine alkali metals such as Na and K.

4.3. Critical literature review for IC

IC was introduced by Small et al. (134) in 1975 who reported an ion exchange experiment employing a suppressor column (dual column). Sodium hydroxide, carbonate and bicarbonate were used as eluents in the determination of anions using a conductivity detector. Following this the technique was extended to cation analysis using a lower exchange capacity resin with a very dilute eluent (135). The precipitation of insoluble hydroxides of many polyvalent metal cations occurs in the suppressor column (136) unless the metal ions are strongly complexed. Hollow-fibre suppressors (137) were developed to overcome this problem.

Fritz et al. (138) developed a system without a suppessor column (one column) using cation-exchange materials of low capacity to separate alkali metals and ammonium cations. They used nitric acid and ethylenediammonium salts as eluents in conjunction with conductivity detection. This development provided another alternative for the elimination of the hydroxide form in suppressed techniques because the non-suppressor techniques depend on electronic instead of chemical suppression. The method requires very low capacity ion-exchange materials, dilute eluent solutions of low equivalent conductivity.

The method of detection employed, whether with or without chemical suppression, determines the type of eluents used. In 1979, Fritz et al. (139) used eluents of low ionic strength to determine inorganic anions using the conductivity cell detection technique. Sevenich and Fritz (140) obtained a good separation of polyvalent metals using complexing anions such as hydroxyisobutyrate, ethylenediammonium or tartrate in conjunction with conductivity detection. Other applications for different type of eluents

were discussed and they used eluents containing tartrate, m-phenylene diamine solution, citrate, oxalate, 2-hydroxyl isobutyrate, or salts of pyridylcarboxylic acid (141-146).

Analysis of alkali metals can be achieved using an isocratic system with suppressed techniques. This analysis has been carried out on mineral water to analyse Na, NH_4^+, K, Mg and Ca using HCl and D,L, 2-3-diaminopropionic acid monochloride as eluents (147) with detection by conductivity. Other publications reporting the analysis of metals by IC have been reported (141-150).

In a further development of IC, monovalent and divalent cations can be determined simultaneously using a cation exchange column (151-156). Group I and II metal cations, in addition to transition metal ions, can be separated in single run using a cation-exchange column, post column derivatisation and detection by UV-Vis (157). At times, this method can be very messy, requiring stringent preparation. Analysis of transition metals and heavy metals can also be done using cation ex-change and ethylene diammonium tartrate with conductivity detection (158). Other publications on analysis of transition metals by post column derivatisation have been reported (159-161).

4.4. Experimental procedures

4.4.1. Ion Chromatography [IC]

4.4.1.1. IC and data output

IC experiments were carried out using a Dionex QIC Analyzer Model DQP-1 (Dionex Corp. Sunnyvale California, 94086, USA), equipped with a 20 μL loop injector and a conductivity detector. The peaks were recorded and integrated by a Hewlett Packard 3395 integration system.

4.4.1.2. Analytical column and guard column

The analytical column employed was an Ion Pac CS10 (4 mm x 250 mm sized with P/N 043015). A Guard Column (Ion Pac CG10 with P/N 043016) was placed before the analytical column to ensure the eluents were free from any impurities.

4.4.1.2.1. Micro Membrane Suppressor

The suppressor used was a Cation Micro Membrane Suppressor model CMMS-1, P/N 037076.

4.4.1.3. Detectors

The system was equipped with a Dionex Conductivity Detector.

4.4.1.4. The standard operating conditions

Eluent flow rate was 1 mL/min., regenerant flow rate was 5 ml/min., detector range was 30 μS full scale and the expected background conductivity was about 1 μS to 5 μS.

4.4.1.5. Chemicals and materials required for IC with Conductivity detection

4.4.1.5.1. Regenerant

100 mmol/L tetrabutylammonium hydroxide (TBAOH) was used as a regenerant for the IC system.

4.4.1.5.2. Chemicals and deionised water

The chemicals and water required for IC were prepared from materials of the highest purity available. Usually the specific resistance of deionised water was at least 17.8 mega ohm-cm and filtered using 0.45 micron filter.

4.4.1.5.3. Stock solution of DAP.HCl.

DL-2, 3-diaminopropionic acid monohydrochloride (DAP.HCl) was employed as a stock solution for the eluents throughout the analysis.

4.4.1.5.4. Stock solution of hydrochloric acid (HCl)

Pure hydrochloric acid stock solution was employed.

4.4.1.6. Eluents preparation:

The following eluents were used to obtain the best resolution of metal cations:

4.4.1.6.1. 40 mmol/L HCl/2mmol/L DAP.HCl

This eluent was prepared as required from the stock solution as outlined in section 4.4.1.5.3. and 4.4.1.5.4.

4.4.1.6.2. 60 mmol/L HCl/6mmol/L DAP.HCl

This eluent was prepared as required from the stock solution as outlined in section 4.4.1.5.3. and 4.4.1.5.4.

4.4.2. Capillary Electrophoresis (CE)

4.4.2.1 Apparatus

The CE system used as stated in section 3.2.1 and connected either with a UV/Vis detector or a conductivity detector. The detector employed was mentioned in section 3.2.3.

4.4.2.2. Capillaries

The application of fused-silica capillary for both conductivity and UV/Vis detection has been discussed in section 3.2.2. The UV/Vis detector employed a wavelength of 214 nm. The separation voltage applied at 30 kV.

4.4.2.3. Standard Operating procedures

At the beginning of each experimental the proper conditioning procedures must be carried out according to section 3.4.1 and 3.4.2 in Chapter 3.

4.4.2.4. Chemicals and Materials

The materials and reagents employed for this investigation is discussed in section 3.3. This section will be discussed on the preparation of buffer for each methods.

4.4.2.4.1. For indirect CE-UV/Vis detection

The combination of 0.5 mol/L acetic acid, 15 mmol/L 4-methylbenzylamine, 50% (v/v) of methanol at pH 4.0 is used to get a separation of metal cations in group I&II metals. 5 mL of acetic acid was taken from stock solution (referred to section 3.5.1.3.), 15 mL of 4-methylbenzylamine was obtained from stock solution (referred to section 3.5.1.5.) and followed by 50 mL (v/v) of methanol were prepared in 100 mL of volumetric flask. The final concentration was adjusted to pH 4.0 according to section 3.6.3.1.

4.4.3. Sample preparation

All of the samples used in this investigation were aqueous, injection of which is not problematic in CE or IC. All of the samples were treated according to the procedures as discussed in section 3.6.2.

4.5. Results and discussion

4.5.1. Metal separations in group I and II by IC

4.5.1.1. Effects of eluent concentrations

Fig. 4.1 shows that both single and standard mixtures of Na, K, Mg and Ca separate faster at higher eluent concentrations compared to lower concentrations.

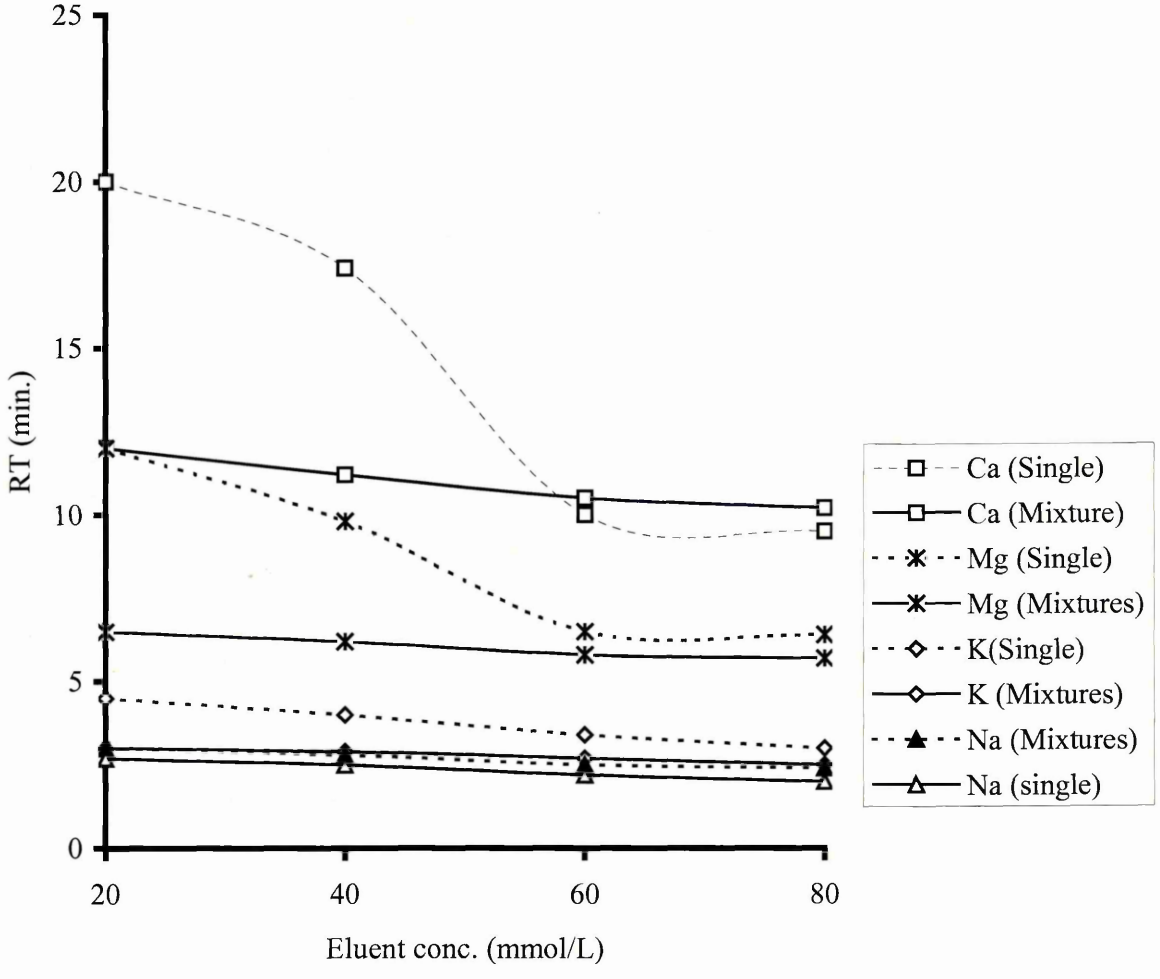


Fig. 4.1. Effects of eluent concentrations on individual and mixtures standard solutions of group I and II metals (10 ppm standard concentrations were used). The eluents employed were 20, 40, 60 and 80 mmol/L of HCl/6 mmol/L DAP.HCl.

The retention time (RT) of single standard was much influenced when the concentration of eluents was increased as shown in Fig. 4.1. However, the retention time (RT) of standard mixtures was not influenced as much when the concentration of eluents was increased. Base on the above experiment, the most suitable eluent concentration was found to be 60 mmol/L HCl/6 mmol/L DAP.HCl and the chromatogram of four metal cations separated as illustrated in Figure 4.2.

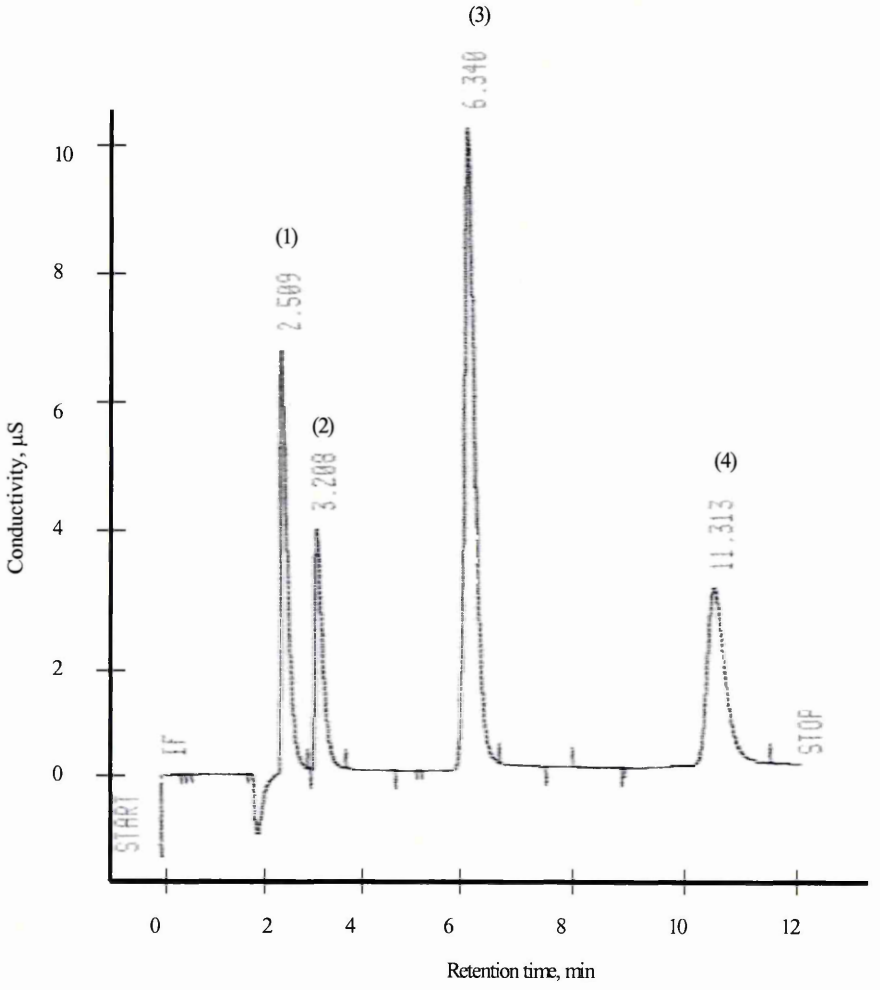


Fig. 4.2. Representative of mixtures standard from group I and II metals (10 ppm standard). The eluents employed was 60 mmol/L of HCl/6 mmol/L DAP.HCl. Peaks: 1=Na (5 ppm); 2=K (5 ppm); 3=Mg (5 ppm); 4=Ca (5 ppm).

This result is in agreement with the finding reported by Thienpont et al. (199) that the eluent concentration used will affects the separation of metal cations. Faster separation elution could have been achieved by increasing the strength of the mobile phase. They employed 4 mmol/L DAP.HCl and 40 mmol/L HCl to get a separation of Ca and Mg in less than 6 min. Meanwhile in another experiment they used 2 mmol/L DAP.HCl and 40 mmol/L HCl to obtain a separation of Mg at 4.2 min and Ca at 9.4 min.

4.5.1.2. Matrix effects

Standards of Na, K, Mg and Ca were mixed together to observe any matrix that may affects retention times. Standard mixtures were prepared at different ranges of analyte concentrations. The results obtained are given in Figs. 4.3 – 4.5.

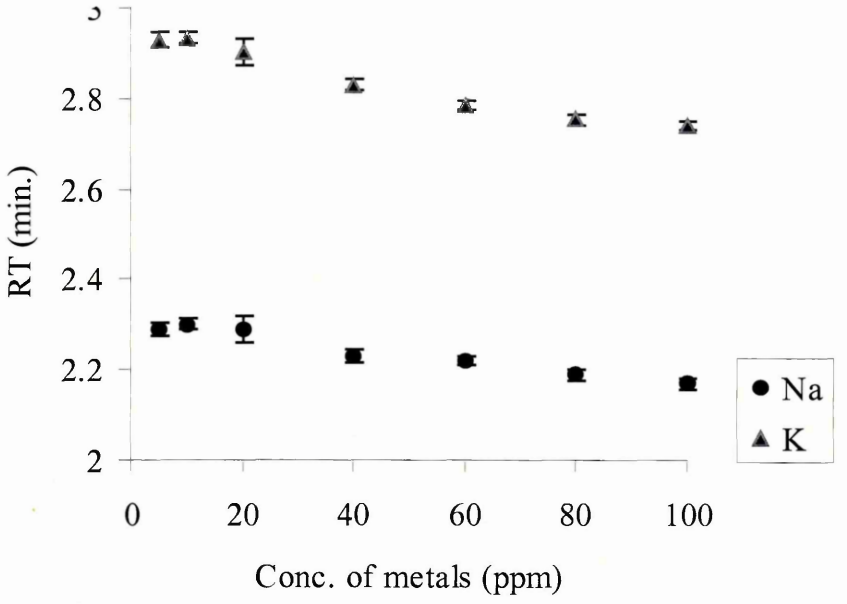


Fig. 4.3. The influence of matrix effect on retention times at different concentrations of standard mixtures of Na and K. The eluent employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

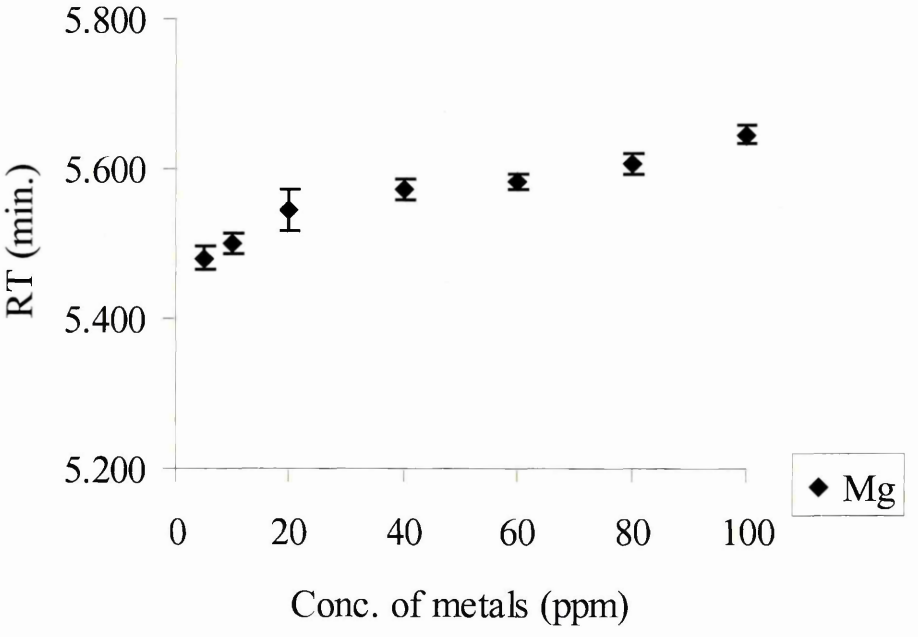


Fig. 4.4. The influence of matrix effect on retention times at different concentrations of standard mixtures of Mg. The eluent employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

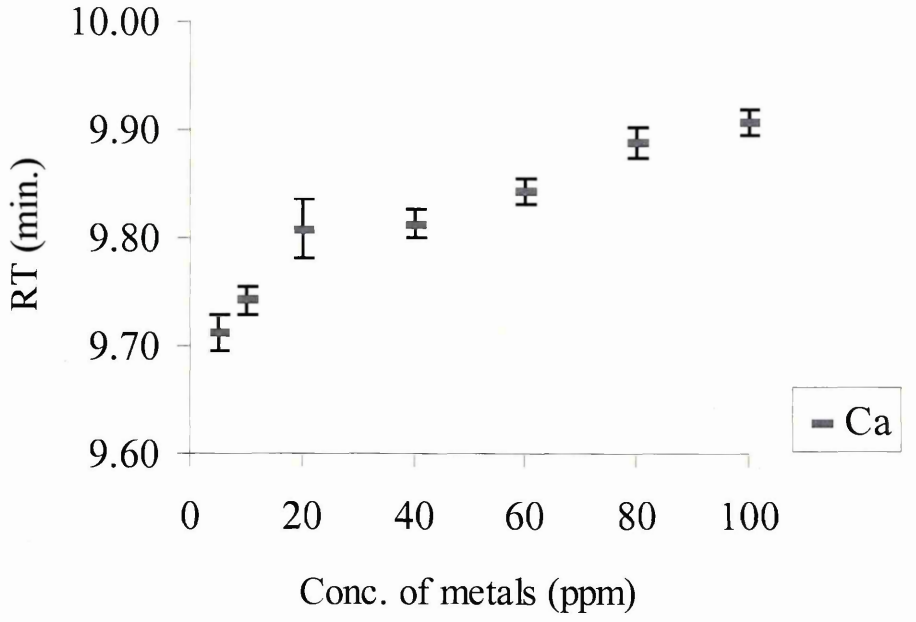


Fig. 4.5. The influence of matrix effect on retention times at different concentrations of Ca. The eluent employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

From the results shown, the retention time of Na and K decreases when the concentration increases (Fig. 4.3). The retention time of group I cations decreases because the monovalent cations achieve high affinities against cation exchanger of the stationary phase (very high of different equilibrium constant, *K*). However the retention time of calcium and magnesium increases (Figs. 4.4 – 4.5) when the concentration increases, therefore will influence divalent cation binding to increase the retention time. It shows that increasing the concentration of analyte components in a standard mixtures was much affected the retention times of metal cations in group I and II. This observation supports the results obtained by Morawski et al. (196) for the analysis of cationic nutrients from foods by ion chromatography. They used 0.1 mmol/L EDTA and 3 mmol/L nitric acid to

analysed Li, Na, NH_4^+, K, Mg, Ca, Sr and Ba. They have found that the presence of multi-analyte at different concentrations will affect decreasing in retention time for each analyte components.

4.5.1.3. Reproducibility

Analysis of metals commenced the same day after sample preparation (i.e, every 0.5 hrs). Ten sectional period were containing mixed standard metals were used. These samples were selected at regular intervals during batch to ensure detection of any concentration trends. Corrected results were subjected to statistical analysis variance (ANOVA) to determine if between sample variability was insignificantly different from within-batch variability (195). Analysis of variance (ANOVA) is used to separate and estimate any variation which is caused by changing the control factor from the variation due to random error. It can thus test whether altering the controlled factor leads to a significant difference between the mean values obtained. In order to verify the reproducibility of analysis, three set of sample solutions of multi-element standards (mixtures standard solution) were injected and the data evaluated statistically. Ten replicates at each concentration was injected with the same set solution of 10 ppm concentration were carried out sequentially. The average and standard deviations (SD) for migration times, absorbance and peak area of metal cations were determined.

4.5.1.3.1. Retention time (RT)

Full summarises reproducibility data for all four metals is shown in Table 4.1.

Elements	Mean result, min. ± SD [a]	ANOVA *F* value[c]
Sodium	2.12 [b] ± 0.03	0.03
Potassium	2.52 [b] ± 0.01	0.33
Magnesium	5.27 [b] ± 0.01	0.27
Calcium	9.83 [b] ± 0.04	0.10

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 4.1. Reproducibility data on retention time for metal cations at different batch samples using 60 mmol/L HCl/6 mmol/L DAP.HCl.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. As can be seen from Table 4.1, the retention times are fairly stable and capable of providing a consistent and reproducible data. This result obtained is in agreement with the findings reported by Jones and Tarter (198) on the analysis of selected transition metal cations using 5 mmol/L oxalic acid, 3.75 mmol/L citric acid at pH 4.37. They observed the reproducibility of retention time ranges from 0.03 to 0.05 of standard deviation. Therefore, the results obtained under this work are consistent with the previous observation in similar field.

4.5.1.3.2. Conductivity

Full summarises reproducibility data for all four metals is shown in Table 4.2.

Elements	Mean result, μS ± SD [a]	ANOVA *F* value[c]
Sodium	1.56 [b] ± 0.02	0.08
Potassium	1.28 [b] ± 0.02	0.14
Magnesium	1.62 [b] ± 0.02	0.23
Calcium	3.62 [b] ± 0.02	0.18

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 4.2. Reproducibility data on conductivity for metal cations at different batch samples using 60 mmol/L HCl/6 mmol/L DAP.HCl.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. The results obtained are given in Table 4.2 shows the conductivity are fairly stable and capable of providing a consistent and reproducible data. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of nickel, zinc and cobalt with the employing of 5 mmol/L oxalic acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of conductivity in this work.

4.5.1.4. Linearity of range

If linearity is in doubt the following test may be applied. Determine for two or three or the highest calibration points the relative deviation of the measured y-value from the calculated line:

$$\text{Deviation (\%)} = \left| \left(\frac{y_i}{bx_i + a} - 1 \right) \right| x100\% \qquad (4.1)$$

If the deviations are less than 5% the curve can be accepted as linear. However, if the deviation is more than 5% then the range is decreased by dropping the highest concentration. Figs. 4.6 – 4.9 shows a linearity of the group I and group II metal cations. There were four concentration levels of multi-element standards used from 1.0 ppm to 80 ppm (except sodium) for the analysis using IC. Ten times at each concentration was injected and duplicates with the same set solution of each concentration were carried out sequentially. Lower concentration than 1 ppm were not tested. The average values of the results in linearity for Na (y = 2E+06x + 4E+06; and R^2 = 0.9977), K (y = 2E+06x + 3E+06; and R^2 = 0.9978), Mg (y = 8E+07x + 1E+08; and R^2 = 0.9969) and Ca (y = 5E+06x - 648987; and R^2 = 0.9987), were acceptable and can be fitted a straight line for the above range.

When as an exercise, this test is applied to calibration curve of Figs. 4.6-4.9 it appears that the deviations of all metals are less than 5% at upper concentration higher than 80 ppm except for Na. The average values of the result in linearity for Na shows the deviations is about 8.4% higher than 60 ppm (y = 2E-06x + 7E-06; and R^2 = 0.9888). Meanwhile, the

non-linearity shows the deviations is about 8.7% at the concentration higher than 80 ppm for K (y = 2E-06x + 8E-06; and R^2 = 0.9896), the deviations is about 5.1% for Mg (y = 7E-07x + 3E-08; and R^2 = 0.9864) and the deviations is about 6.1% for Ca (y = 4E-06x + 1E-07; and R^2 = 0.9768). It was could be due by various factors during the experiments were carried out. The IC system used was shared with other users which operated with various type of samples. A conductivity cell may be contaminated or accumulated by other metal salts after their analysis. If some of them not properly flushed and removed from the system, this will affect the linearity of detector. This behaviour could be contributed to the poor linear relationship for most metal cations especially at high concentration.

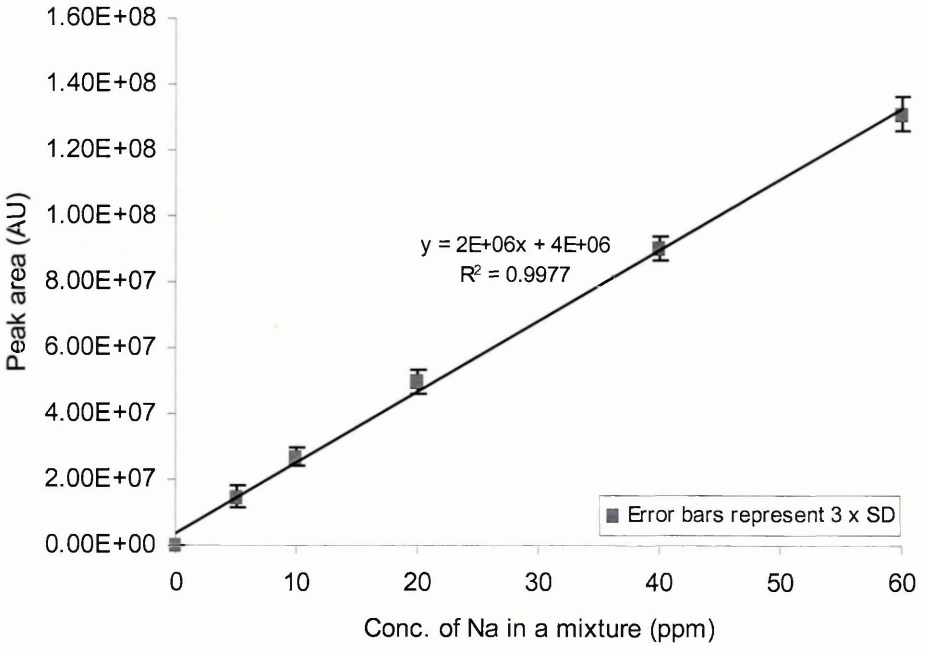


Fig 4.6. Linearity of Na in group I&II. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

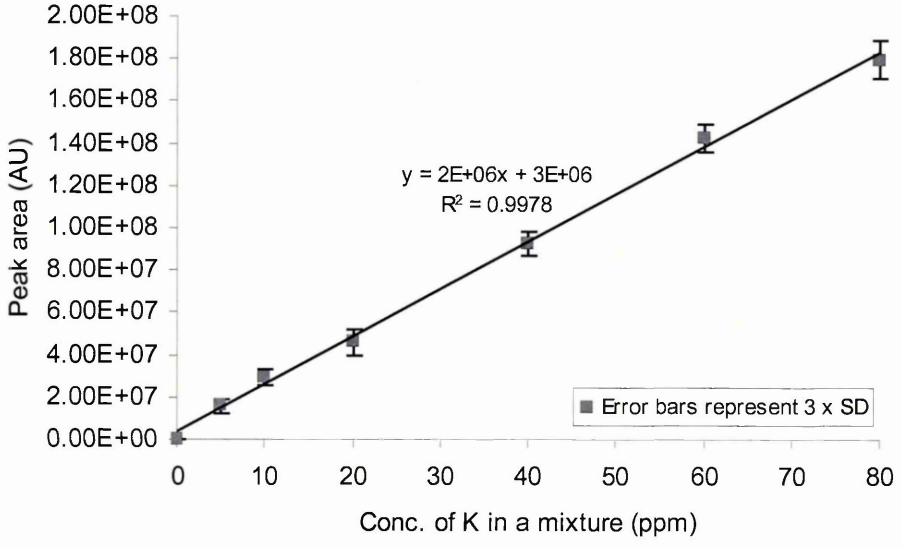


Fig 4.7. Linearity of K in group I&II. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

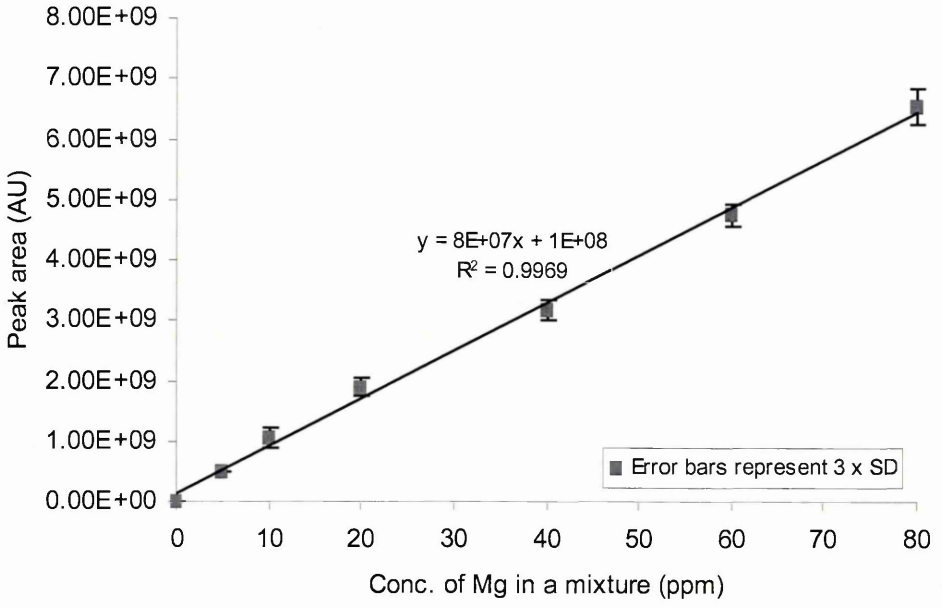


Fig. 4.8 Linearity of Mg in group I&II. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCL

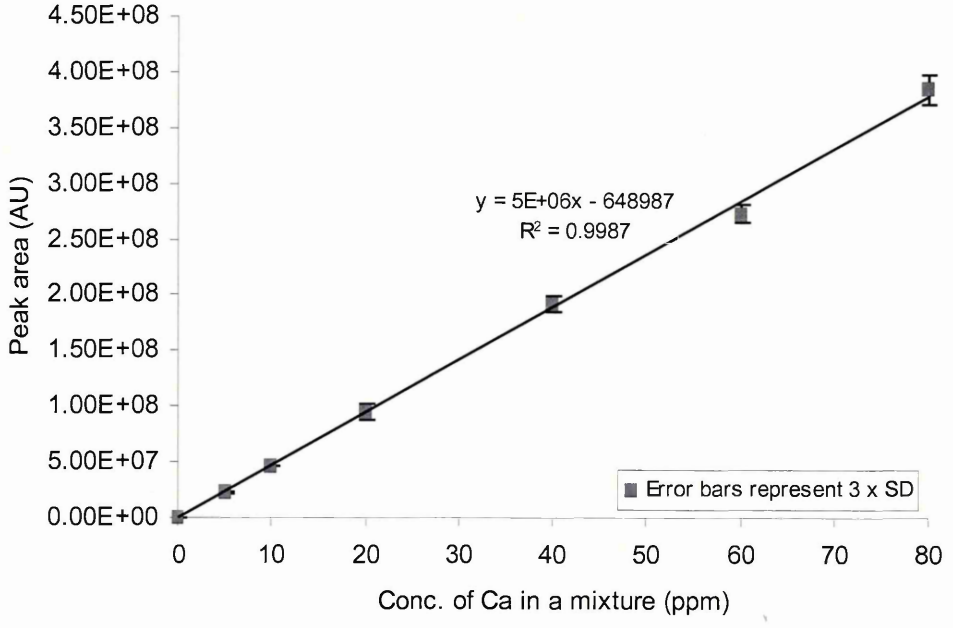


Fig 4.9. Linearity of Ca in group I&II. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

The linearity range obtain for all metal cations are found to be compared favourably even better with those data obtained in the observation by Sevenich and Fritz (140) on the addition of complexing agents in IC for separation of polyvalent metal ions. They have reported the linearity for magnesium from 1.0 to 15 ppm, calcium from 2 to 30 ppm and zinc from 2 to 14 ppm. The combination of 4 mmol/L EDTA and 3 mmol/L α - hydroxyisobutyrate at pH 4.5 was employed for thid investigation. In another development, Morawski et al. (196) also reported that the linearity for Li, Na, K, Mg, Ca, Sr and Ba were obtained over a range of 0 to 10 ppm for monovalent cations and 0 to 20 ppm for divalent cations. They used 0.1 mol/L EDTA, 3 mmol/L nitric acid to get a separation of metal cations in their investigations. The data obtained also demonstrated that the calibration curve for metal cations can be fitted a straightline for quantitative analysis.

4.5.1.5. Limits of detection (LOD)

The method by which detection limits were determined was by making serial dilutions of a standard solution. Minimum detectable concentrations for metal cations in group I and group II, based on a peak signal (conductance) to noise (3:1), are given in Table 4.3. The eluent employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

Metal cations	Mean of detection limit, (ppm ± SD)
Sodium	0.20[a] ± 0.08
Potassium	0.30[a] ± 0.10
Magnesium	0.10[a] ± 0.09
Calcium	0.20[a] ± 0.05

Notes: [a] = mean of 10 replicates

Table 4.3. Limit of detection for group I and II metals. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

Limits of detection are found to be in the sub ppm level but not as good compared to to values obtained by Morawski et al. (196) in the analysis of cationic nutrients using 0.1 mmol/l EDTA and 3 mmol/L nitric acid. They obtained limit of detection in the range of 0.005 ppm for Na, 0.02 ppm for K, 0.005 ppm for Mg and 0.01 ppm for Ca. Although the limit of detection in this work is slight higher compared to the previous work but the method developed is possible for the quantitative analysis.

4.5.2. Studies of metal separation in group I and II by CE

4.5.2.1. Effect of buffer concentration

Related works by Lin et al. (58) and Beck and Engelhardt (200) had shown the separation was most efficient if CE was carried out when the mobility of the analytes matched with the carrier electrolyte. They have found that a weekly complexing reagent including imidazole has been found to be satisfactory for this purpose. However, in the absence of a complexing reagent, only five peaks were found, Na and Mg could not be resolved. In this work acetic acid was employed as complexing reagent to observe the separation of group Iand group IIU metal cations. The concentration of complexing reagents is a very important parameter in ensuring the complete separation of metal cations. Migration times of metals in group I&II is not much influenced by changing the complexing reagent because their migration is more dependent on the differences in electrophoretic mobilities. The migration times of transition metals are much more reliant on the concentration of the complexing reagent. This is because the formation of the metal complex is affected by the concentration of complexing reagent present. The influence of complexing reagent concentration versus analyte migration times is shown in Fig. 4.10.

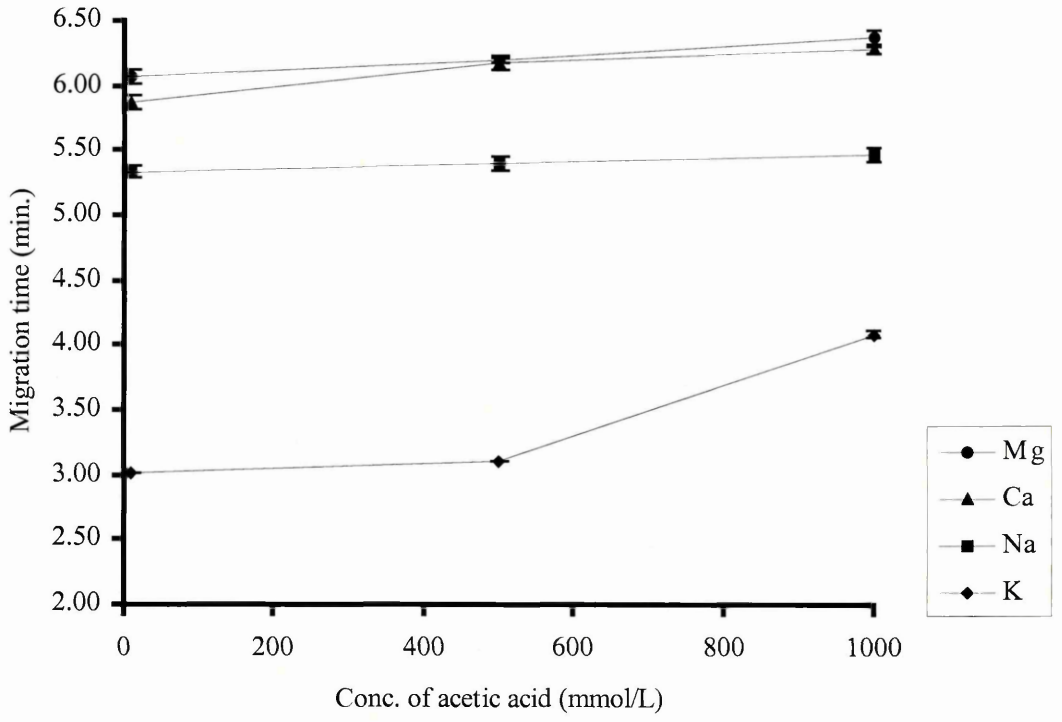


Fig. 4.10. Effect of changing the concentration of acetic acid. The buffer employed was 15 mmol/L 4-methylbenzylamine and 50% MeOH (pH .4.0).

Most metals in group I and group II could not be resolved without the present of more than 10 mmol/L acetic acid. At the lower concentration some metals e.g. K, Na, Ca and Mg were not observed and it is assumed that they co-migrated. They appear separated at concentrations higher than 10 mmol/L of acetic acid in the buffer solution. The best separation of metal cations obtained was at about 500 mmol/L of acetic acid. This work also confirmed observations of Lin et al. (58) for the employing of monoprotic acid as a complexing reagent in their investigation. They found that by changing the concentration of acetic acid used in the buffer solution may affects the separation of the six metals ion include K, Ba, Ca, Na, Mg and Li.

4.5.2.2. Effect of pH

Changing the pH of the buffer changed only the migration time span (the separation time was shorter at higher pH owing to the increase in the electroosmotic flow). It was reported (58,200) that all six metal ions (Li, Na, K, Mg, Ca and Ba) were well separated when the buffer pH was adjusted to higher pH at about 6.0 as compared to pH 4.0. In this work, acetic acid was used as a complexing reagent to get a separation of metals in group I and group II. The combination of 500 mmol/L acetic acid and 15 mmol/L 4-methylbenzylamine with 50% methanol was employed to observe the separation of metal cations at different pH. Under this conditions, the pH value was increased from pH 3.0 to pH 4.5 to study the separation of metal cations. The separation of metal cation in group I and group II at different pH is shown in Fig 4.11. Under normal conditions the metal cations will migrate faster at higher pH and slower at lower pH. In this investigation the migration times at pH 3.0 are slower than pH 4.5 because at higher pH the electroosmotic flow is significantly greater than at lower pH. This study showed that the best separation of metals in group I and group II was almost completed at pH 4.0.

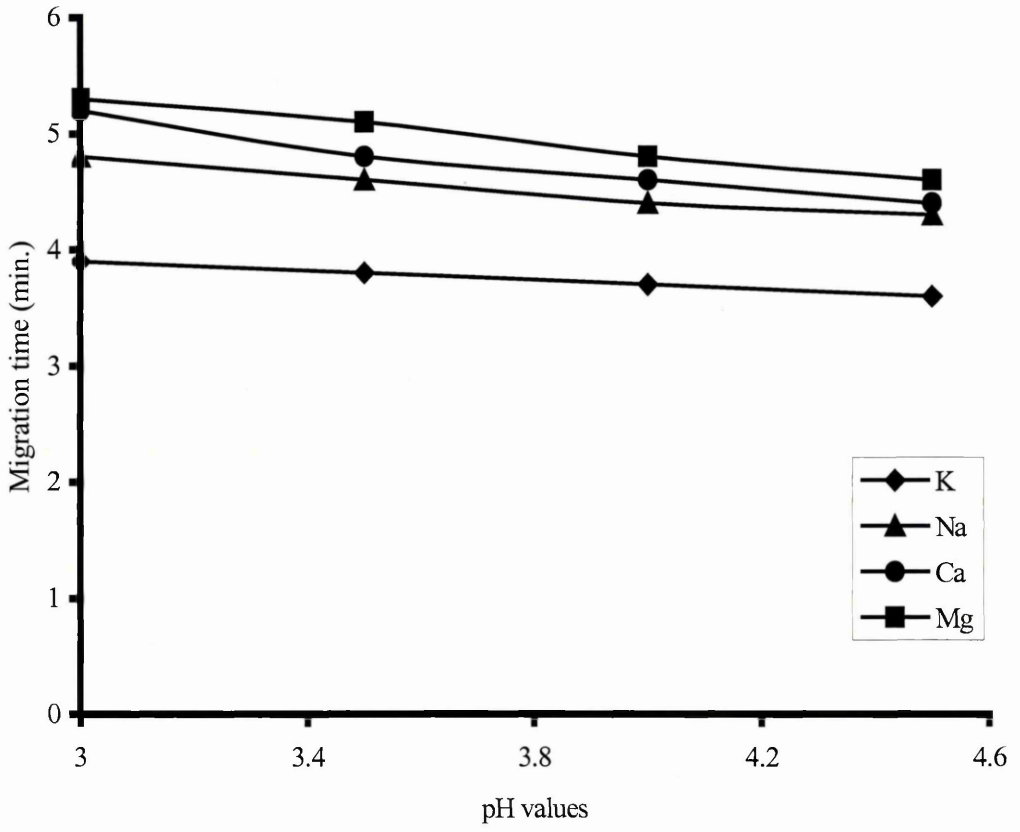


Fig. 4.11. Effect of pH adjusted on the electropherograms in the presence of 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol.

The slope of migration times versus pH not so much affected by increasing from pH 3.0 to pH 4.5. These were indicated that the separation of metal cations in group I&II are not so much influenced by changing the pH buffer. The migration order was: potassium, sodium, calcium then magnesium. It was noted that the acetic acid added to adjust the buffer pH affected the resolution. The above experiment indicated that the optimum pH was found at 4.0 to obtain a good separation with a reasonable analysis times for most cations. Weston et al. (22) was observed a similar trend for the effect of pH on the migration time of eleven alkali, alkaline earth and transition metals of the altering the pH. The combination of 6.5 mmol/L HIBA at pH 4.4 was employed in their investigation.

They found that as the pH is lowered, the migration time is increase, due to decrease in the EOF. Likewise, as the pH increased, the migration times decease, due to increase in the EOF. Weston et al. (23) also found that K and NH_4^+ co-elute at pH 6.15 owing to their identical equivalent ionic conductivity. By altering the pH of the electrolyte, the ionization of alkali metals and their mobilities will be essentially unaffected. However, as the pH of electrolyte is increased, the NH_4^+ becomes progressively less protonated (pK_b of NH_4^+ = 4.75) and its apparent mobility decreases. At pH 8.5, the apparent mobilities of K and NH_4^+ become sufficiently different to permit an effective separation.

4.5.2.3. Effect of the addition of organic solvents

In this investigation the combination of acetic acid and 4-methylbenzylamine was employed to study the effects of changing the percentage of methanol for the separation of metal cations from group I and II. At first, 15 mmol/L 4-methylbenzylamine and 500 mmol/L acetic acid was run at pH around 4.0. Selectivity was observed for group I and group II metals but the resolution was very poor. The resolution of metal cations began to improve when methanol was added to the buffer. The resolution of metals increased as the amount of methanol added increased. The separation of metal cations in group I, II is shown in Fig. 4.12.

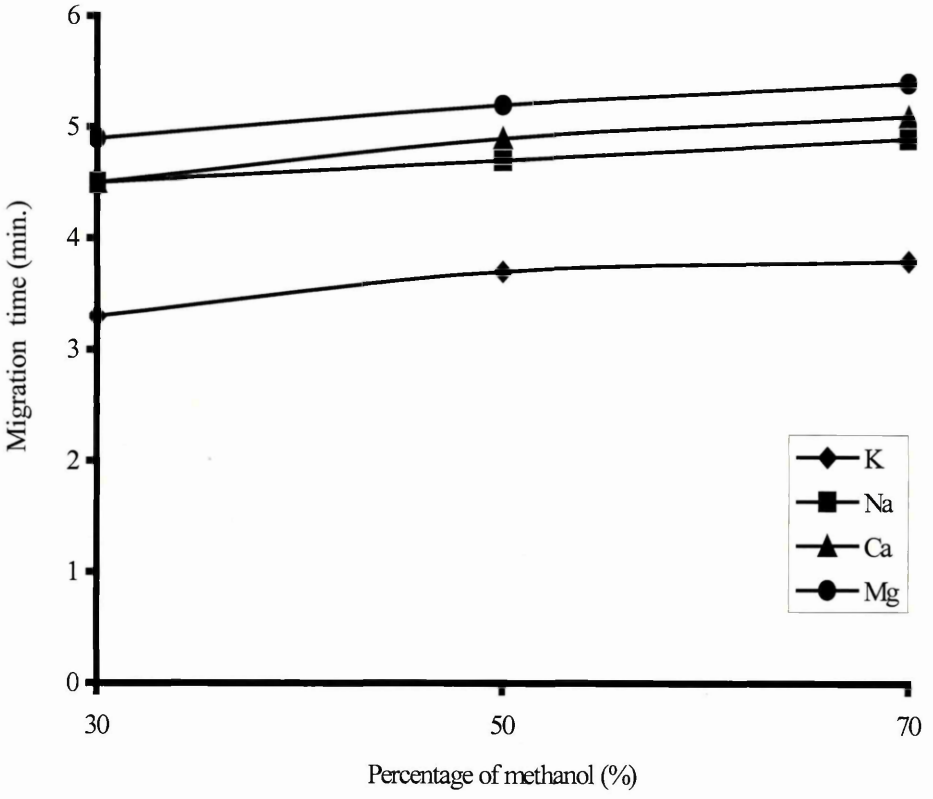


Fig. 4.12. Effect of different concentrations of methanol in buffer solution. Electrophoretic separation of four metal cations using 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine, various percentage of methanol (pH 4.0).

The separation was improved when 30% methanol was used while the pH was maintained at pH 4.0. The resolution of the electropherogram increased when the amount of methanol was increased from 30 % up to 70% of buffer composition. Most metals in group I and group II were well separated with good resolution after the addition of methanol. It was noted that increasing the amount of methanol in the buffer solution improved the resolution of metal cations. Based on the above experiment, the optimum concentration of methanol in the buffer composition was found at 50%. At this concentration most metal cations were well separated with a good resolution. This results supports the investigation by Yang et al. (62) on the simultaneous separation of ammonium and alkali, alkaline earth

and transition metal ions in aqueous. They have found that with the absence of methanol in background electrolyte, Li, Ni and Zn are co-migration but after the addition of methanol they can get a separation of Li, Ni and Zn. However they observe this effect of methanol up to 30% at the maximum amount for improving on the electrophoretic mobilities of the separation of cations. Other reported by Shi and Fritz (30) also was noted that the electrophoretic mobility decreases almost linearly as the percentage of methanol was added in the buffer solution. However they only experimented the methanol up to 20 % under their investigation. They obtained a good resolution by increasing the methanol concentration in the separation of eight metal cations using phthalic acid.

4.5.2.4. Reproducibility

A buffer composition of 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0 was employed to study the reproducibility of metals from group I and group II. In this study the standard mixtures containing 4 metals from group I and group II were injected to observe the reproducibility of migration time and absorbance. These metals were include K, Na, Ca and Mg. The procedure used was followed the outlined in Section 4.5.1.3. for ANOVA calculation.

4.5.2.4.1 Migration time (MT)

Full summarises reproducibility data for all four metals is shown in Table 4.4.

Elements	Mean result, min. ± SD [a]	ANOVA F value[c]
Potassium	3.65 [b] ± 0.05	0.06
Sodium	4.54 [b] ± 0.06	0.06
Calcium	4.85 [b] ± 0.07	0.02
Magnesium	5.26 [b] ± 0.09	0.05

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 4.4. Reproducibility data on migration time for metal cations at different batch samples using 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0.

Since the calculated value of F from series observations is less than the critical value of F, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 4.4 shows that the migration times were stable and capable of providing consistent and reproducible data. These values obtained for reproducibility on migration time is consistent with the results of Weston et al. (23) in their investigation on the analysis of K, Ca, Na and Mg using the buffer solution of UV Cat-2, tropolone, HIBA at pH 3. They have reported the reproducibility in less 2% of relative standard deviation (RSD) compared to less than 1.7% of RSD in this work. However the results obtained in this work were not as good as the results obtained by Weston et al. (64) on the analysis of K, Na, Ca and Mg using the buffer solution of 5 mmol/L, UV Cat-1 and 6.5 mmol/L HIBA at neutral pH. They have reported RSD on migration time was 0.34 for K, 0.37 for Na, 0.37 for Ca and 0.83 for Mg. Meanwhile Lin et al. (58) reported the RSD for migration time was 0.8 for K, 0.59 for Ca, 0.58 for Na and 0.47 for Mg. They have employed 3

mmol/l succinic acid with 5 mmol/L imidazole. Other reported by Riviello and Harrold (71) on RSD for migration time was 0.28 for K, 0.25 for Na, 0.26 for Ca and 0.25 for Mg.

4.5.2.4.2. Absorbance

Full summarises reproducibility data for all four metals is shown in Table 4.5.

Elements	Mean result, AU (x 10^{-3}) ± SD [a]	ANOVA *F* value[c]
Potassium	0.93 [b] ± 0.02	0.53
Sodium	1.87 [b] ± 0.04	0.15
Calcium	2.63 [b] ± 0.06	0.12
Magnesium	4.22 [b] ± 0.10	0.03

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 4.5. Reproducibility data on absorbance for metal cations at different batch samples using 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. The results obtained are given in Table 4.5 shows that the absorbance are fairly stable and capable for providing a consistent and reproducible data. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of Ni, Zn and Co with the employing of 5 mmol/L oxalic

acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of absorbance in this work.

4.5.2.5. Linearity

Figs. 4.13 – 4.16 show a linearity study for the group I and group II metal cations. The procedure used for linearity of the graph was followed as outlined in Sectioned 4.5.1.4. There were five concentration levels of multi-element standards used from 1.0 ppm to 100 ppm for the analysis using CE. Ten times at each concentration was injected and duplicates with the same set solution of each concentration were carried out sequentially. Lower concentration than 1 ppm were not tested. The average values of the results in linearity for K ($y = 7E\text{-}05x + 0.0001$; and $R^2 = 0.9959$), Na ($y = 7E\text{-}05x + 0.0002$; and $R^2 = 0.9953$), Ca ($y = 5E\text{-}05x + 0.0001$; and $R^2 = 0.9976$) and Mg ($y = 7E\text{-}05x + 0.0001$; and $R^2 = 0.9978$), were acceptable and can be fitted a straight line for the above range.

The non-linearity observed at concentration higher than 100 ppm with the deviations is about 6.9% for K ($y = 7E\text{-}05x + 0.0002$; and $R^2 = 0.9921$), the deviations is about 6.3% for Na ($y = 6E\text{-}05x + 0.0004$; and $R^2 = 0.9796$), the deviations is about 5.9% for Ca ($y = 5E\text{-}05x + 0.0002$; and $R^2 = 0.9931$) and the deviations is about 5.4% for Mg ($y = 6E\text{-}05x + 0.0002$; and $R^2 = 0.9822$). In general the sensitivity may not be constant over a long range for K, Na, Ca and Mg at higher concentrations by saturation of the signal (absorbance). The linearity achievable with UV detection in non-ideal optical cell such as a round capillary for all metals may be limited. Instrumental deviation s from Beer's Law

ultimately limit quantitative analysis. At high analyte concentrations, the deviation usually takes the form of decreased slope of the calibration curve (Figs. 4.13 - 4.16). The linear detection range is mainly limited by stray light reaching the detector. Ideally, all light should pass through the centre of the capillary, not through the wall. Note that the linear detection range is significantly lower than that observed in other established analytical instrument such as liquid chromatograph due to the small size and curvature of the capillary. The improvement in sensitivity for good linearity may be overcome by using other method e.g. different shape of capillary, stacking and standard enrichment etc.

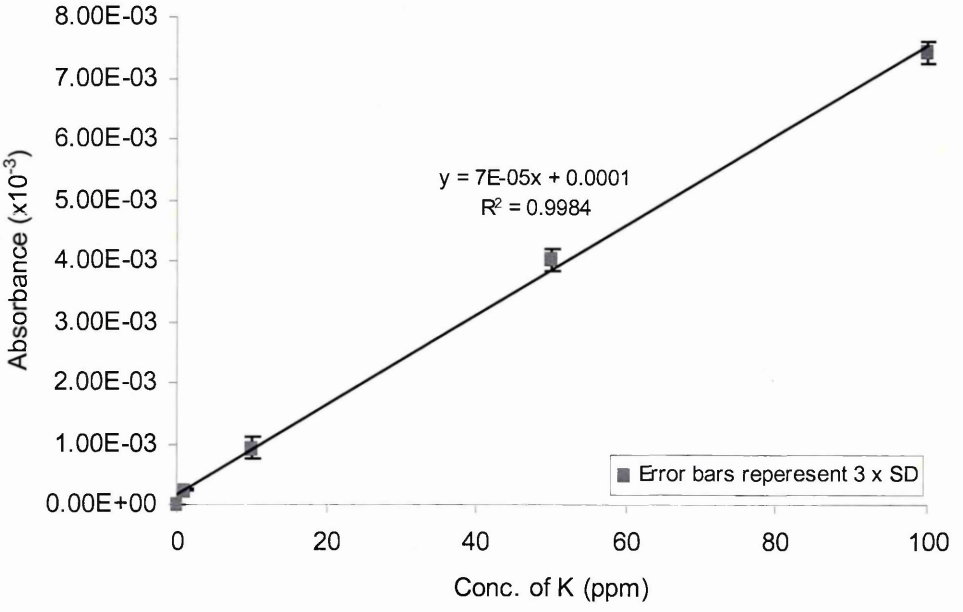


Fig. 4.13. Linearity range of K from group I&II. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0).

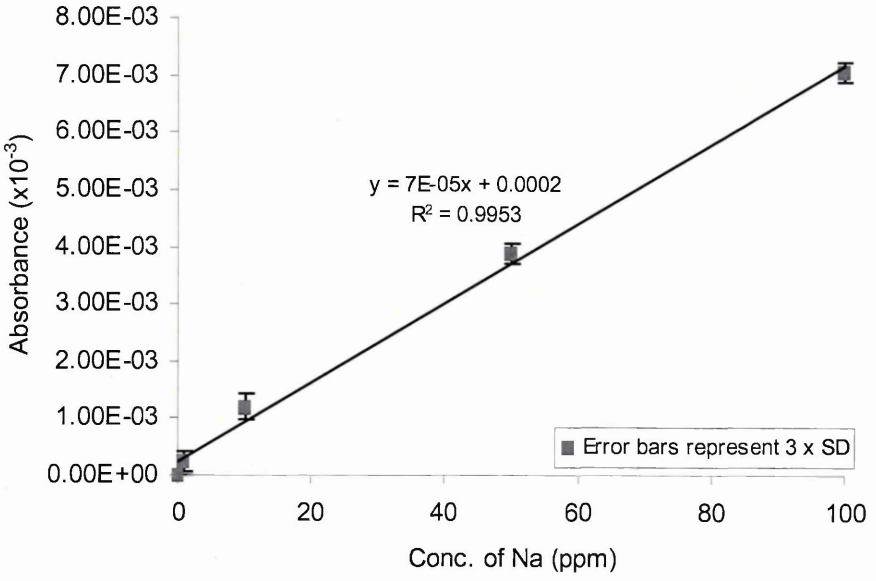


Fig. 4.14. Linearity range of Na from group I&II. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0).

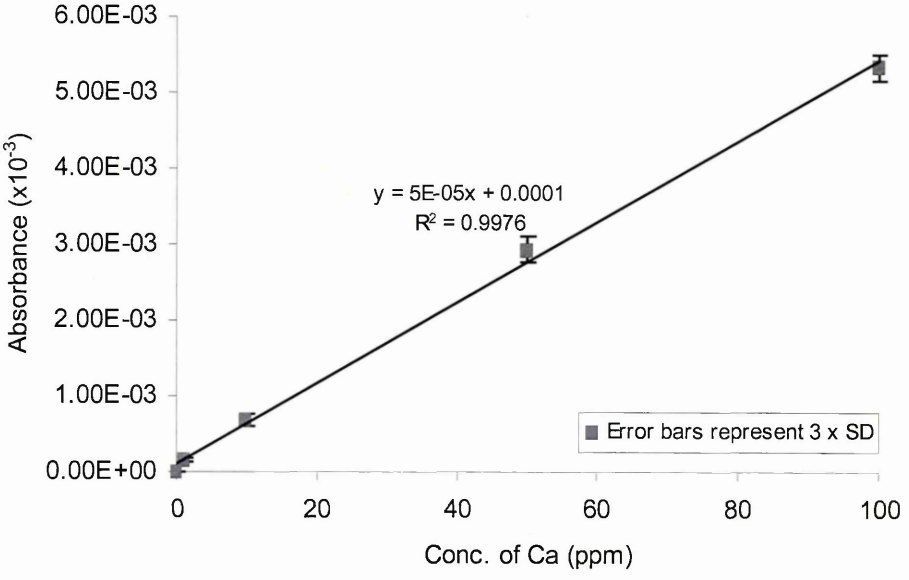


Fig. 4.15. Linearity range of Ca from group I&II. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0).

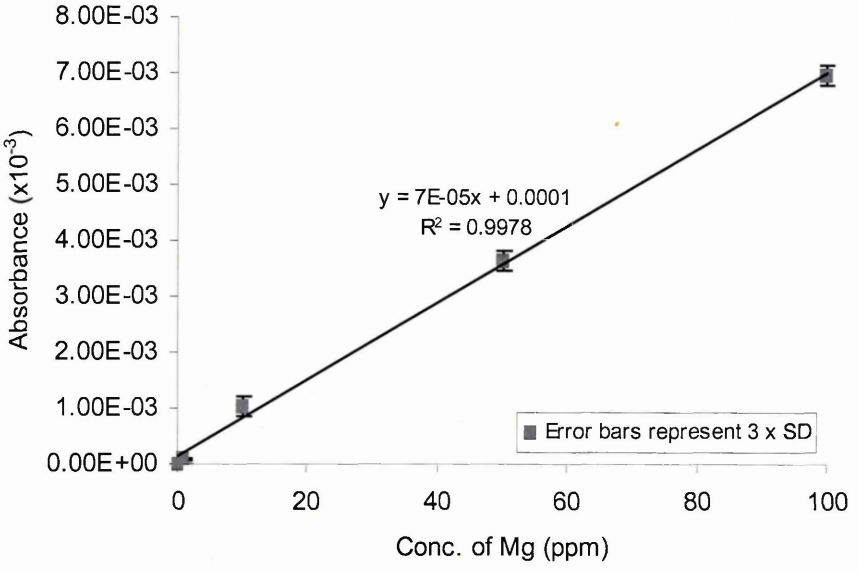


Fig. 4.16. Linearity range of Mg from group I&II. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0).

The overall linearity for K, Na, Ca and Mg that are found to be up to 100 ppm in this work are excellent compared to the previous work in similar field. Juang and Wu (186) reported the linear relationships between the peak area and concentration up to 50 ppm for K, Na, Ca and Mg. Meanwhile, Lin et al. (58) also reported the calibration graphs expressed as peak area versus concentration range between 0.1 to 10 ppm for Li but other metal such as K, Na and Mg exhibit hyperbolic-shape curves, It is apparent that the calibration sensitivity is much better in the lower concentration range. Korbeda et al. (56) reported the linear calibration curves between 5 to 50 ppm for the separation of K, Na, Ca and Mg. They used the buffer solution of 5 mmol/L UV-Cat1, 6.5 mmol/L HIBA at pH

4.4. In general, the linearity obtained is possible to get a straight line for quantitative analysis.

4.5.2.6. Limit of detection (LOD)

Serial dilutions of a mixture standard solutions were made to determine the detection limits of the system. Minimum detectable concentrations for metal cations based on a peak signal (absorbance) to noise (3:1) are given in Table 4.6. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0.

Metal cations	Mean of detection limit, (ppm ± SD)
Potassium	0.98[a] ± 0.12
Sodium	0.94[a] ± 0.15
Calcium	0.76[a] ± 0.10
Magnesium	0.80[a] ± 0.09

Notes: [a] = min. of 10 replicates injections

Table 4.6. Limit of detection for group I&II metals.

These values obtained in this work are consistent with the usual range as reported in several investigations in similar work. Lee and Lin (57) employed 10 mmol/L pyridine, 12 mmol/L glycolic acid at pH 4.0 obtained the limit of detection is 0.02 ppb for Na, 0.2 for Mg, 0.4 ppb for K and 0.4 ppb for Ca. Meanwhile, Weston et al. (64) is used UV Cat-1, 65 mmol/L HIBA at pH 4.4 reported the values of limit of detection is 0.222 ppm for K,

0.102 ppm for Ca, 0.096 ppm for Na and 0.055 ppm for Mg. Riviello and Harrold (71) using 4.0 mmol/L cupric sulphate, 4 mmol/L formic acid and 4 mmol/L 18-crown-6 at pH 3.0 obtained the limit of detection is 1 ppm for K, 0.4 ppm for Na, 0.33 for Ca and 0.32 for Mg.

4.5.3. Analysis of mineral water

4.5.3.1. Separation performance by IC

Calibration curves obtained for Na, K, Mg and Ca are given in Figs. 4.17 - 4.20. The procedure used for linearity of the graph was followed as outlined in Sectioned 4.5.1.4. There were five concentration ranges of multi-element standards used from (0 - 40) ppm for this analysis. Ten times at each concentration was injected and duplicates with the same set solution of each concentration were carried out sequentially. The graph is plotted after subtracting the blank value to give a curve passing through zero. The average values of the results in linearity for Na ($y = 3E+06x + 693808$; and $R^2 = 0.9984$), K ($y = 3E+06x + 910892$; and $R^2 = 0.9982$), Mg ($y = 1E+07x + 173333$; and $R^2 = 0.9999$) and Ca ($y = 5E+06x - 1E+06$; and $R^2 = 0.9998$) were acceptable and can be fitted a straight line for the above range.

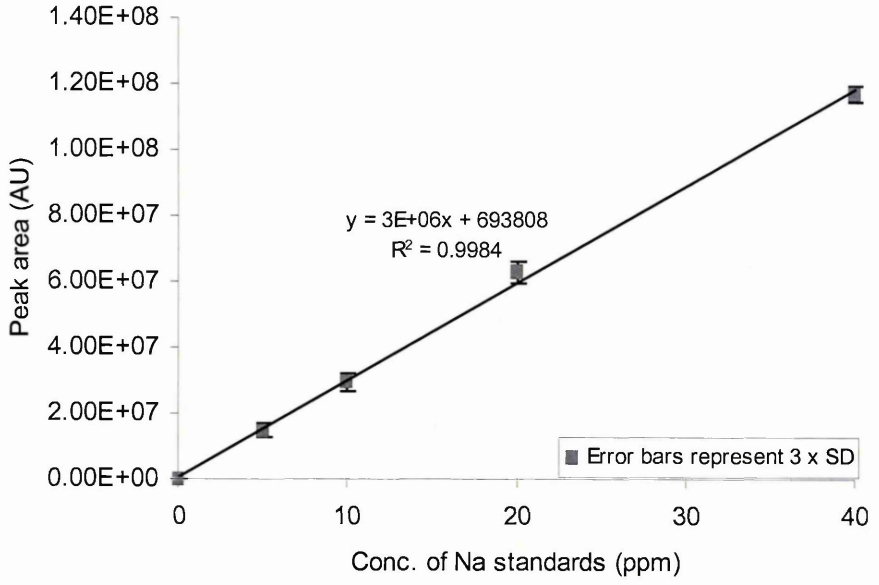


Fig. 4.17. The calibration curve of standard Na used for the investigation of group I&II metal cations in mineral water. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

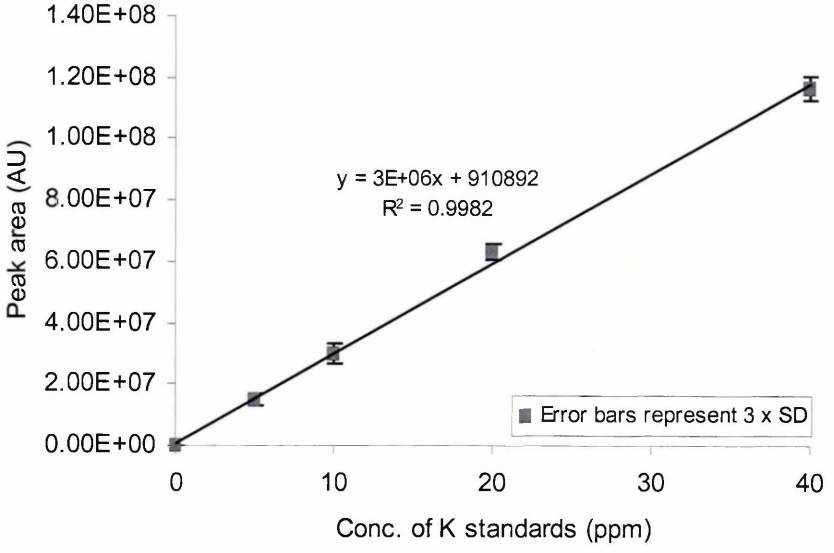


Fig. 4.18. The calibration curve of standard K used for the investigation of group I&II metal cations in mineral water. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

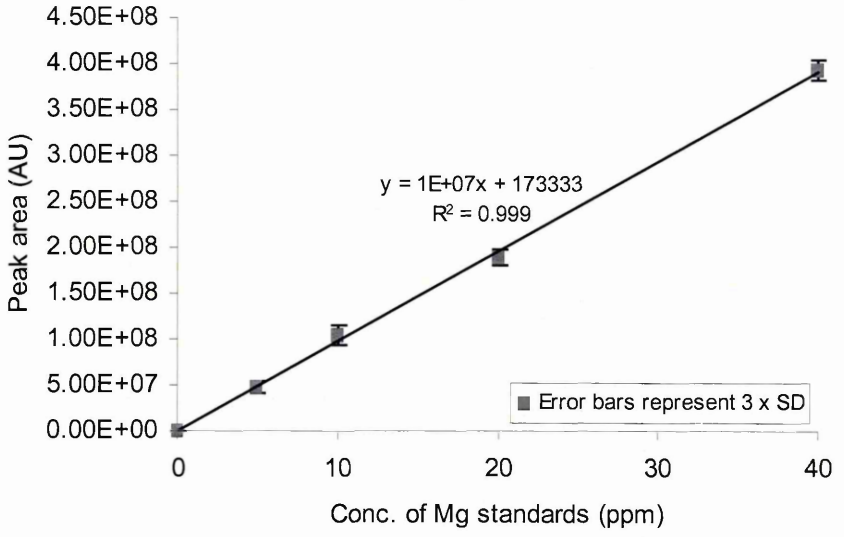


Fig. 4.19. The calibration curve of standard Mg used for the investigation of group I&II metal cations in mineral water. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

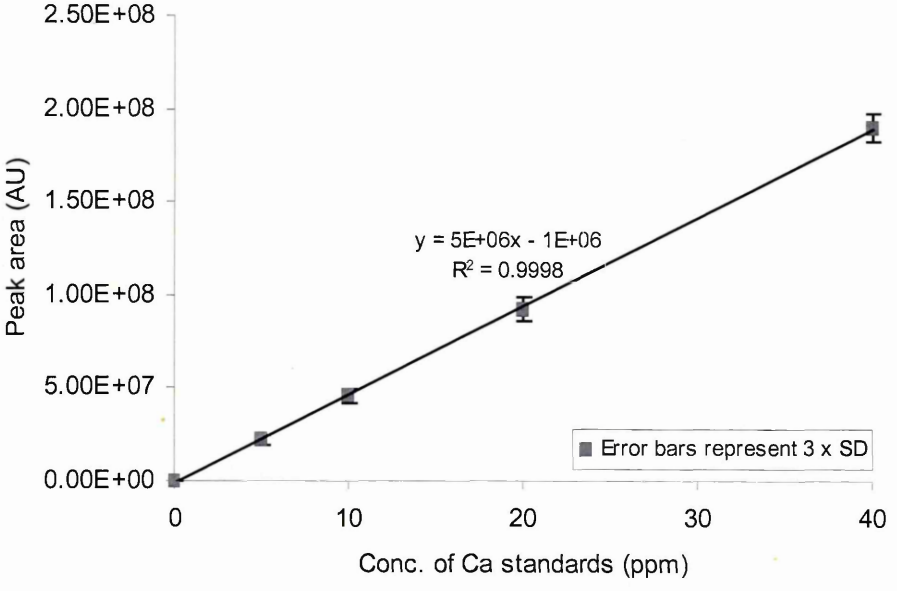


Fig. 4.20. The calibration curve of standard Ca used for the investigation of group I&II metal cations in mineral water. The eluents employed was 60 mmol/L HCl/6 mmol/L DAP.HCl.

As can be seen from Figs 4.17-4.20, a good calibration curve were plotted and than employed to determines the concentration level of metal cations in mineral water. Under this work only four metals from group I & II include Na, K, Mg and Ca were observed for IC with conductivity detection. All metals were separated in less than 11 minutes using 60 mmol/L HCl/6 mmol/L DAP.HCl. The successful separation of 4 metal cations is shown in Fig. 4.21. The migration order follows the sequence Na, K, Mg and Ca. All peaks are completely baseline resolved.

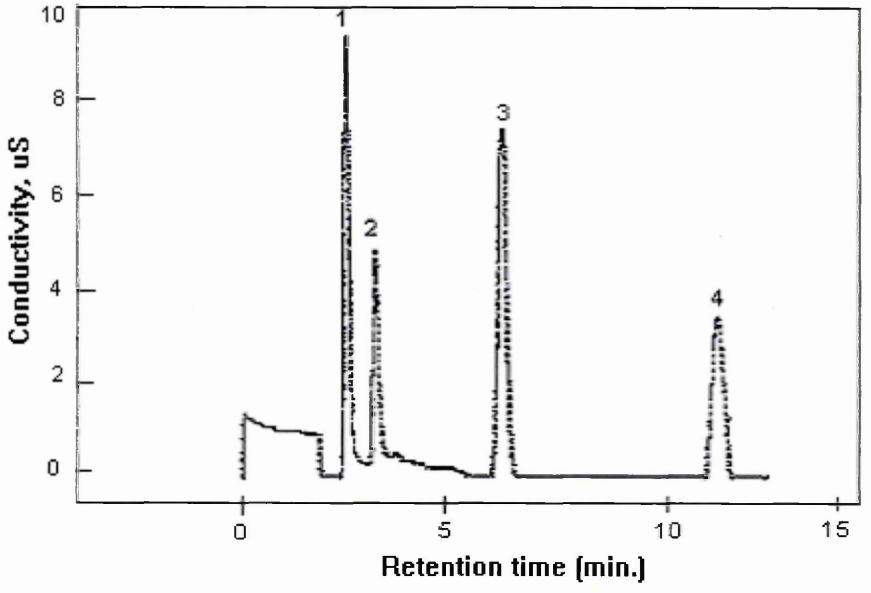


Fig. 4.21. IC analysis of Volvic mineral water. Eluents, 60 mmol/L HCl with 6 mmol/L DAP.HCl; flow rate 1 mL/min., regenerant 100 mmol/L TBAOH; injection volume 20 µL; Conductivity detector; Peaks: 1=Na (9.4 ppm); 2=K (5.7 ppm); 3=Mg (6.1 ppm); 4=Ca(7.9 ppm).

Fig. 4.21 shows a representative chromatogram of Volvic mineral water. Group I and group II metals were well separated by using a combination of 60 mmol/L HCl /6 mmol/L DAP.HCl. Two values of DAP.HCl eluents were tried to obtain a good separation of metal cations . The chromatogram shows that by using 60 mmol/L HCl/6 mmol/L DAP.HCl a faster separation compared to the lower concentration was obtained. The metal cations will be appeared at less than 11 minutes while using 40 mmol/L HCl/2 mmol/L DAP.HCl they came out after 38 minutes. The peaks become more symmetric with increasing concentration. The buffer concentration has a great effect on the peak shape. If we compare the results with those achieved under the same conditions in previous studies, in spite of the use of a significantly longer capillary, the increases in buffer concentration case much more symmetrical peaks. The plate number increases together with buffer concentration. However, this optimisation step is limited. A high buffer concentration causes an increase of noise, and temperature and, accordingly, Joule heating increases. Plate numbers achieved for each metals are listed in Table 4.7.

Metal cations	Number of theoretical plates per meter (N/m)
Sodium	299,301
Potassium	152,244
Magnesium	274,172
Calcium	144,786

Table 4.7. Peak efficiency for 60 mmol/L HCl/6 mmol/L DAP.HCl using IC-conductivity detection.

This result is in agreement with the finding reported by Thienpont et al. (199) that the eluent concentration used will affects the separation of metal cations. Faster separation elution could have been achieved by increasing the strength of the mobile phase. They employed 2 mmol/L DAP.HCl and 40 mmol/L HCl to get a separation of calcium and magnesium in less than 9.4 min with IC system via conductivity detection. The retention times is this work considerably lower than with a conventional column combination using the same eluents (approximately 19 min for calcium and 16 min for magnesium) another experiment by manufacturer (201). This observation supports the results obtained by Morawski et al. (196) for the analysis of cationic nutrients in foods by IC system. They used 0.1 mmol/L EDTA and 3 mmol/L nitric acid with conductivity detection to analyse cations from various sample matrices such as pretzels (salted), parsley (dried), bread crumbs, parmesan cheese and peanut butter. All metals cations including Na, K, Mg and

Ca were eluted after 20 min. The resolution for each metals is no so good and baseline resolved). This finding also demonstrated that the IC method used in this work is suitable for quantitative analysis and comparably good with previous works on IC system.

4.5.3.2. Separation performance by CE

Calibration curves obtained for Na, K, Mg and Ca are given in Figs. (4.22 - 4.25). The procedure used for linearity of the graph was followed as outlined in Sectioned 4.5.1.4. There were five concentration ranges of multi-element standards used from (0 - 20) ppm for this analysis. Ten times at each concentration was injected and duplicates with the same set solution of each concentration were carried out sequentially. The graph is plotted after subtracting the blank value to give a curve passing through zero. The average values of the results in linearity for K ($y = 9E\text{-}05x + 6E\text{-}05$; and $R^2 = 0.9944$), Na ($y = 0.0003x + 9E\text{-}05$; and $R^2 = 0.9962$), Ca ($y = 0.0003x + 0.0002$; and $R^2 = 0.9938$) and Mg ($y = 0.0005x + 0.0003$; and $R^2 = 0.9939$) were acceptable and can be fitted a straight line for the above range.

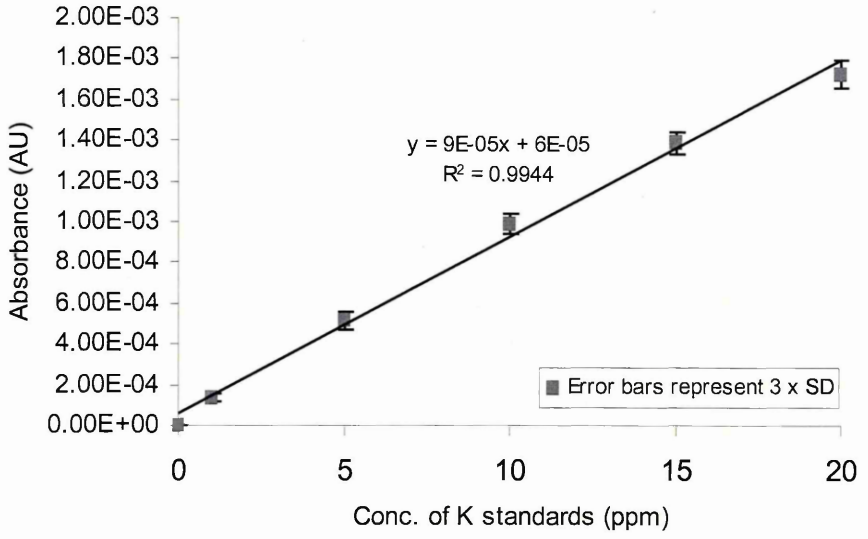


Fig. 4.22. The calibration curve of standard K used for the investigation of group I&II metal cations in mineral water. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine, 50% methanol (pH 4.0).

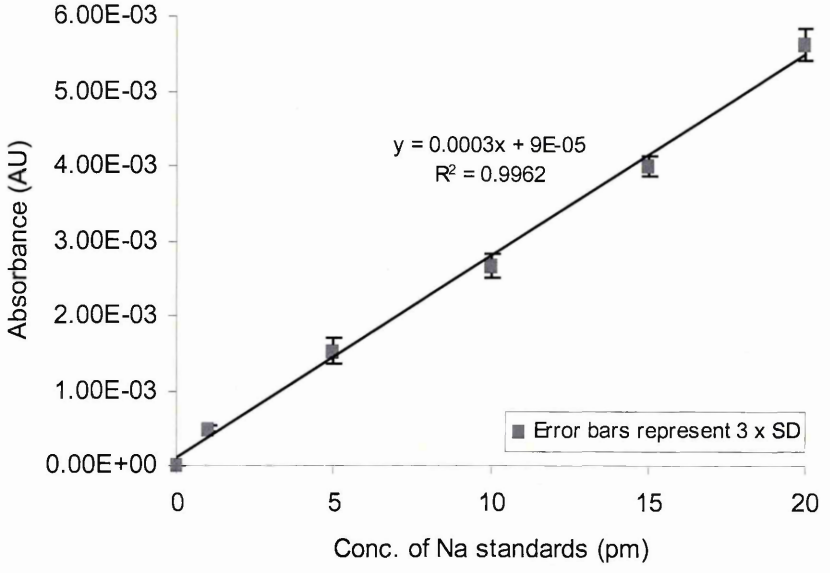


Fig. 4.23. The calibration curve of standard Na used for the investigation of group I&II metal cations in mineral water. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine, 50% methanol (pH 4.0).

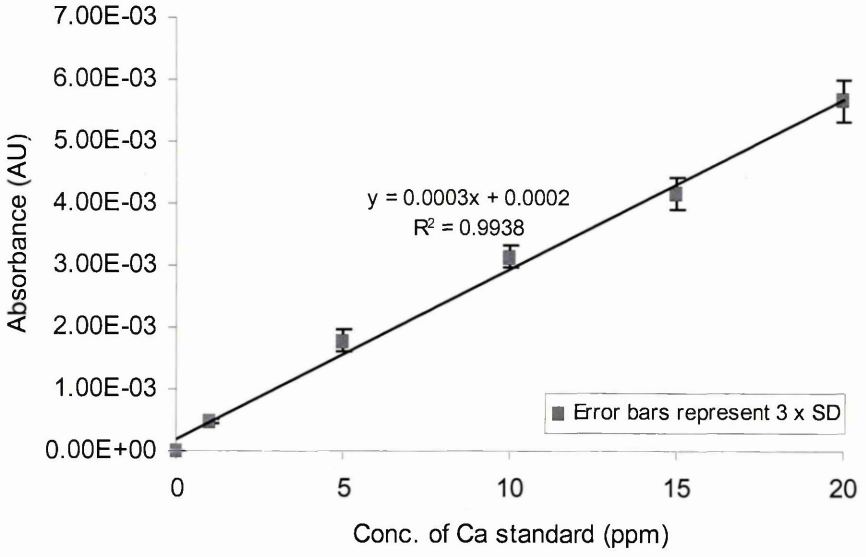


Fig. 4.24. The calibration curve of standard Ca used for the investigation of group I&II metal cations in mineral water. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine, 50% methanol (pH 4.0).

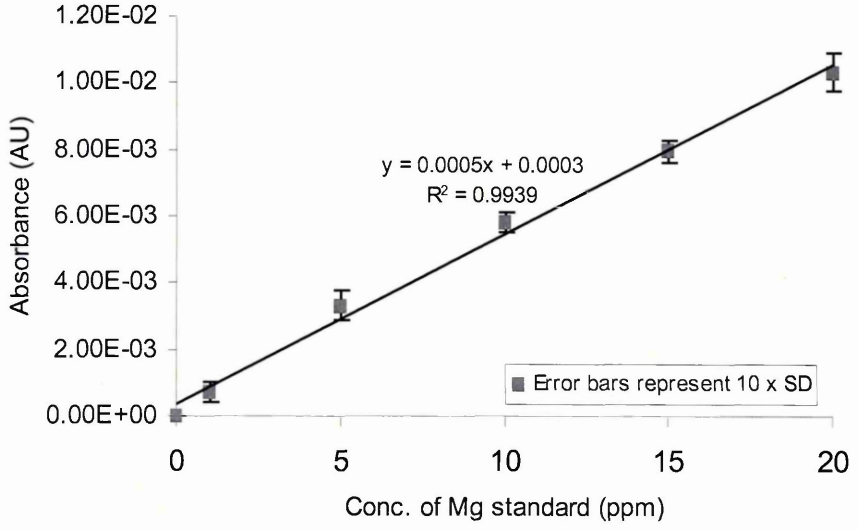


Fig. 4.25. The calibration curve of standard Mg used for the investigation of group I&II metal cations in mineral water. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine, 50% methanol (pH 4.0).

As can be seen from Figs 4.22-4.25, a good calibration curve were plotted and than used to determines the concentration level of metal cations in Volvic mineral water. Fig 4.26

shows a representative chromatogram of the separation of metal cations in group I and group II for Volvic mineral water. The buffer employed using 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0).

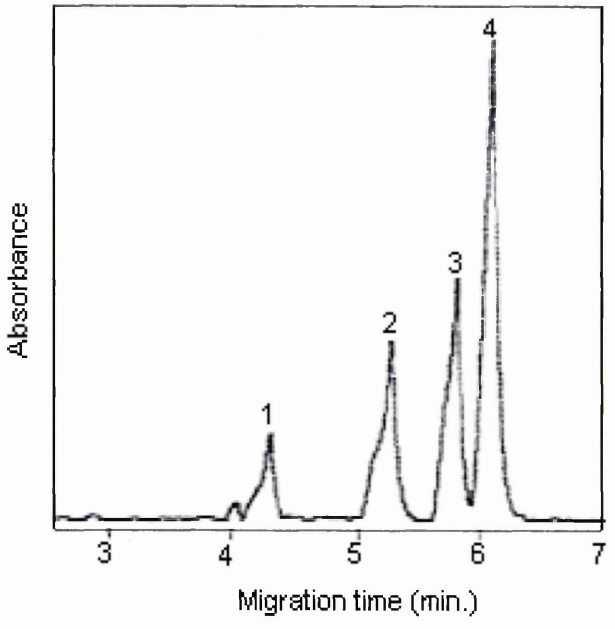


Fig. 4.26. CE analysis of Volvic mineral water. The buffer employed was 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0). Applied voltage 30 kV; Hydrodynamic injection with 30s of length injection; pressure applied was 20 mBar; UV/Vis detector; Peaks: 1=K (5.7 ppm); 2=Na (9.4 ppm); 3=Ca (7.9 ppm); 4= Mg (6.1 ppm).

Four metals from group I & II include K, Na, Ca and Mg were observed for CE with UV/Vis detection. Four metals were separated in less than 6.5 minutes using 500 mmol/L

acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0. The successful separation of 4 metal cations is shown in Fig. 4.26. The migration order follows the sequence K, Na, Ca and Mg. All peaks are completely baseline resolved. The peaks become more symmetric with increasing concentration. The buffer concentration has a great effect on the peak shape. If we compare the results with those achieved under the same conditions in previous studies, in spite of the use of a significantly longer capillary, the increases in buffer concentration case much more symmetrical peaks. The plate number increases together with buffer concentration. However, this optimisation step is limited. A high buffer concentration causes an increase of noise, and temperature and, accordingly, Joule heating increases. Plate numbers achieved for each metals are listed in Table 4.8.

Metal cations	Number of theoretical plates per meter (N/m)
Potassium	319,393
Sodium	343,153
Calcium	286,543
Magnesium	204,723

Table 4.8. Peak efficiency for 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0 using CE-UV/Vis detection.

These values obtained for the overall migration time is consistent with the results of Weston et al. (23) in the analysis of group I and group II metal cations for commercial cough syrup using the buffer solution of 5mmol/L UV-Cat1, 6.5 mmol/L HIBA at pH 4.2. They reported the overall migration time in less 5 min to get a separation of K, Ca, Na and Mg compared to less than 6.5 min in the analysis of a Volvic mineral water. They obtained a good peak shape with baseline resolution between the majority of the peaks. Another excellent result was obtained by Weston et al. (64) on the analysis of a standard solution of K, Na, Ca and Mg using 5 mmol/L UV Cat-2 and tropolone at pH 4. They reported the overall migration time in less than 3 min to get a separation of K, Ba, Sr, Ca, Na, Mg and Li. The mobility of UV Cat-2 is similar to that sodium, resulting in an almost symmetrical peak shape for sodium. The metals migrating faster than sodium show a little degree of fronting, while the metals migrating slower than sodium show a little tailing. Under these conditions of more closely matched mobilities, the concentration of the analytes may even approach the concentration of the background electrolyte without electromigration dispersion producing a significant effect. Meanwhile Lin et al. (58) reported the migration time in less than 2 min for potassium, barium, calcium, sodium, magnesium and lithium. They used acetic, glycolic, lactic, HIBA, oxalic malonic, malic, tartaric, succinic and citric acid as a complexing reagent for the separation six alkali and alkaline erath metal cations. They found that, if the ions that co-migrate closer to the carrier imidazole have better N values that ranging from 16, 000/m to 760, 000/m. Thus the fronting K and the trailing Li both have poorer N values than the other ions and are affected less by the type of acid present. The N values for Ca and Mg varied most significantly; they are particularly low in oxalic acid and citric acid, in which the stability constants of the complexing

reagent with these two ions is high. In citric acid, Na has the best N, but all other divalent ions have the poorest N. In glycolic acid, Mg has the highest N of all, reaching 760, 000/m.

4.6. Conclusion

Most of the development and applications in CE have concerned organic compounds and biological macromolecules, but there have been a few reports discussing the separation of small inorganic ions. CE offers great potential for the separation of inorganic ions particularly metal cations. The method using CE also is quite straightforward as compared to ion chromatography methods. Most metal cations in mineral water, river water and rain water can be separated by CE with indirect UV/Vis absorbance detection in a single run.

The separation of inorganic ions by ion chromatography methods is reasonably well-documented. Initially, work using established methods was undertaken to develop the necessary skills required for sample handling. The use of 60 mmol/L HCl/6 mmol/L DAP.HCl was demonstrated to obtain a separation of metal cations in from group I and group II in less than 11 minutes. Alteration of buffer concentration had not much influence in the retention time, but would be affected the area and absorbance for most metal cations. Reproducibility of retention time and peak absorbance for metal cations from group I and group II metals was good ($F_{obs.} < F_{crit.}$). Limits of detection obtained were from high ppb to low ppm range, with good linearity (correlation coefficients ranges 1 - 80 ppm except for Na with $R^2 > 0.99$).

The use of 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0) for the separation of metal cations in sample of mineral water by UV/Vis detector was demonstrated. Separation of K, Na, Ca and Mg could be obtained in less than 6.5 min. The use of acetic acid in-conjunction with 4-methylbenzylamine also showed that the

analysis can be done faster for the separation of metal cations in group I and group II when compared with ion chromatography methods. Reproducibility of retention time and peak absorbance for metal cations from group I and group II metals was good ($F_{obs.} < F_{crit.}$). Limits of detection obtained were from high ppb to low ppm range, with good linearity (correlation coefficients ranges 1 - 100 ppm with $R^2 > 0.99$). The method used is quiet straightforward to operate especially for the analysis of metals in the sample of mineral water.

CHAPTER 5

SEPARATION OF METAL CATIONS BY CE USING A UV-VIS DETECTOR

5.1. Introduction

The separation of metal cations in CE relies on the differences in their effective electrophoretic mobilities. Most of the metals in group I&II are able to be separated without forming metal complexes. The separation of metals from the transition series may be difficult to obtain without complexation due to the similarity in their electrophoretic mobilities. There are many ways to enhance the separation of metals in group I, II and transition metals including partial complexation, complexation with macrocyclic ligands, solvation, ion pairing and pre-column complexation. Most of these methods have already been discussed in Chapter 2. This chapter will focus on the use of a organic acids and acetylacetone (acac) as complexing reagents for the separation of metal cations from group I, II and transition metals.

This work investigates whether oxalic acid, acetylacetone and acetic acid can be used to obtain a separation of metals in a single experiment. K, Na, Ca, Mg, Co, Ni, Al, Cr, Fe, Zn, Cu, Mn, Ba, Cd and Pb are used to verify the separation capability of the methods. Ammonium is also included in this investigation to observe its separation in the presence

of metals. The effects of concentration, pH, viscosity and analyte on the migration times were studied. The optimised methods were used to establish the reproducibility, linearity and limit of detection of each method.

5.2. Experimental procedures

5.2.1. Instrumentation

The CE instrument used was described in section 3.2.1 in conjunction with a UV/Vis detector also mentioned in section 3.2.3. Indirect UV/Vis was employed at a wavelength of 214 nm with 4-methylbenzylamine as chromophore. A positive separation voltage of between 20 kV to 30 kV was used throughout the analysis. The fused-silica capillary was described in section 3.2.2 and the length of each capillary was fixed to ensure that reproducibility of migration times was maintained in all experiments.

5.2.2. Materials and reagents

The reagents used were as referred to in section 3.3. Working standard solutions containing 10 ppm of each metal cation were prepared according to the following steps. 10 mL was taken from stock solution of each metal as described in section 3.3 and then made-up to 100 mL with deionised water in a volumetric flask. Ammonium samples were prepared from ammonium stock solutions as mentioned in section 3.3. The percentage of methanol and acetonitrile were freshly prepared as required in accordance with the procedure outlined section 3.3.

5.2.3. Capillary preparation and cleaning

The fused silica capillary employed was as described in section 3.2.2. The conditioning step was discussed in section 3.4.1, washing procedures were as in section 3.4.2 and buffer replenishment procedures as in section 3.4.3. These steps were performed each time prior to an analysis being carried out.

5.2.4. Injection procedures

In this study, the hydrodynamic injection mode was used to affect sample loading. The sample solution was injected through the capillary inlet for a duration and at a pressure dependent on the concentration of sample employed. The procedure used was followed the outlined in section 3.6.1.

5.2.5. Buffer preparation

The oxalic acid, acac, acetic acid and 4-methylbenzylamine solutions used in this investigations were prepared by mixing appropriate amounts of the stock buffer solutions as outlined in section 3.5.1 and corresponding volumes of reagent methanol in a 100-ml volumetric flask. The pH adjustment, and degassing and filtration methods employed were followed the procedure as outlined in section 3.6.3 and 3.6.4.

5.3. Results and discussion

5.3.1. Studies of metal separations using an oxalate buffer

The effects of diprotic oxalic acid on the separation of metal cations were complex, depending on the pH. The complexation of metal ions is influenced by pH (129); a pH range between 3.5 to 4.4 (pK_a of oxalic acid = 1.23, 4.19) was studied with oxalic acid added to the buffer (162).

The structure of oxalic acid is shown below:

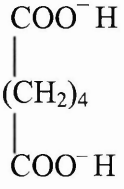


Fig. 5.1. Structure of oxalic acid

5.3.1.1. Effect of changing buffer concentration

In this experiment the concentration of 4-methylbenzylamine was fixed at 6.9 mmol/L and the concentration of an oxalic was increased from 1 mmol/L to 10 mmol/L while the buffer pH was maintained at 4.0. The variation of migration times versus concentration is shown in Fig. 5.2 and shows that when the concentration of oxalic acid is increased, the migration times of group I&II metals and Cd initially fell and then increased. The result was an agreement with the previous investigation (58) using oxalic acid demonstrated that the separation between Li and Ca was very poor at high concentration (3.4 mmol/L) but

can be improved when the buffer concentration reduced to low concentration (1.9 mmol/L). The changes in migration times of group I&II and transition metals were seen to all be affected similarly and not much dependent on the altering of the concentration of oxalic acid. Fig. 5.2 indicates that co-elution of several species occurs at 1 mmol/L oxalic acid.

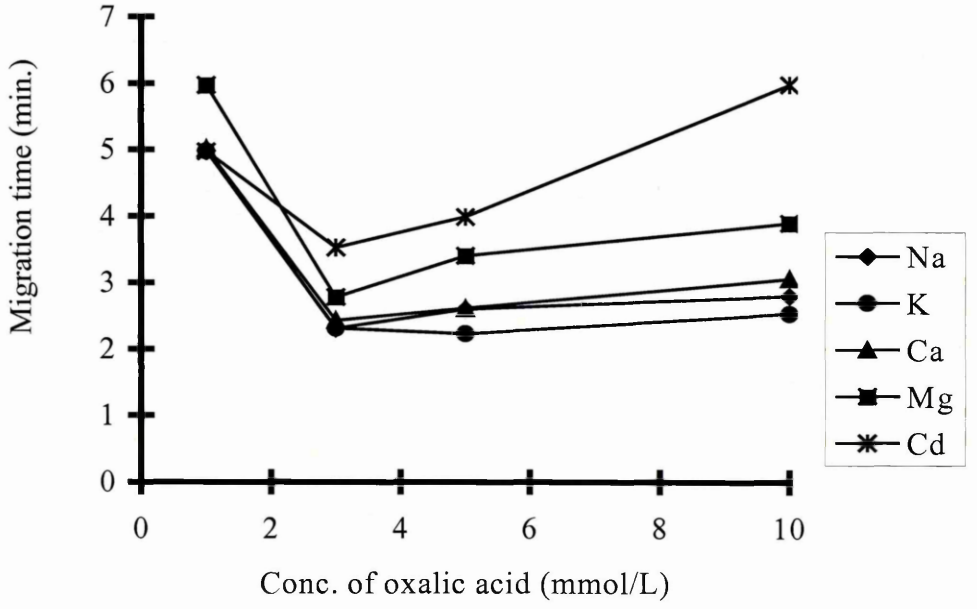


Fig. 5.2. Effects of changing the concentration of oxalic acid. The buffer employed was 6.9 mmol/L 4-methylbenzylamine at pH 4.0.

Based on the above study the optimum concentration of oxalic acid employed was found at 5 mmol/L. At this concentration most metal cations were well separated with a good resolution. This work also confirmed observations of Lin et al. (58) for the employing of monoprotic acid as a complexing agent in their investigation. They found that by changing

the concentration of acetic acid used in the buffer solution may affects the separation of the metal cations include K, Ba, Ca, Na, Mg and Li. However they do not investigate the separation of transition metals in their work using the above buffer conditions.

5.3.1.2. Effect of pH

In the separation of metal cations the effective mobility of an ion is the summation of EOF and electrophoretic mobility. In this study a weak complexing agent is used to enhance any differences in the electrophoretic mobilities. The net mobility is the weighted average of the mobility of the free metal ion and it's various complexes.

The effect of pH on the migration times of metal cations was investigated and the data are shown in Figs. 5.3 and 5.4. The buffer concentration of 4-methylbenzylamine was maintained at 6.9 mmol/L due to a stable baseline at this concentration. Meanwhile oxalic acid was kept constant at 5 mmol/L in order to study the affect on pH. The pH was increased using N,N-diethylethanolamine and decreased with acetic acid to the appropriate pH value. The migration times of the 5 metal ions were observed over a particular range of pH. The voltage was maintained at 20 kV. A separation was achieved when using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine with a migration order of K, Na, Ca, Mg and Cd. At pH of 3.5 the Na and Ca co-elute. Fig. 5.3 shows that after the pH buffer is increased from pH 3.5 to pH 5.0, the migration times of all solutes decrease due to an increase in the EOF. The previous work using oxalic acid as a buffer component (58) has been shown that the resolution between Li and Ca was poor at pH 3.0 but were well separated when the pH buffer was changed to 4.0. Weston et al.

(22) was observed a similar trend for the effect of pH on the migration time of eleven alkali, alkaline earth and transition metals of the altering the pH. The combination of 6.5 mmol/L HIBA at pH 4.4 was employed in their investigation. They found that as the pH is lowered, the migration time is increase, due to decrease in the EOF. Likewise, as the pH increased, the migration times decrease, due to increase in the EOF. In another experiment, Weston et al. (23) also found that K and NH_4^+ co-elute at pH 6.15 owing to their identical equivalent ionic conductivity. By altering the pH of the electrolyte, the ionization of alkali metals and their mobilities will be essentially unaffected. However, as the pH of electrolyte is increased, the NH_4^+ becomes progressively less protonated (pK_b of NH_4^+ = 4.75) and its apparent mobility decreases. At pH 8.5, the apparent mobilities of K and NH_4^+ become sufficiently different to permit an effective separation.

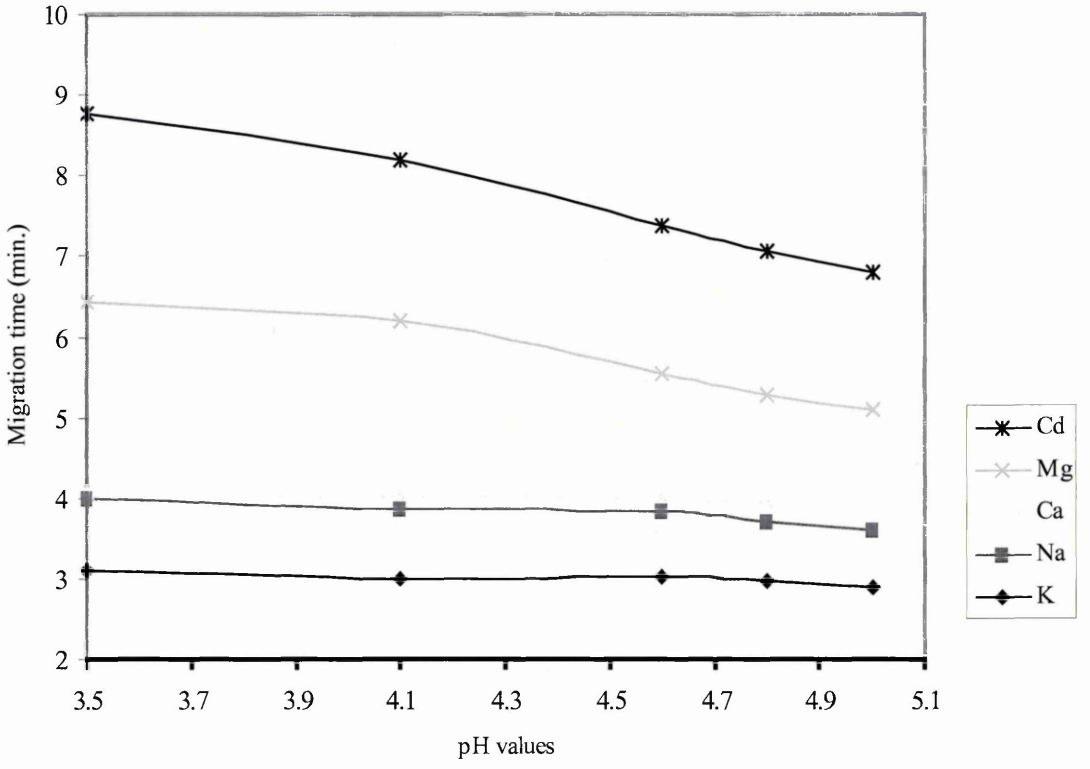


Fig 5.3. Effect of increasing pH. The buffer employed was 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine.

Several metal cations co-eluted and moved slower towards the detector at pH 3.5 and no peaks were observed at pH less than 3. At this stage, most of the group I, II and Cd are unable to separate due to the low degree of ionisation of silanol groups at pH below 3. Even though there is no EOF the cations will migrate towards the cathode.

Fig. 5.4 shows that when the pH of the buffer is decreased, the migration times of group I&II metals and Cd increase due to an decrease in the EOF. This is because the velocity of the EOF relies on the number of dissociated silanol groups on the capillary

wall. The negatively charged silanoate group will be generated more as the pH of the buffer increases.

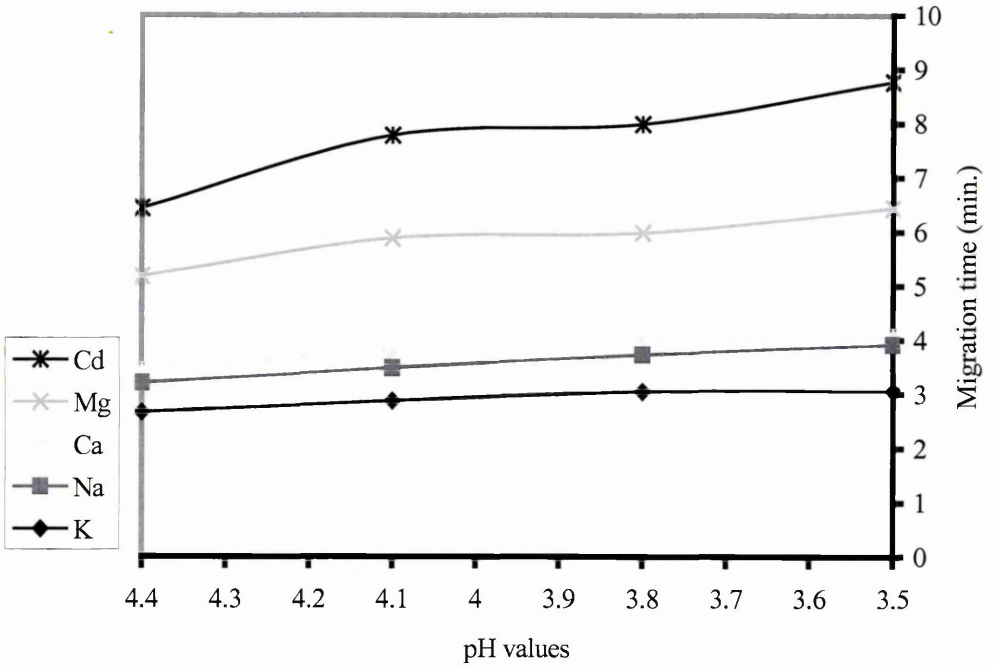


Fig. 5.4. Effect of decreasing pH. The buffer employed was 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine.

In general, Figs. 5.3 and 5.4 indicate that there are two groups of metals affected by changing the buffer pH. First, group I metals are not much influenced by the change of pH as shown by the slope of migration times versus pH. The second observation e.g. the slope for calcium, magnesium and cadmium is marked by changed as the pH is increases or decreases. Usually in the separation of most alkali and alkaline earth cations, complexation does not occur and the slope can be predicted to follow the changes in mobility of the EOF with the altering of the buffer pH. The migration times of divalent

cations and Cd are more affected because of increased tendency to complex with oxalic acid. This degree of complexation is affected by the pH of the solution. The effects of diprotic acids e.g. oxalic acid, on the separation of divalent metals were complex depending also on the pH. The acid dissociation constants, pK_1 and pK_2 of oxalic acid is 1.23 and 4.19. Apparently, this was due to the complex formation of divalent ions and cadmium with the ionic conjugate base. It was indicated that the optimum pH was found at 4.0 to obtain a good separation with a reasonable analysis times for metal cations.

5.3.1.3. Matrix effects

The procedure used was followed the outlined in Section 4.5.1.3. for ANOVA calculation. The results obtained are given in Tables 5.1 - 5.3 and data evaluated statistically.

5.3.1.3.1. Migration time (MT)

Full summarises reproducibility data for all five metals is shown in Table 5.1.

Elements	Single stds.		Mixed stds.	
	Mean result, min. ± SD [a]	ANOVA *F* value[c]	Mean result, min. ± SD [a]	ANOVA *F* value[c]
Potassium	3.50 [b] ± 0.01	0.07	4.24 [b] ± 0.05	1.09
Sodium	4.60 [b] ± 0.01	0.25	5.14 [b] ± 0.08	1.65
Calcium	5.51 [b] ± 0.07	0.06	5.84 [b] ± 0.10	1.24
Magnesium	6.52 [b] ± 0.09	0.63	7.03 [b] ± 0.17	1.48
Cadmium	9.52 [b] ± 0.09	0.42	9.16 [b] ± 0.25	0.90

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.1. Matrix effects on the migration times data for single and mixed standards solution at different batch samples using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine at pH 4.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.1 shows that migration times are stable and capable to provide a consistent and reproducible data for both single standard and mixtures standard solution. In general, the migration times and absorbance are quite consistent and reproducible with good standard deviation. However, the migration times of all metal cations in mixed standard solutions were slightly slower when compared to metal cations in a single standard solution. The differences is due to matrix effect of metal cations of group I, II and transition metals when they were mixed together in a single run. This observation

supports the results obtained by Morawski et al. (196) for the analysis of cationic nutrients from foods by ion chromatography. They used 0.1 mmol/L EDTA and 3 mmol/L nitric acid to analyse Li, Na, NH_4^+, K, Mg, Ca, Sr and Ba. They have found that the presence of multi-analyte at different concentrations will affect decreasing in retention time for each analyte components. However, they have not observed any matrix effects on migration time for transition metals in their investigation.

5.3.1.3.2. Absorbance

Full summarises reproducibility data for all five metals is shown in Table 5.2.

Elements	Single stds.		Mixed stds.	
	Mean result, AU(x10^{-3}) ± SD [a]	ANOVA *F* value[c]	Mean result, AU(x10^{-3}) ± SD [a]	ANOVA *F* value[c]
Potassium	1.12 [b] ± 0.01	0.08	1.41 [b] ± 0.04	0.02
Sodium	1.91 [b] ± 0.02	1.80	2.65 [b] ± 0.04	0.90
Calcium	5.32 [b] ± 0.13	0.02	5.83 [b] ± 0.10	1.36
Magnesium	3.53 [b] ± 0.04	1.55	3.92 [b] ± 0.10	0.91
Cadmium	1.11 [b] ± 0.02	1.80	1.60 [b] ± 0.03	0.04

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.2. Matrix effects on absorbance data for single and mixed standard solution at different batch samples using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine at pH 4.

Since the calculated value of F from series observations is less than the critical value of F, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.2 shows that absorbance were stable and able to provide consistent and reproducible data for both single standard and mixed standard solution except for Cd. The high standard deviation for cadmium may be due to the stability of formation complex was much influenced in mixed standard solutions compared to single standard solutions. This observation supports the results obtained by J. Morawski et al. (196) for the analysis of cationic nutrients from foods by ion chromatography. They used 0.1 mmol/L EDTA and 3 mmol/L nitric acid to analyse Li, Na, NH_4^+, K, Mg, Ca, Sr and Ba. They have found that the presence of multi-analyte at different concentrations will affect decreasing in retention time for each analyte components. However, they have not observed any matrix that will affects absorbance of the transition metals in their investigation. This finding also shows that the absorbance for each metal cations does not influenced by matrix effect.

5.3.1.3.3. Peak area

Full summarises reproducibility data for all five metals is shown in Table 5.3.

Elements	Single stds.		Mixed stds.	
	Mean result, peak area ($x10^6$) ± SD [a]	ANOVA *F* value[c]	Mean result, peak area ($x10^6$) ± SD [a]	ANOVA *F* value[c]
Potassium	0.06 [b] ± 0.03	1.80	0.10 [b] ± 0.04	1.55
Sodium	0.13 [b] ± 0.01	1.80	0.17 [b] ± 0.01	0.01
Calcium	0.23 [b] ± 0.13	1.06	0.20 [b] ± 0.10	0.01
Magnesium	0.42 [b] ± 0.01	1.80	0.46 [b] ± 0.02	1.51
Cadmium	0.04 [b] ± 0.02	0.90	0.06 [b] ± 0.02	1.03

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.3. Matrix effects on the peak area data for single and mixed standard solution at different batch samples using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine at pH 4.0.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.3 shows that peak area are stable and capable to provide a consistent and reproducible data for both single standard and mixtures standard solution. This observation supports the results obtained by J. Morawski et al. (196) for the analysis of cationic nutrients from foods by ion chromatography. They used 0.1 mmol/L EDTA and 3 mmol/L nitric acid to analyse Li, Na, NH_4^+, K, Mg, Ca, Sr and Ba. They have found that the presence of multi-analyte at different concentrations will affect decreasing in retention time for each analyte components. This work also demonstrated that the peak area of each metal cations obtained does not affected by matrix effect.

5.3.1.4. Reproducibility

A buffer composition of 5 mmol/L oxalic acid, 6.9 mmol/L 4-methylbenzylamine at pH 4.0 was employed to study the reproducibility of metals from group I and group II and cadmium. The procedure used was followed the outlined in Section 4.5.1.3. for ANOVA calculation. The results obtained are given in Tables 5.4 - 5.6. In this study the standard mixtures containing 5 metals from group I, group II and Cd were injected to observe the reproducibility of migration time, absorbance and peak area. These metals were include K, Na, Ca, Mg and Cd.

5.3.1.4.1. Migration time (MT)

Full summarises reproducibility data for all five metals is shown in Table 5.4.

Elements	Mean result, min. ± SD [a]	ANOVA *F* value[c]
Potassium	4.43 [b] ± 0.03	0.92
Sodium	5.32 [b] ± 0.08	0.01
Calcium	5.92 [b] ± 0.11	0.68
Magnesium	6.62 [b] ± 0.15	0.97
Cadmium	8.57 [b] ± 0.29	0.01

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.4. Reproducibility data on the migration time for metal cations at different batch samples time using 5 mmol/L oxalic acid, 6.9 mmol/L 4-methylbenzylamine at pH 4.0.

Since the calculated value of F from series observations is less than the critical value of F, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.4 shows that the migration times were stable and able to provide consistent and reproducible data. This result obtained was agreed with the findings reported by Jones and Tarter (198) on the analysis of selected transition metal cations using 5 mmol/L oxalic acid, 3.75 mmol/L citric acid at pH 4.37. They observed the reproducibility of retention time ranges from 0.03 to 0.05 of standard deviation.

5.3.1.4.2. Absorbance

Full summarises reproducibility data for all five metals is shown in Table 5.5.

Elements	Mean result, AU($x10^{-3}$) ± SD [a]	ANOVA F value[c]
Potassium	1.34 [b] ± 0.09	1.59
Sodium	2.68 [b] ± 0.10	0.01
Calcium	5.70 [b] ± 0.18	1.35
Magnesium	3.52 [b] ± 0.08	1.26
Cadmium	0.52 [b] ± 0.03	1.02

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.5. Reproducibility data on the absorbance for metal cations at different batch samples using 5 mmol/L oxalic acid, 6.9 mmol/L 4-methylbenzylamine at pH 4.0.

Since the calculated value of F from series observations is less than the critical value of F, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.5 shows that the absorbance were stable and able to provide consistent and reproducible data for K, Na, Ca, Mg and Cd. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of Ni, Zn and Co with the employing of 5 mmol/L oxalic acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of absorbance in this work.

5.3.1.4.3. Peak area

Full summarises reproducibility data for all five metals is shown in Table 5.6.

Elements	Mean result, peak area ($x10^6$) ± SD [a]	ANOVA F value[c]
Potassium	0.08 [b] ± 0.10	1.72
Sodium	0.21 [b] ± 0.02	0.91
Calcium	0.19 [b] ± 0.01	0.90
Magnesium	0.14 [b] ± 0.02	1.80
Cadmium	0.03 [b] ± 0.03	2.22

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.6. Reproducibility data on peak area for metal cations at different batch samples using 5 mmol/L oxalic acid, 6.9 mmol/L 4-methylbenzylamine at pH 4.0.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.6 shows that the peak area are stable and enable to provide a consistent and reproducible data for K, Na, Ca, Mg and Cd. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of Ni, Zn and Co with the employing of 5 mmol/L oxalic acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of peak area in this work.

5.3.1.5. Linearity

Figs. 5.5 – 5.9 show linearity for group I and group II metal cations. The procedure used for linearity of the graph was followed as outlined in Sectioned 4.5.1.4. Five concentrations at low levels of multi-element standards were used from 0 ppm to 1 ppm for the analysis using IC. Ten times at each concentration was injected and duplicates with the same set solution of each concentration were carried out sequentially. Concentrations lower than 0.2 ppm were not tested. The average values of the results in linearity for K ($y = 0.0019x + 2E\text{-}05$; and $R^2 = 0.9983$), Na ($y = 0.0034x + 9E\text{-}05$; and $R^2 = 0.9929$), Ca ($y = 0.007x + 3E\text{-}05$; and $R^2 = 0.9957$), Mg ($y = 0.0066x - 0.0002$; and $R^2 = 0.9966$) and Cd ($y = 0.0012x + 2E\text{-}05$; and $R^2 = 0.9938$) were acceptable and can be fitted a straight line for the above range.

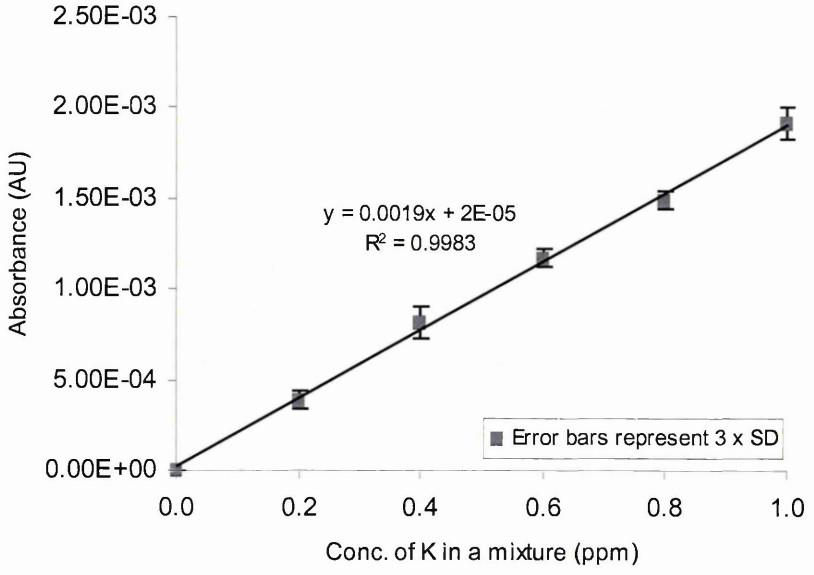


Fig 5.5. Linearity of K based on the peak response (absorbance). The buffer employed was 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine (pH 4.0).

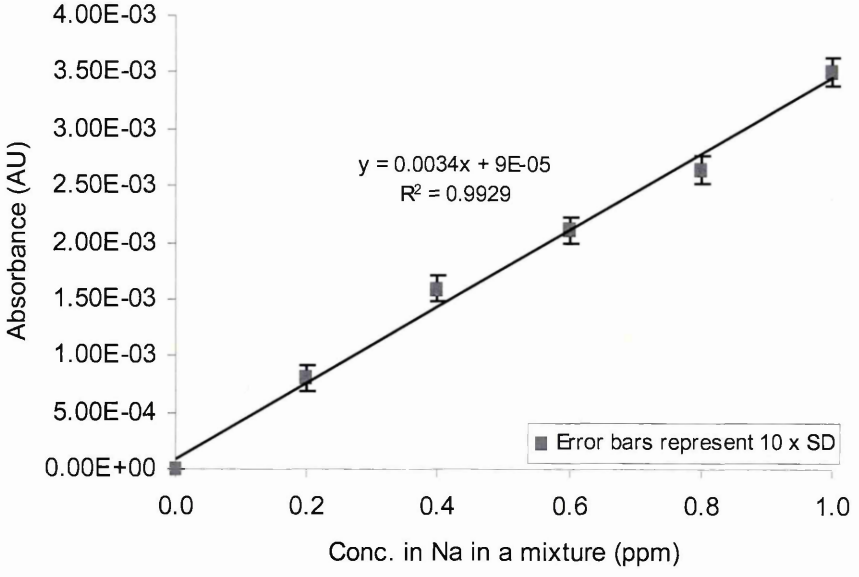


Fig 5.6. Linearity of Na based on the peak response (absorbance). The buffer employed was 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine (pH 4.0).

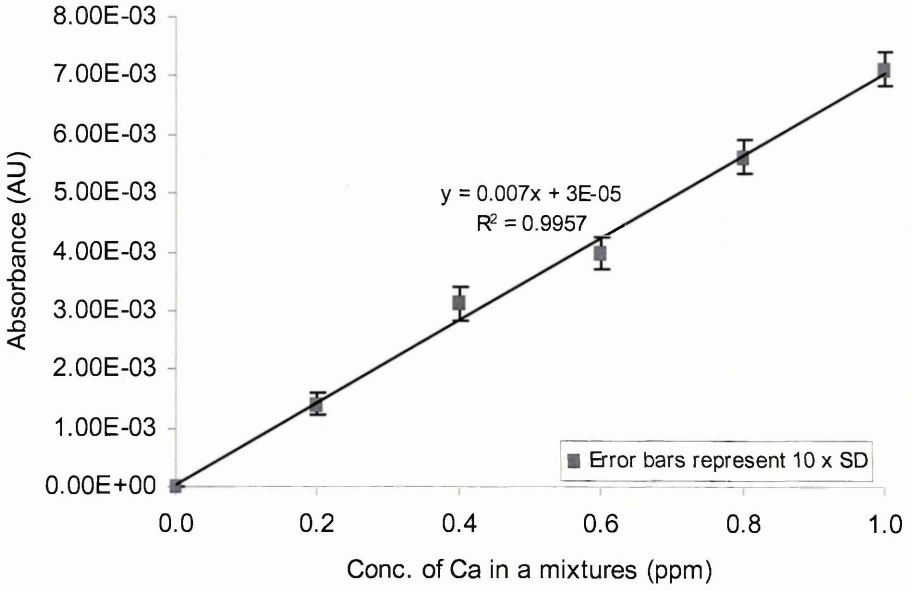


Fig 5.7. Linearity of Ca based on the peak response (absorbance). The buffer employed was 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine (pH 4.0).

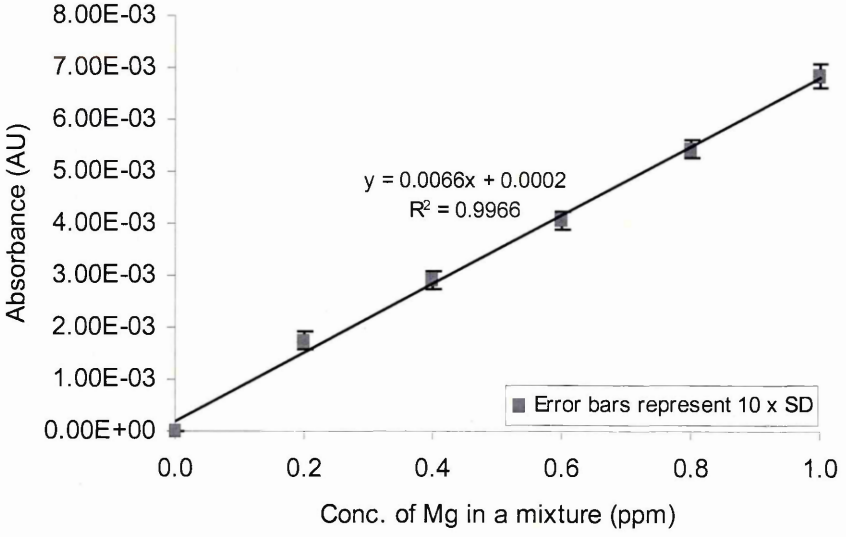


Fig 5.8. Linearity of Mg based on the peak response (absorbance). The buffer employed was 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine (pH 4.0).

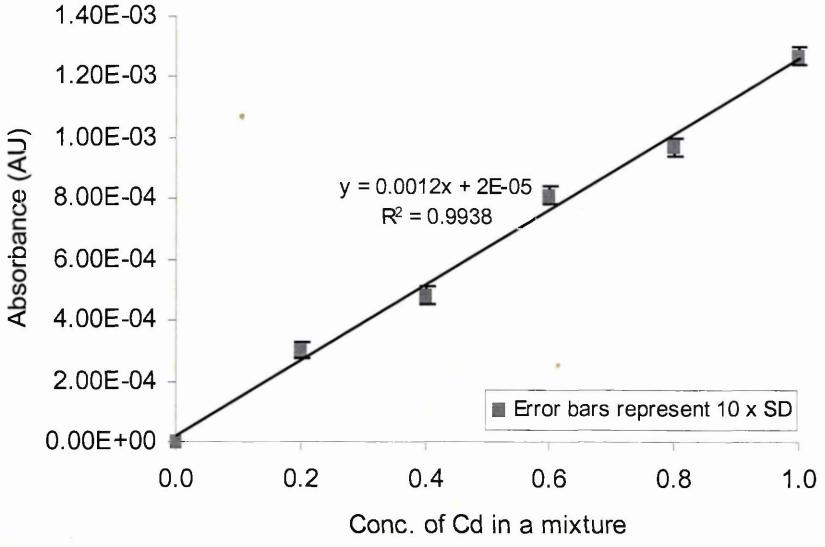


Fig 5.9. Linearity of Cd based on the peak response (absorbance). The buffer employed was 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine (pH 4.0).

The overall linearity of K, Na, Ca, Mg and Cd are found from 0 to 1 ppm in this work are not so good (the correlation coefficients, $R^2 < 0.99$) at lower concentration range but not tested at higher range as compared to the previous work. Shi and Fritz (29) reported a good linear calibration curves were obtained for the metal cations in the 0.4 to 10 ppm concentration range. They used the buffer solution of 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3 for the separation of eight metal cations include K, Na, Pb, Mn, Co, Ni, Zn and Cd. However they obtain a relatively poor correlation coefficient of linear regression for zinc. Meanwhile, Shi and Fritz (30) obtained a linear calibration curves for the metal cations from 0.1 to 10 ppm for the separation of Sr, Mg, Mn, Co and Zn. They used 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 0.6 mmol/L 18-crown-6, 10% methanol at pH 4.3. In another development, Yang et al. (63) reported the linear calibration curves up to 20 ppm was obtained for the separation of K, Na, Ca, Mg and Mn.

They also found that at high concentrations peak distortion occurred owing to overloading, which caused insufficient selectivity of separation, so that the calibrations were no longer useful. At pH 4.5 the calibration line for manganese was linear only 6 ppm owing to the insufficient separation between manganese and magnesium. Recently, N. Shakulashvili et al. (191) reported a good linear calibration curves from 0.75 to 80 ppm for the separation of sixteen metal cations using 10 mol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. Based on the above linearity, a further development may required to obtained good correlation coefficients (R^2) and linearity at higher range for quantitative analysis.

5.3.1.6. Limit of detection

A number of serial dilutions of a standard mixture of metals solution were made to determine the detection limits of the system. Minimum detectable based on a peak signal (absorbance) to noise ratio of are 3:1, given in Table 5.7.

Metal cations	Mean of detection limit[a] (ppm ± SD)
Potassium	0.20[a] ± 0.05
Sodium	0.10[a] ± 0.05
Calcium	0.50[a] ± 0.06
Magnesium	0.40[a] ± 0.08
Cadmium	0.10[a] ± 0.07

Notes: [a] = mean of 10 replicates

Table 5.7. Limit of detection for group I, II metals and Cd.

Limits of detection are found to be in the sub ppm level but not as good compared to values obtained by Lee and Lin (57) in the analysis a mixture of nineteen metal ions (Li, Na, K, Cs, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ag, Al and Pb) using 10 mmol/L pyridine, 12 mmol/L glycolic acid at pH 4.0. They obtained limit of detection in the range of 0.02 ppb for Na up to 0.208 ppm for Cr. Meanwhile, Yang et al. (62) obtained the limit of detection are as follows: 0.4 ppb for Li and Mg, 1 ppb for NH_4^+ and Ca, 2.5 ppb for Na, 10 ppb for K, 120 ppb for Sr, Mn, Cr and Zn, 500 ppb for Ba and Ni and 1000 ppb for Cu. The values for limit of detection are based on three times the

baseline noise. They use 5 mmol/L imidazole, 6.5 mmol/L HIBA, 20% methanol and 1.33 mmol/L 18-crown-6 at pH 4.5. Shi and Fritz (29) also reported the limit of detection in the range 0.05 to 0.5 ppm for the separation of twenty seven alkali, alkaline earth, transition and rare earth metal cations. Light ions are in the low detection limit range, and heavy ions are in the high detection limit range. They employed 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. Based on the above data obtained, a further development need to be carried out to improve the limit of detection of each metal cations for quantitative analysis.

5.3.1.7. Effects of oxalic acid on separation performance

The electropherogram in Fig. 5.10 represents an example of a good separation of K, Na, Ca, Mg and Cd using indirect UV-Vis detection. In this experiment 5 mmol/L 4-methylbenzylamine and 6.9 mmol/L oxalic acid was employed as the buffer composition at a pH of 4.0. All metals, K, Na, Ca, Mg and Cd were successfully separated in a single run at a concentration of 10 ppm each.

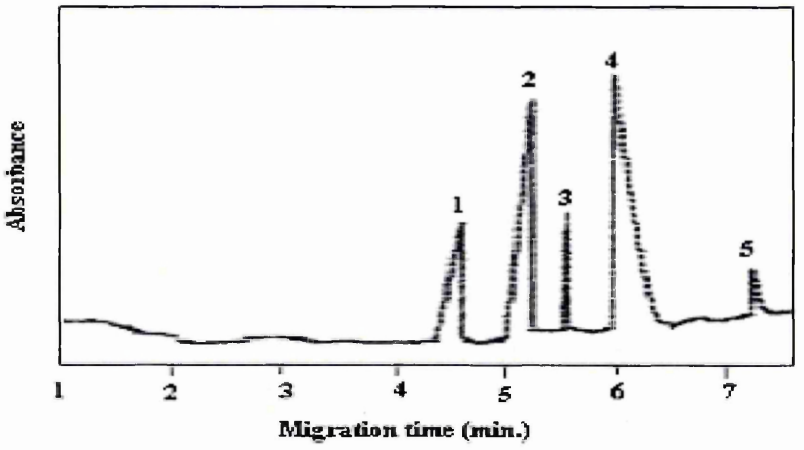


Fig. 5.10. CE separation of metal ions using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine adjusted to pH 4.0; capillary length 60 cm x 50 micron I.D; Voltage 20 kV; UV-Vis detection at 214 nm; Peaks; 1=K; (10 ppm); 2=Na (10 ppm); 3=Ca (10 ppm); 4=Mg (10 ppm); 5= Cd (10 ppm).

Separation of Co from Cd was not possible when using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine at pH 4.0. In a later development the pH of this buffer was increased to pH 5.0 in order to get a separation of cobalt from cadmium as shown in Fig. 5.11. The migration order of the separation is K, Na, Ca, Mg, Co and Cd. The peak heights between group I&II metals and transition metals is slightly different due to the degree of complexation with oxalic acid. Six metals from group I, II and transition metals include K, Na, Ca, Mg and Cd were observed for CE-UV/Vis detection. Six metals were separated in less than 8.5 minutes using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine at pH 5.0. The successful separation of six metal cations is shown in Fig. 5.11. The migration order follows the sequence include K, Na, Ca, Mg, Co and Cd. All peaks are completely baseline resolved. All metals including K, Na, Ca and Mg peaks that migrated more slowly showed peak trailing and had broader peak widths. Meanwhile,

Cu and Cd migrated the slowest and had the highest retention (about 8 to 10 minutes vs. 4 to 6.5 min for other peaks) with considerable peak trailing. It was demonstrated that both Cu and Cd are not so sensitive as the peak height very small compared to other metals under the same conditions. Presumably the last two ions formed complexes with oxalic acid that have higher stability constants.

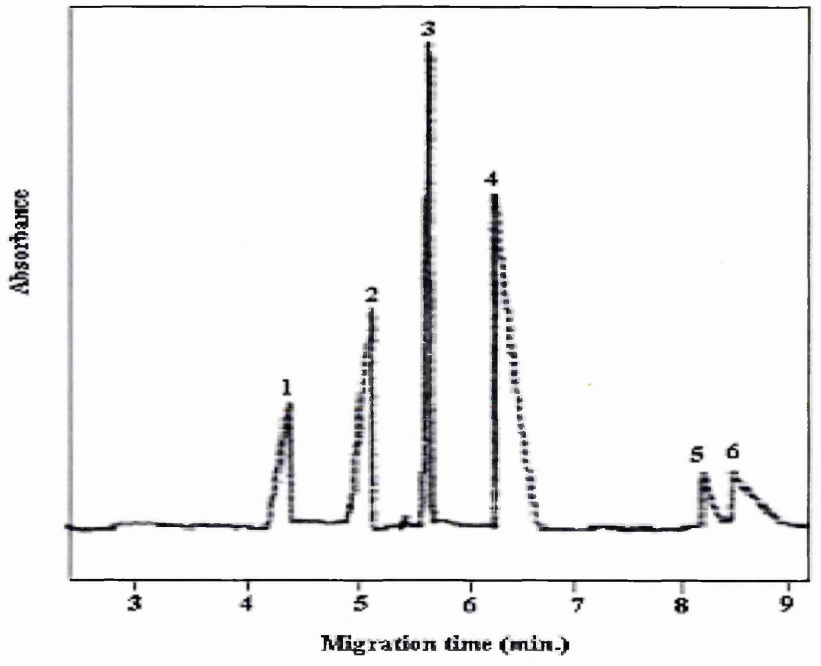


Fig. 5.11. CE separation of metal ions using 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine adjusted to pH 5.0; capillary length 60 cm x 50 micron I.D; Voltage 15 kV; UV-Vis detection at 214 nm; Peaks; 1=K; (10 ppm); 2=Na (10 ppm); 3=Ca (10 ppm); 4=Mg (10 ppm); 5= Co (10 ppm). 6= Cd (10 ppm).

Both electropherograms in Figs. 5.10 and 5.11 clearly show that the adjustment of pH buffer is required for the separation of Co from Cd. The peaks become more symmetric with increasing concentration. The background carrier electrolyte and buffer concentration

has a great effect on the peak shape. If we compare the results with those achieved under the same conditions in previous studies, in spite of the use of a significantly longer capillary, the increases in buffer concentration case much more symmetrical peaks. The resolution depended on the mobility of the background carrier electrolyte used. Therefore, the resolution of last two peaks was poor because the mobility of 4-methylbenzylamine was too fast, and hence incompatible with these two metals. These finding is not as good in term of number of analytes separated and overall run time compared to those observation by Shi and Fritz (29). They obtained eight metal cations including K, Na, Pb, Mn, Co, Ni, Zn and Cd in less than 6 minutes using 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3. They have a good peak shape and baseline resolved. In another development they have separated twelve metal cations using 2.5 mmol/L tartaric acid, 6 mmol/L *p*-toluidine, 20 % methanol at pH 4.8 in less than 8 min. Resolution is excellent with a steady, flat baseline.

Plate numbers achieved for each metals are listed in Table 5.8.

Metal cations	Number of theoretical plates per meter (N/m)
Potassium	240,044
Sodium	265,047
Calcium	173,595
Magnesium	116,738
Cadmium	52,341

Table 5.8. Peak efficiency for 5 mmol/L oxalic acid and 6.9 mmol/L 4-methylbenzylamine (pH 4.0) using CE-UV/Vis detection.

From Table 5.8, Na has the highest number of theoretical plates (N), about 265,047 followed by K, Ca, Mg and Cd because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as compared to Cd has the lowest N value, about 52,341. This values obtained was acceptable as compared to N value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the N value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. The result obtained shows that the peak efficiencies for each metal cations are suitable for qualitative analysis and may required further development for quantitative analysis.

5.3.2. Acetylacetone as a complexing reagent

5.3.2.1. Development of buffer composition

Varying concentrations of acetylacetone (acac) and 10 mmol/L 4-methylbenzylamine were used over a concentration range from 1 mmol/L to 10 mmol/L of complexing reagent. This study will focus on the effect of acac, methanol (the use of methanol without acac) and the effect of pH. These were investigated in order to obtain the optimum combination that would provide separation of a mixture transition metals and group I &II metals.

The first experiment was carried out with 10 mmol/L 4-methylbenzylamine without acac at unadjusted pH about 9.1. At this point most of the metal cations were unidentified. Certain metal cations separated after the pH was reduced to pH about 4.5, though most of them co-eluted. These results show that the separation of metal cations was not possible without the presence of acac. In the second experiment, 5 mmol/L acac and 10 mmol/L 4-methylbenzylamine were used at pH about 4.5 but some metals still co-eluted. In the third experiment, 5mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol were used at pH about 4.0. Most of the metal cations separated except iron which co-eluted between sodium and calcium. This separation is shown in Fig. 5.12. The elution order was K, Na, Ca, Mg, Co, Ni, Cd and Pb.

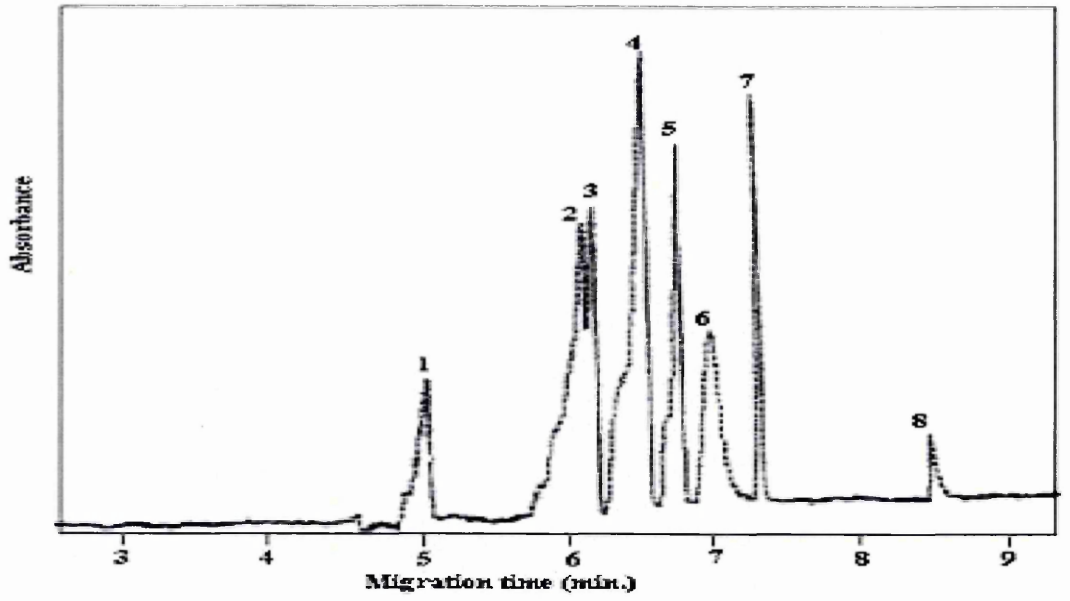


Fig. 5.12. Electrophoretic separation of eight metal cations using 5 mmol/L acac and 10 mmol/L 4-methylbenzylamine, 50% methanol (pH 4.0). 1=K; 2=Na; 3=Ca; 4=Mg; 5=Co; 6=Ni; 7=Cd; 8=Pb.

In order to separate sodium from calcium the same buffer composition of 5mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0 was employed. However the separation of sodium, calcium and iron was not possible under these conditions. In another experiment, when the concentration of acac was increased to 10 mmol/L, complete separation of all metal cations in group I and II were obtained except iron as can be seen in Fig. 5.13. Iron co-eluted with calcium.

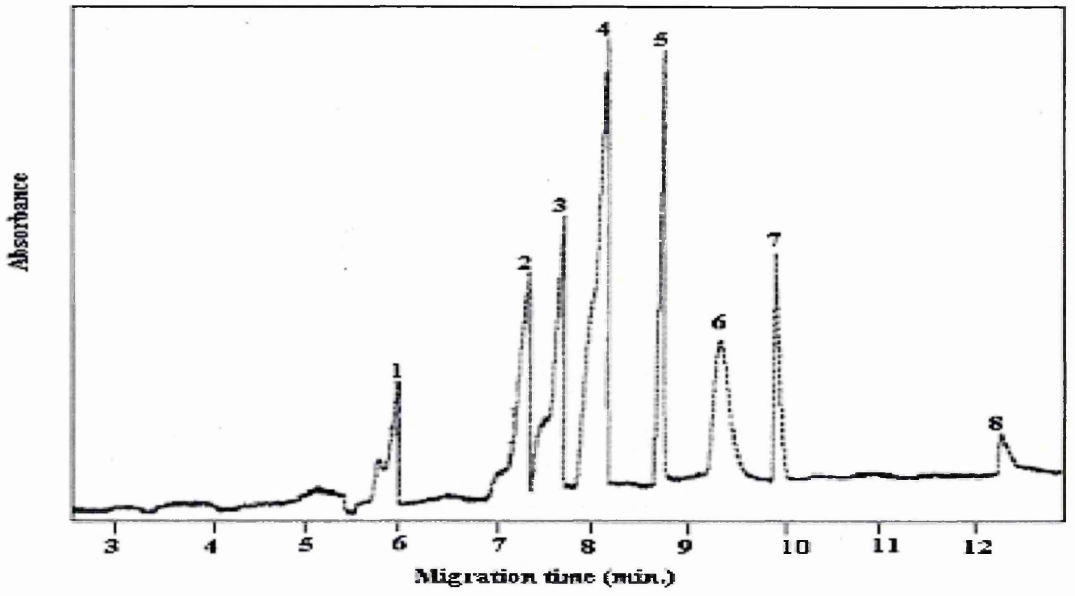


Fig. 5.13. Electrophoretic separation of eight metal cations using 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0). 1=K; 2=Na; 3=Ca; 4=Mg; 5=Co; 6=Ni; 7=Cd; 8=Pb.

5.3.2.2. Reproducibility

A buffer composition of 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0 was employed to study the reproducibility of analysis of the selected metals from group I and group II and transition metals. The procedure used was followed the outlined in Section 4.5.1.3. for ANOVA calculation. The average (mean) and standard deviations (SD) obtained are given in Tables 5.8 - 5.10. In this study the standard mixtures containing eight metals from group I, group II and transition metals were injected to observe the reproducibility of migration time, absorbance and peak area. These metals were K, Na, Ca, Mg, Co, Ni, Cd and Pb.

5.3.2.2.1. Migration time

Full summarises reproducibility data for all five metals is shown in Table 5.9.

Elements	Mean result, min. ± SD [a]	ANOVA *F* value[c]
Potassium	6.11 [b] ± 0.10	0.94
Sodium	7.54 [b] ± 0.13	0.95
Calcium	7.95 [b] ± 0.14	0.93
Magnesium	8.43 [b] ± 0.15	1.26
Cobalt	9.03 [b] ± 0.16	1.21
Nickel	9.60 [b] ± 0.17	1.86
Cadmium	10.06 [b] ± 0.17	0.96
Lead	12.54 [b] ± 0.23	1.89

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.9. Reproducibility data on the migration time for metal cations at different batch samples using of 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.9 shows that the migration times were stable and able to provide consistent and reproducible data. This result obtained was agreed with the findings reported by Jones and Tarter (198) on the analysis of selected transition metal cations using 5 mmol/L oxalic acid, 3.75 mmol/L citric acid at pH 4.37. They observed the

reproducibility of retention time ranges from 0.03 to 0.05 of standard deviation. Therefore, results obtained under this work are consistent with their observation in similar field.

5.3.2.2.2. Absorbance

Full summarises reproducibility data for all five metals is shown in Table 5.10.

Elements	Mean result, AU(x10^{-3}) ± SD [a]	ANOVA *F* value[c]
Potassium	1.70 [b] ± 0.06	1.80
Sodium	3.45 [b] ± 0.05	1.04
Calcium	4.33 [b] ± 0.25	1.18
Magnesium	6.95 [b] ± 0.13	1.45
Cobalt	6.70 [b] ± 0.17	1.80
Nickel	2.03 [b] ± 0.11	0.90
Cadmium	3.50 [b] ± 0.05	0.01
Lead	0.60 [b] ± 0.01	0.01

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.10. Reproducibility data on absorbance for metal cations at different batch samples using of 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ

significantly. Table 5.10 shows that the absorbance were stable and able to provide consistent and reproducible data for K, Na, Ca, Mg, Co, Ni, Cd and Pb. This result obtained was agreed with the findings reported by Jones and Tarter (198) on the analysis of selected transition metal cations using 5 mmol/L oxalic acid, 3.75 mmol/L citric acid at pH 4.37. They observed the reproducibility of retention time ranges from 0.03 to 0.05 of standard deviation.

5.3.2.2.3. Peak area

Full summarises reproducibility data for all five metals is shown in Table 5.11.

Elements	Mean result, peak area ($x10^6$) ± SD [a]	ANOVA *F* value[c]
Potassium	0.03 [b] ± 0.01	1.80
Sodium	0.11 [b] ± 0.01	0.91
Calcium	0.14 [b] ± 0.050	0.01
Magnesium	0.26 [b] ± 0.004	1.58
Cobalt	0.11 [b] ± 0.002	1.80
Nickel	0.10 [b] ± 0.007	1.80
Cadmium	0.06 [b] ± 0.002	0.01
Lead	0.02 [b] ± 0.002	0.02

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.11. Reproducibility data on peak area for metal cations at different batch samples using 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0.

Since the calculated value of *F* from series observations for all metals is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.11 shows that the peak area for K, Na, Ca, Mg, Co, Ni, Cd and Pb are fairly good for providing a consistent and reproducible data. This result obtained was agreed with the findings reported by Jones and Tarter (198) on the analysis of selected transition metal cations using 5 mmol/L oxalic acid, 3.75 mmol/L citric acid at pH 4.37. They observed the reproducibility of retention time ranges from 0.03 to 0.05 of standard deviation. Therefore, results obtained under this work are consistent with their observation in similar field and may improved further for the quantitative analysis.

5.3.2.3. Linearity

Figs. 5.14 – 5.21 show linearity with group I and group II metal cations. The procedure used for linearity of the graph was followed as outlined in Sectioned 4.5.1.4. There were five concentrations at low levels of multi-element standards used from 1 ppm to 25 ppm for the analysis. Ten times at each concentration was injected and duplicates with the same set solution of each concentration were carried out sequentially. Lower concentration than 1 ppm were not tested. The average values of the results in linearity for K ($y = 7E\text{-}05x + 3E\text{-}05$; and $R^2 = 0.9976$), Na ($y = 0.0001x + 0.0002$; and $R^2 = 0.9918$), Ca ($y = 0.0001x + 0.0002$; and $R^2 = 0.9936$), Mg ($y = 0.0003x + 0.0003$; and $R^2 = 0.9932$), Co ($y = 0.0003x + 0.0002$; and $R^2 = 0.9931$), Ni ($y = 0.0003x + 0.0001$; and $R^2 = 0.9933$), Cd ($y = 1E\text{-}04x + 4E\text{-}05$; and $R^2 = 0.9959$) and Pb ($y = 3E\text{-}05x + 2E\text{-}05$; and $R^2 = 0.9962$) were acceptable and a straight line can be fitted for the above range. However the linearity for

the above 25 ppm and lower than 1 ppm for each metal cations may need to improve for the quantitative analysis. The buffer conditions used in this investigation must be properly optimised and controlled (i.e. pH) in order to obtain a good linear relationship for each metal cations.

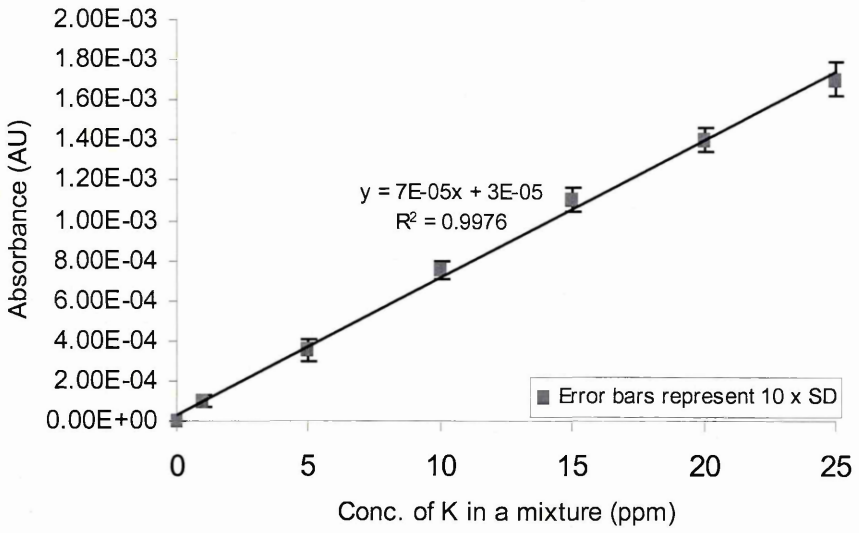


Fig. 5.14. Linearity of K with 10 mmol/L 4 methylbenzylamine, 10 mol/L acac and 50% methanol (pH 4.0) at 30 kV.

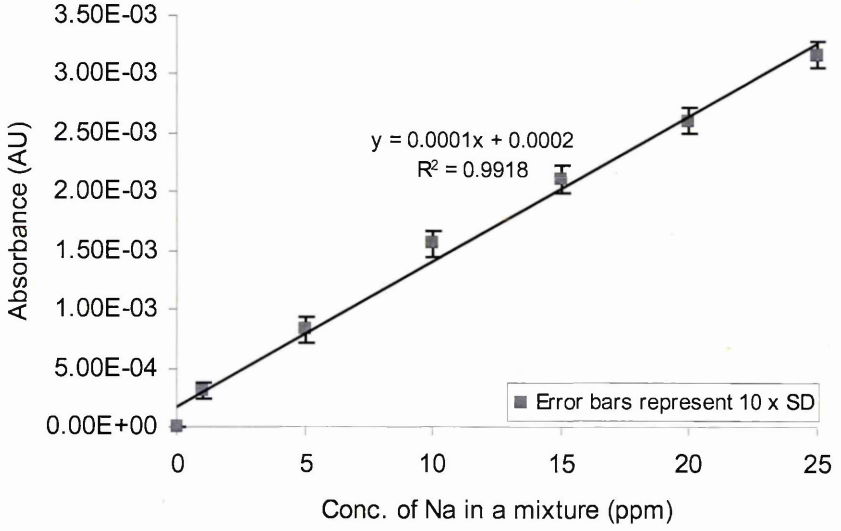


Fig. 5.15. Linearity of Na with 10 mmol/L 4 methylbenzylamine, 10 mmol/L acac and 50% methanol (pH 4.0) at 30 kV.

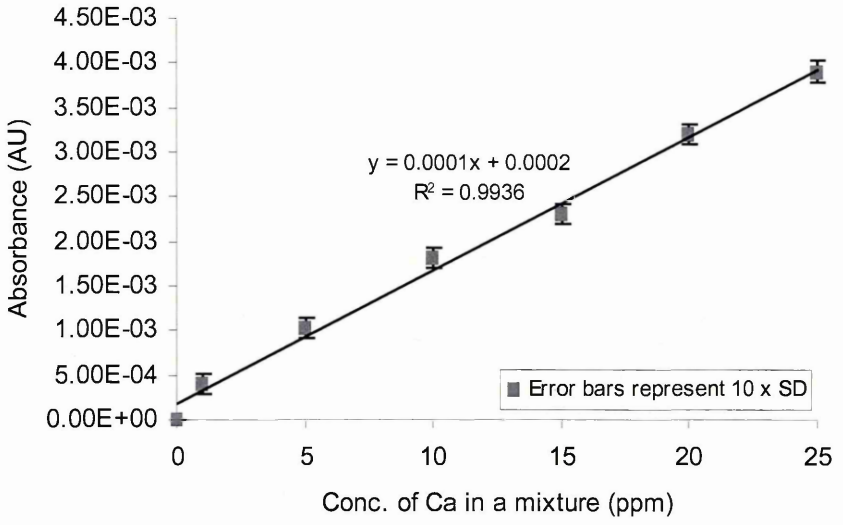


Fig. 5.16. Linearity of Ca with 10 mmol/L 4 methylbenzylamine, 10 mmol/L acac and 50% methanol (pH 4.0) at 30 kV.

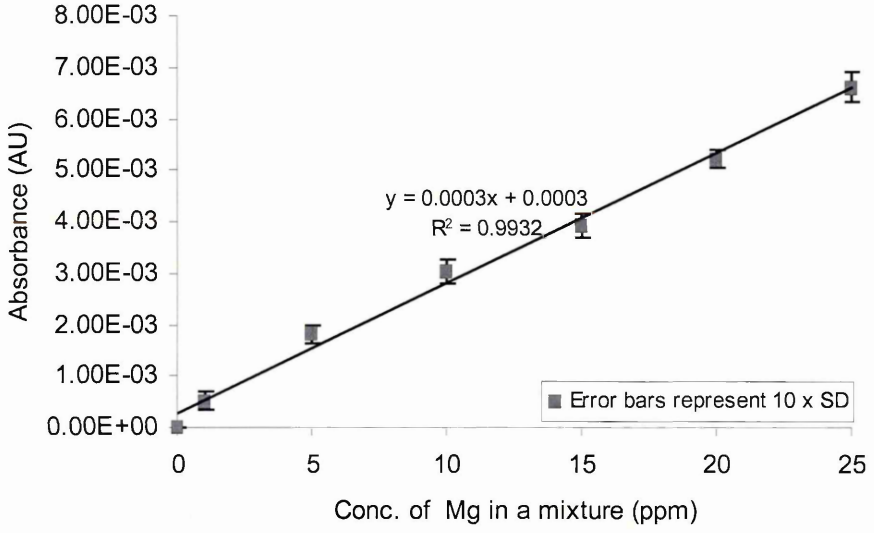


Fig. 5.17. Linearity of Mg with 10 mmol/L 4 methylbenzylamine, 10 mmol/L acac and 50% methanol (pH 4.0) at 30 kV.

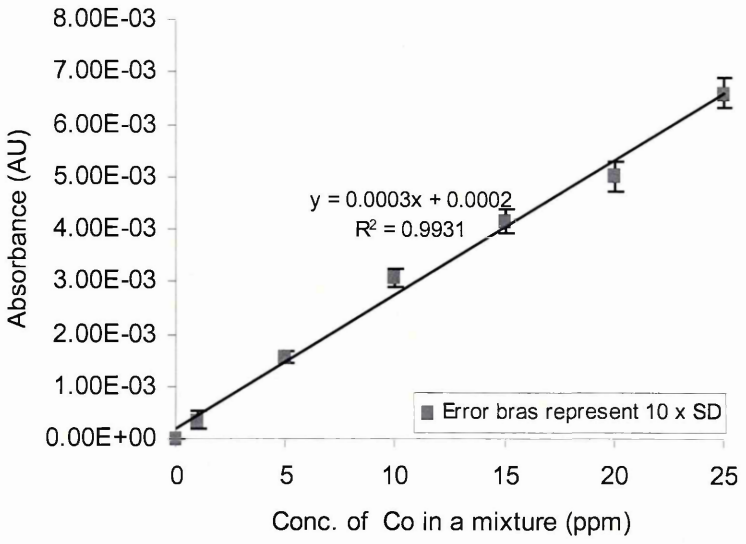


Fig. 5.18. Linearity of Co with 10 mmol/L 4 methylbenzylamine, 10 mmol/L acac and 50% methanol (pH 4.0) at 30 kV.

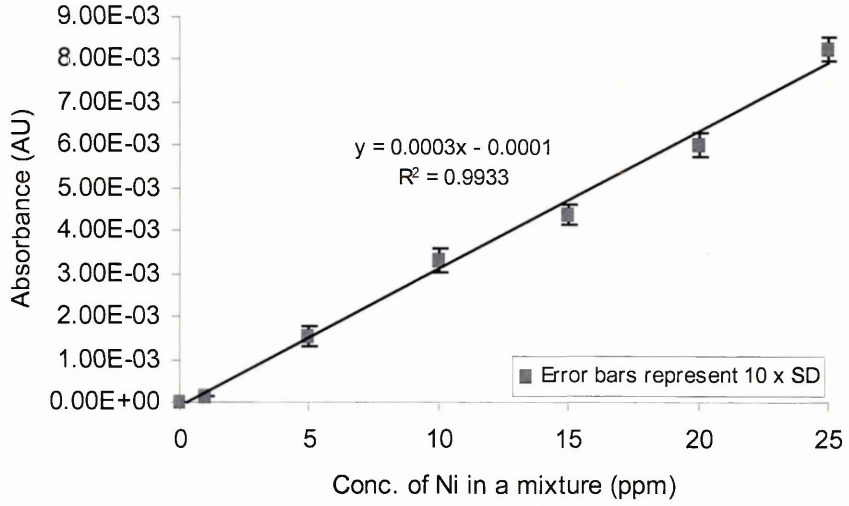


Fig. 5.19. Linearity of Ni with 10 mmol/L 4 methylbenzylamine, 10 mmol/L acac and 50% methanol (pH 4.0) at 30 kV

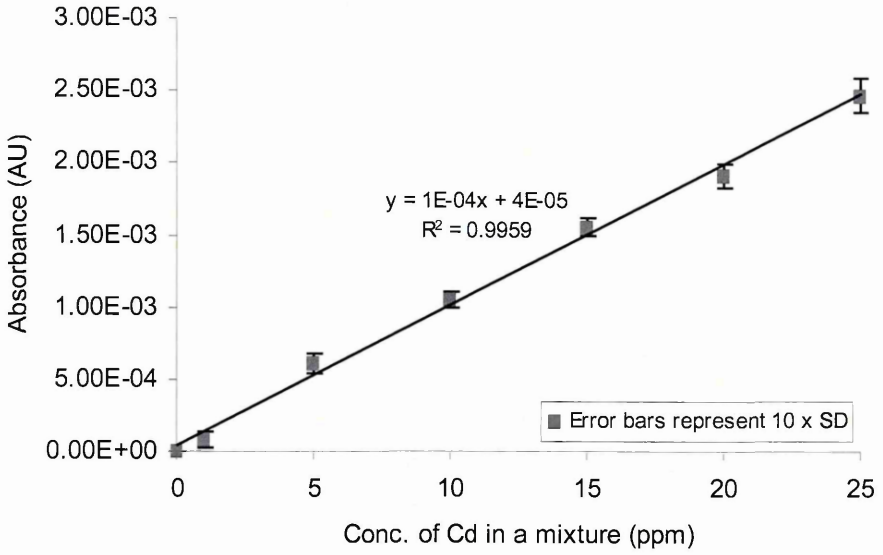


Fig. 5.20. Linearity of Cd with 10 mmol/L 4 methylbenzylamine, 10 mmol/L acac and 50% methanol (pH 4.0) at 30 kV.

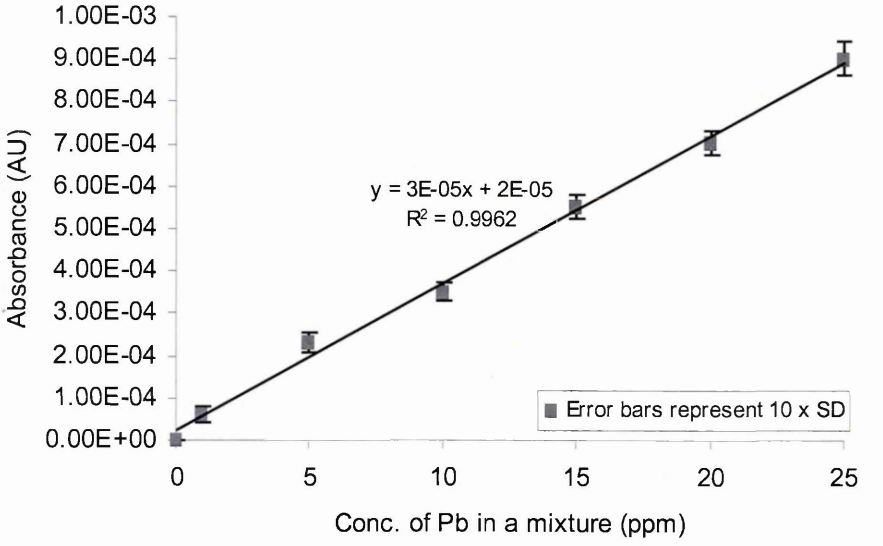


Fig. 5.21. Linearity of Pb with 10 mmol/L 4 methylbenzylamine, 10 mmol/L acac and 50% methanol (pH 4.0) at 30 kV.

The overall linearity of ten metals from group I, group II and transition metals that are found from 0 to 25 ppm in this work are not so good (the correlation coefficients, $R^2 <$ 0.99) compared to the previous work in similar field. Shi and Fritz (29) reported a good linear calibration curves were obtained for the metal cation studies in the 0.4 ppm to 10 ppm concentration range. They used the buffer solution of 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3 for the separation of eight metal cations include K, Na, Pb, Mn, Co, Ni, zinc and Cd. However they obtain a relatively poor correlation coefficient of linear regression for zinc. Meanwhile, Shi and Fritz (30) obtained a linear calibration curves for the metal cations from 0.1 to 10 ppm for the separation of Sr, Mg, Mn, Co and Zn. They used 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 0.6 mmol/L 18-crown-6, 10% methanol at pH 4.3. In another development, Yang et al. (63) reported the linear calibration curves up to 20 ppm was obtained for the separation of K, Na, Ca, Mg and Mn. They also found that at high concentrations peak distortion occurred owing to overloading, which caused insufficient selectivity of separation, so that the calibrations were no longer useful. At pH 4.5 the calibration line for manganese was linear only 6 ppm owing to the insufficient separation between manganese and magnesium. Recently, N. Shakulashvili et al. (191) reported a good linear calibration curves from 0.75 to 80 ppm for the separation of sixteen metal cations using 10 mol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. Based on the above data for linear relationship, it has shown that the method used not suitable for quantitative analysis.

5.3.2.4. Limit of detection (LOD)

Serial dilutions of standard solution at sub ppm level were made to determine the detection limits of the system. Minimum detectable concentrations for metal cations in group I and II and transition metals , based on a peak signal (absorbance) to noise (3:1), are given in Table 5.12. The buffer employed was 10 mmol/L acac and 10 mmol/L 4 methylbenzylamine, 50% methanol at pH 4.0.

Metal cations	Mean of detection limit[a] (ppm ± SD)
Potassium	0.68[b] ± 0.15
Sodium	0.76[b] ± 0.10
Calcium	0.50[b] ± 0.09
Magnesium	0.57[b] ± 0.09
Cobalt	0.21[b] ± 0.08
Nickel	0.25[b] ± 0.06
Cadmium	0.17[b] ± 0.05
Lead	0.30[b] ± 0.06

Notes: [a] = mean of 10 replicates injection

Table 5.12. Limit of detection for group I and II metals and transition metals. The buffer employed was 10 mmol/L acac, 10 mmol/L 4 methylbenzylamine and 50% methanol (pH 4.0).

Limits of detection are found to be in the sub ppm level but not as good compared to values obtained by Lee and Lin (57) in the analysis a mixture of nineteen metal ions (Li,

Na, K, Cs, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ag, Al and Pb) using 10 mmol/L pyridine, 12 mmol/L glycolic acid at pH 4.0. They obtained limit of detection in the range of 0.02 ppb for sodium up to 0.208 ppm for chromium. Meanwhile, Yang et al. (62) obtained the limit of detection are as follows: 0.4 ppb for Li and Mg, 1 ppb for NH_4^+ and Ca, 2.5 ppb for Na, 10 ppb for K, 120 ppb for Sr, Mn, Cr and Zn, 500 ppb for Ba and Ni and 1000 ppb for Cu. The values for limit of detection are based on three times the baseline noise. They use 5 mmol/L imidazole, 6.5 mmol/L HIBA, 20% methanol and 1.33 mmol/L 18-crown-6 at pH 4.5. Shi and Fritz (29) also reported the limit of detection in the range 0.05 to 0.5 ppm for the separation of twenty seven alkali, alkaline earth, transition and rare earth metal cations. Light ions are in the low detection limit range, and heavy ions are in the high detection limit range. They employed 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. N. Shakulashvili et al. (191) obtained the limit of detection in the range 0.1 for K and 0.454 ppm for Cu in a mixture of 16 metal ion which is separated using 10 mmol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. A further development may be required to improve the limit of detection and for the possibility of quantitative analysis.

5.3.2.5. Effects of acetylacetone on separation performance

Eight metals from group I, II and transition metals include K, Na, Ca, Mg, Co, Ni, Cd and Pb were observed. Eight metals were separated in less than 8.5 minutes using 10 mmol/L acac, 10 mmol/L 4 methylbenzylamine and 50% methanol at pH 4.0. The successful separation of eight metal cations is shown in Fig. 5.12. The migration order follows the include K, Na, Ca, Mg, Co, Ni, Cd and Pb. All peaks are completely baseline resolved

except for Na and Ca. The peaks become more symmetric with increasing concentration. The buffer concentration has a great effect on the peak shape. If we compare the results with those achieved under the same conditions in previous studies, in spite of the use of a significantly longer capillary, the increases in buffer concentration case much more symmetrical peaks. We believe that this can be explained by the very high level of sorption phenomenon in the These finding is not as good in term of number of analytes separated and overall run time compared to those observation by Shi and Fritz (29). They obtained eight metal cations including K, Na, Pb, Mn, Co, Ni, Zn and Cd in less than 6 minutes using 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3. They have a good peak shape and baseline resolved. In another development they have separated twelve metal cations using 2.5 mmol/L tartaric acid, 6 mmol/L *p*-toluidine, 20 % methanol at pH 4.8 in less than 8 min. Resolution is excellent with a steady, flat baseline. However an excellent separation of twenty seven metal cations has been achieved by Shi and Fritz (29) in a single run in less than 6 min. They used 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. Among those metal cations that were separated including K^{+}, Ba^{2+}, Sr^{2+}, Na^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^{+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Cu^{2+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+} . The average deviation in peak height from one run to another was approximately ± 5% or smaller at the ion concentration used.

Plate numbers achieved for each metals are listed in Table 5.13.

Metal cations	Number of theoretical plates per meter (N/m)
Potassium	223,749
Sodium	201,621
Calcium	193,267
Magnesium	189,301
Cobalt	190,904
Nickel	191,128
Cadmium	209,883
Lead	178,163

Table 5.13. Peak efficiency for 10 mmol/L acac, 10 mmol/L 4 methylbenzylamine and 50% methanol (pH 4.0) using CE-UV/Vis detection.

From Table 5.13, K has the highest number of theoretical plates (N), about 223,749 followed by Cd, Na, Ca, Ni, Co, and Mg because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as acceptable to Pb has the lowest N value, about 178,163. This values obtained was good as compared to N value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation.

In another development, Simunicova et al. (68) obtained the *N* value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of *N* between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. The result obtained shows that the peak efficiencies for each metal cations are suitable for quantitative analysis.

5.3.3. The use of acetic acid as a complexing reagent

5.3.3.1. Effect of changing the concentration

The concentration of complexing reagents is a very important parameter in ensuring the complete separation of metal cations. Migration times of metals in group I&II are not much influenced by changing the complexing reagent because their migration is more dependent on the differences in electrophoretic mobilities. The migration times of transition metals are much more reliant on the concentration of the complexing reagent. This is because the concentration of complexing reagent present will affects the formation of metal complex as mentioned in the previous work by Lin et al. (58). They found that in the absence of a acetic acid, only five peaks (K, Ba, Ca, Li and Na-Mg)) were found but Na and Mg could not be resolved. The influence of complexing reagent concentration versus analyte migration times is shown in Fig. 5.22. In general, the migration time was shorter in the absence of a complexing reagent at the same pH.

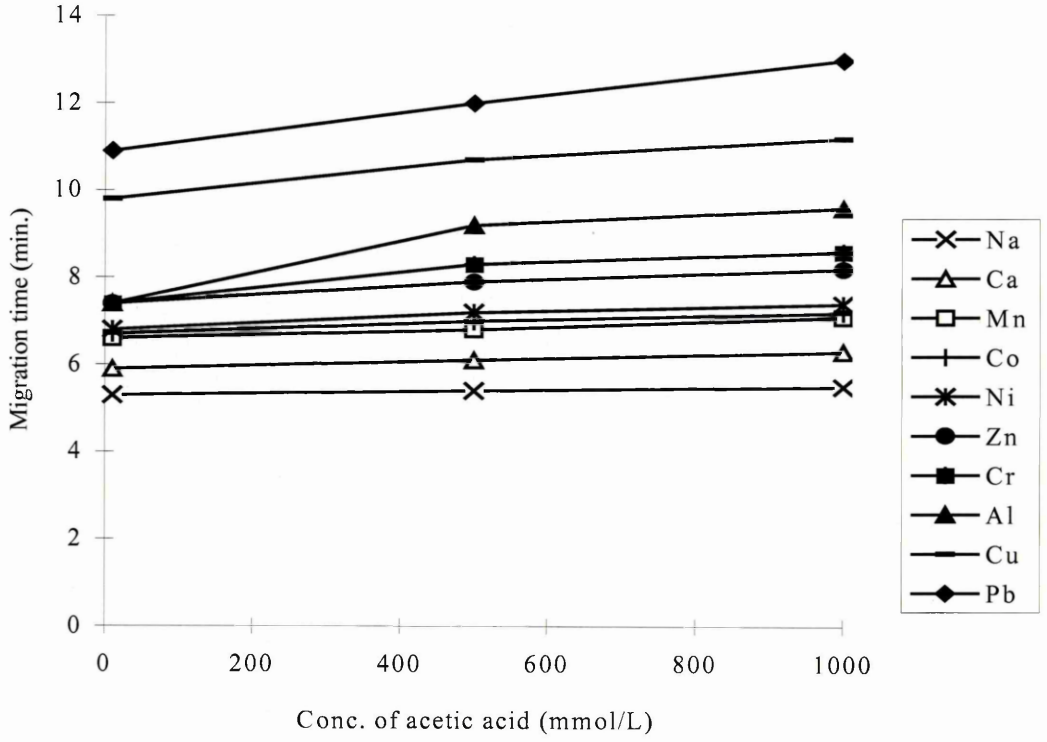


Fig. 5.22. Effect of changing the concentration of acetic acid. The buffer employed was 10 mol/L 4-methylbenzylamine and 50% MeOH.

The effect of acetic acid concentration on the separation of metal cations is also shown in Fig. 5.23. Some of the transition metals could not be resolved at lower concentration as compared at higher concentration. At the lower concentration some metal e.g. Cr and Pb were not observed and it is assumed that they co-eluted. They appear reported at the higher concentration. The best separation of metal cations obtained was at about 20 mmol/L acetic acid. This work also confirmed observations of Lin et al. (58) for the employing of monoprotic acid as a complexing reagent in their investigation. They found that by changing the concentration of acetic acid used in the buffer solution may affects the separation of the metal cations in group I and group II include K, Ba, Ca, Na, Mg and Li.

However they do not investigate the separation of transition metals in their work using the above buffer conditions.

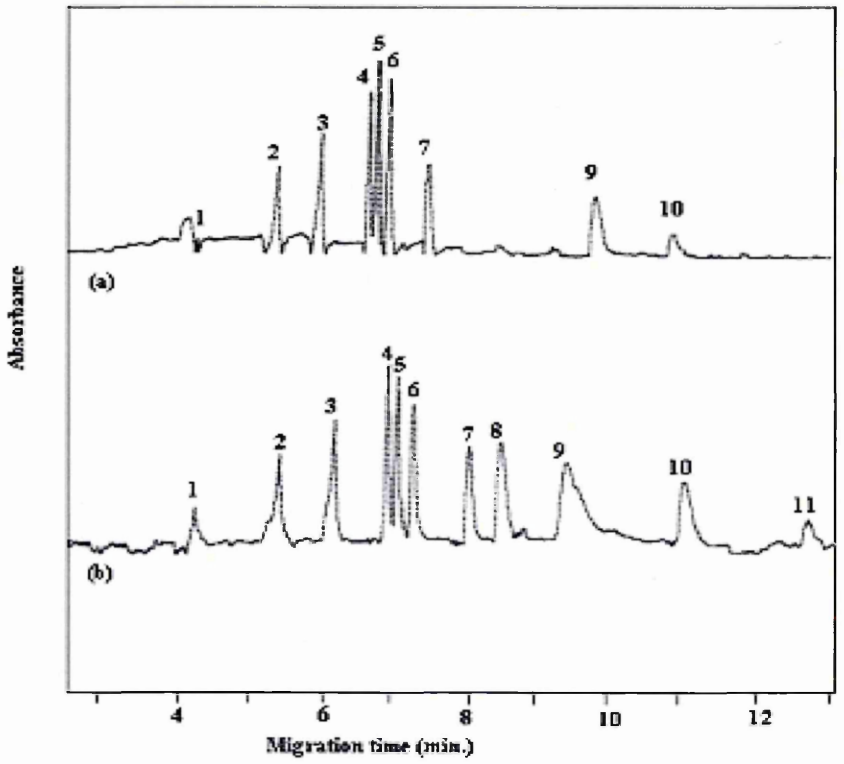


Fig. 5.23. Effect of increasing the concentration acetic acid with 10 mmol/L 4-methylbenzylamine, 50% MeOH at pH 3.5. The concentration of acetic acid employed for both experiments were (a) 10 mmol/L and (b) 20 mmol/L with a same conditions of the buffer composition. 1=K; 2=Na; 3; Ca; 4=Mn; 5=Co; 6=Ni; 7=Zn; 8=Cr; 9=Al; 10=Cu; 11=Pb.

The electropherogram shown in Fig. 5.24 was obtained using a buffer composition of 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine, 50% methanol at pH 3.5. The fourteen metal cations and ammonium were separated in 14 minutes. However, it was still not possible to separate iron from manganese under these conditions.

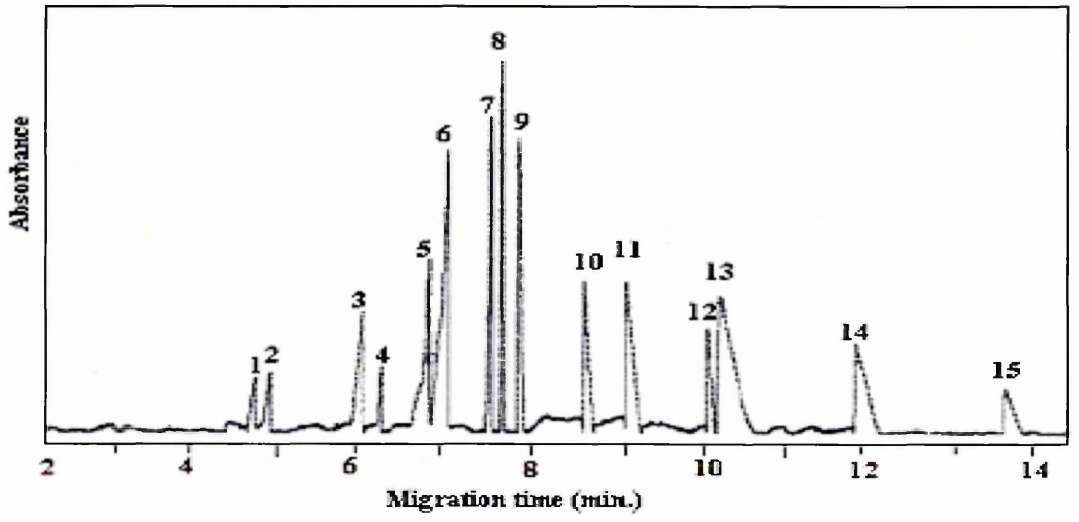


Fig. 5.24. Electrophoretic separation of fourteen metal cations and ammonium using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5). 1=NH_4^+; 2=K; 3; Ba; 4=Na; 5=Ca; 6=Mg; 7=Mn; 8=Co; 9=Ni; 10=Zn; 11=Cr; 12=Cd; 13=Al; 14=Cu; 15=Pb.

Based on the above study the optimum concentration of acetic acid was found at 20 mmol/L. At this concentration most metal cations were well separated with a good resolution.

5.3.3.2. Effect of pH

In this work, acetic acid was used as a complexing reagent to get a separation of NH_4^+ and 14 metal cations. Acetic acid is a monoprotic acid which pK_a value about 4.75 (162). The combination of 20 mmol/L acetic acid and 10 mmol/L 4-methylbenzylamine with 50% methanol was employed to observe the separation of metal cations at different pH. Under this conditions, the pH value was reduced from pH 4.0 (unadjusted pH) to pH 3.5 to study the separation of metal cations. This investigation was also to verify whether ammonium can be separated from potassium with the presence of other metal cations. The separation of metal cations from groups I&II and transition metals at different pH's is shown in Fig 5.25. Under normal conditions usually the metal cations will migrate faster at higher pH and slower at the lower pH. In this investigation the migration times at pH 4.0 are higher than pH 3.5 because the capillary employed is flushed with NaOH before the experiment at pH 3.5 was carried out. However this study showing the best separation of metals in group I and II and transition metals were almost complete at pH 3.5.

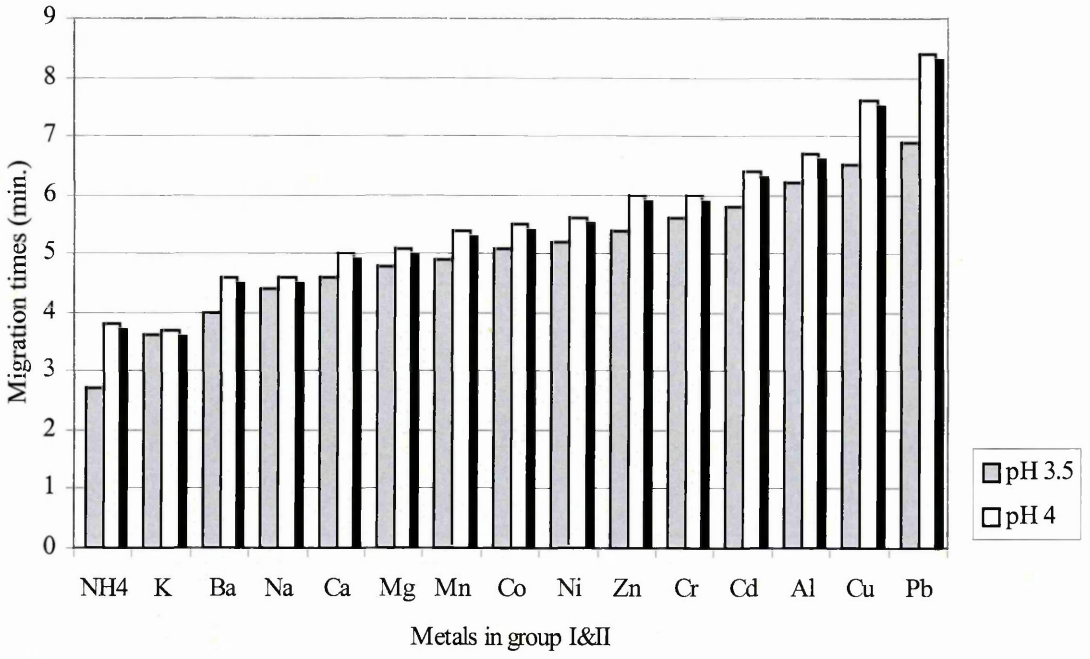


Fig. 5.25. Effects of pH using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol. Voltage used was 30 kV (The migration times at pH 3.5 are slower than pH 4 due to the capillary being flushed with NaOH before the experiment was carried out).

The slope of migration times versus pH not so much affected by reducing from pH 4.0 to pH 3.5. This indicates that the separation of metal cations in group I&II are not much influenced by changing the pH of the buffer. An effect of pH on the separation of transition metals may be expected due to complexation with acetic acid. This effect can also be observed from the slope of the migration times of Zn, Cr, Cd, Al, Cu and Pb in Fig. 5.25.

The separation obtained at different pH is also clearly shown in Fig 5.26. Fourteen metals in mixed standards solution were injected into CE but only twelve metals separated at pH 4.0. Barium did not separate from sodium while Zn co-migrated with Cr. The separation of FE was also impossible to obtain under this conditions.

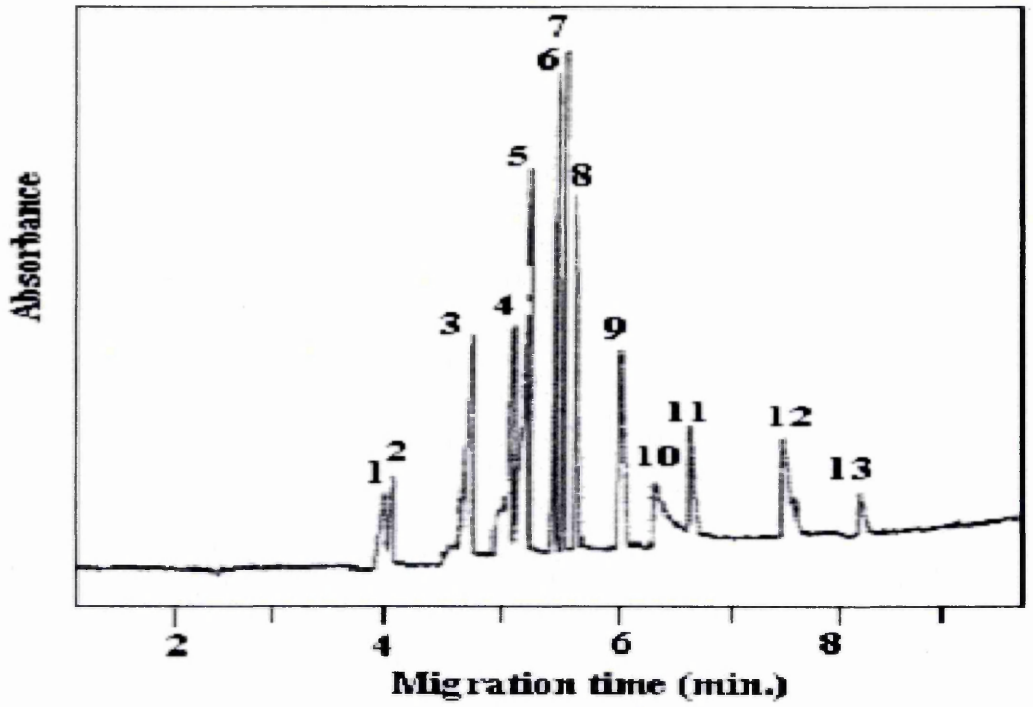


Fig. 5.26. Electrophoretic separation of twelve metal cations and ammonium using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0). 1=NH_4^+; 2=K; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Co; 8=Ni; 9=Cr; 10=Cd; 11=Al; 12=Cu; 13=Pb.

The number of metal separations increased to fourteen when the pH was reduced to 3.5. Ammonium was also separated under this conditions as shown in Fig. 5.27. The elution order was: NH_4^+, K, Ba, Na, Ca, Mg, Mn, Co, Ni, Zn, Cr, Cd, Al, Cu and Pb. However Fe still did not separate. In general, alterating the pH of the buffer changed only the migration time span (the separation time was shorter at higher pH owing to the increase in the electroosmotic flow); it could not resolve the barium and zinc peak or alter the migration order. It was noted that the acetic acid added to adjust the pH buffer may affect the resolution.

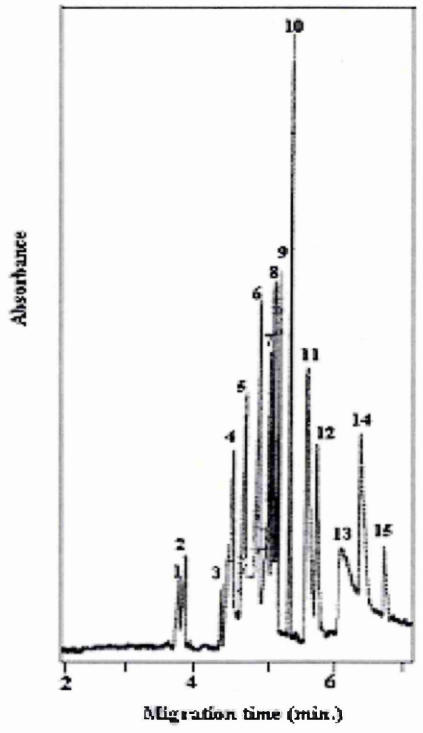


Fig. 5.27. Electrophoretic separation of fourteen metal cations and ammonium using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5). 1=NH_4^+; 2=K; 3; Ba; 4=Na; 5=Ca; 6=Mg; 7=Mn; 8=Co; 9=Ni; 10=Zn; 11=Cr; 12=Cd; 13=Al; 14=Cu; 15=Pb.

The above experiment indicated that the optimum pH was 3.5 to obtain a good separation with a reasonable analysis times for most metal cations. A. Weston et al. (22) was observed a similar trend for the effect of pH on the migration time of eleven alkali, alkaline earth and transition metals of the altering the pH. The combination of 6.5 mmol/L HIBA at pH 4.4 was employed in their investigation. They found that as the pH is lowered, the migration time is increase, due to decrease in the EOF. Likewise, as the pH increased, the migration times decease, due to increase in the EOF. In another experiment, Weston et al. (23) also found that potassium and ammonium co-elute at pH 6.15 owing to their identical

equivalent ionic conductivity. By altering the pH of the electrolyte, the ionization of alkali metals and their mobilities will be essentially unaffected. However, as the pH of electrolyte is increased, the NH_4^+ becomes progressively less protonated (pK_b of NH_4^+ = 4.75) and its apparent mobility decreases. At pH 8.5, the apparent mobilities of K and NH_4^+ become sufficiently different to permit an effective separation.

5.3.3.3. Effect of the addition of organic solvents

The use of organic solvents in the buffer solution has been investigated (41,163), and it has been demonstrated that, in general, the electroendoosmotic flow decreases with increasing fraction of organic solvent. This behaviour can be explained by changes of the electric properties of the electric double layer and the charge generation at the fused silica surface. The organic solvents are added to the running buffer to improve analyte solubility and detection sensitivity, to control electroosmotic flow (EOF) and to provide additional selectivity. Organic solvents may influence electrophoretic behaviour as follows:- (i) change the viscosity of the buffer solution; (ii) solvate analyte ions (163); and (iii) effect the chemical equilibrium between buffer and analytes (164,165).

In this investigation the combination of acetic acid and 4-methylbenzylamine was employed to study the effects of changing the percentage of methanol. At first , 10 mmol/L 4-methylbenzylamine and 20 mmol/L acetic acid was used without adjustment the pH (pH 4.0). However no separation was observed under these conditions, all peaks co-eluted together i.e. no separation. As shown in Fig. 5.28, separation of metal cations was observed at pH 3.5 but the resolution is still very poor.

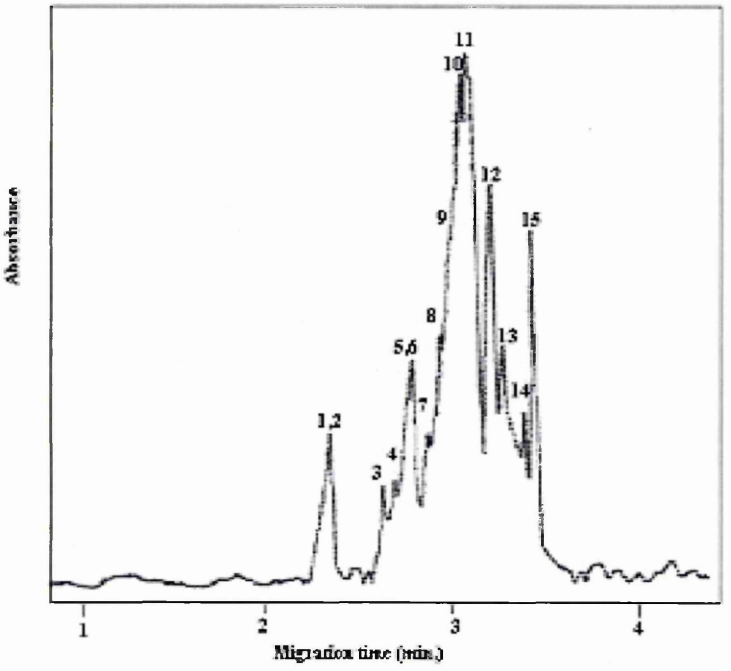


Fig. 5.28. Electrophoretic separation of fourteen metal cations and ammonium using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine, without methanol (pH 3.5). 1=NH_4^+; 2=K; 3; Ba; 4=Na; 5=Ca; 6=Mg; 7=Mn; 8=Co; 9=Ni; 10=Zn; 11=Cr; 12=Cd; 13=Al; 14=Cu; 15=Pb.

Separation of metal cations began to occur when methanol was added to the buffer. The number of metals that were resolved increased as the amount of methanol added increased, as shown in Fig. 5.29.

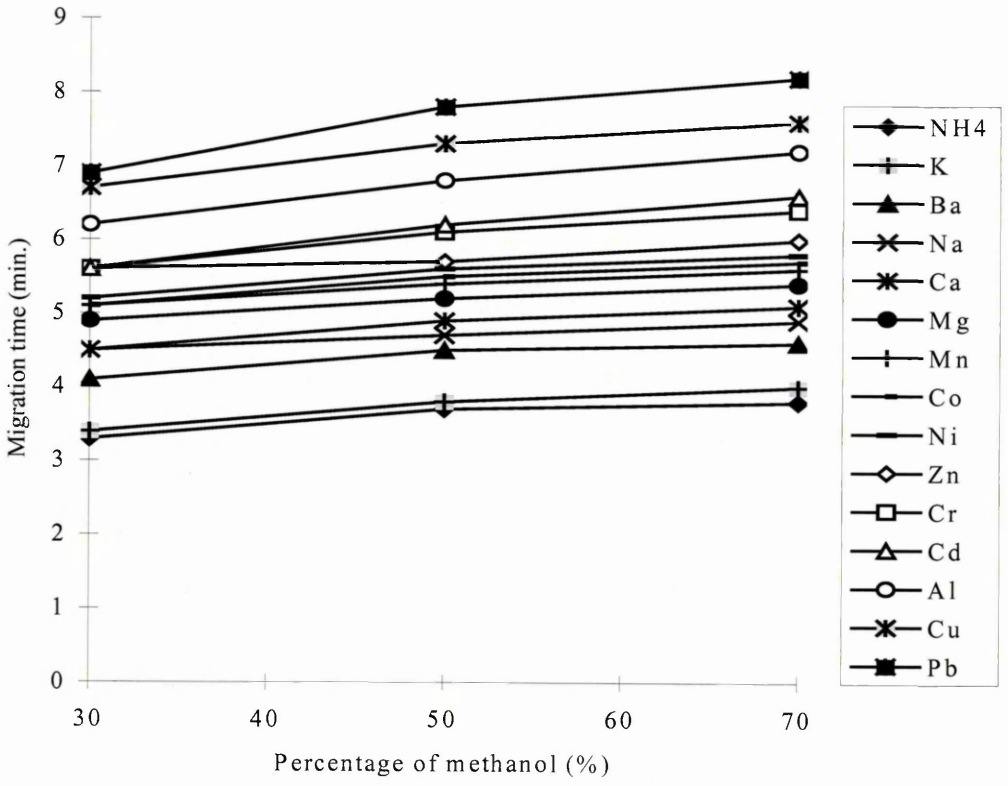


Fig. 5.29. Effect of different concentration of methanol in buffer solution. Electrophoretic separation of fourteen metal cations and ammonium using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine, various percentage of methanol (pH 3.5).

Based on the above observation, the separation of metal cations was improved when the methanol concentrations increased from 30% to 70% meanwhile the pH was maintained at 3.5. Most metal cations were well separated as the percentage of methanol in the buffer solution increased. The separation of metal cations in group I, group II and transition metals is shown in Fig. 5.30.

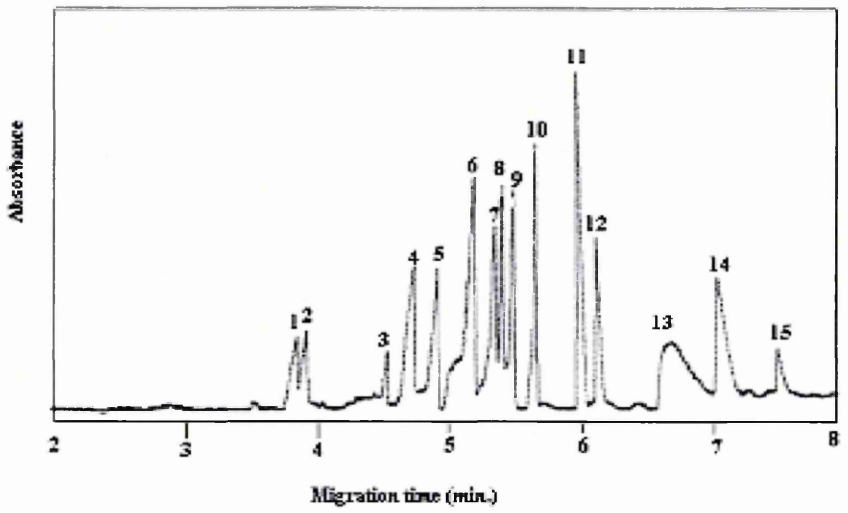


Fig. 5.30. Electrophoretic separation of fourteen metal cations and ammonium using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine, 50% methanol (pH 3.5). 1=NH_4^+; 2=K; 3; Ba; 4=Na; 5=Ca; 6=Mg; 7=Mn; 8=Co; 9=Ni; 10=Zn; 11=Cr; 12=Cd; 13=Al; 14=Cu; 15=Pb.

This shows that increasing the amount of methanol in the buffer solution will improve the resolution of metal cations. The effect of adding acetonitrile was also observed but the separation was not as good as with methanol. The baseline was noisy and unstable. Based on the above experiment the optimum concentration of methanol in the buffer composition was found to be 50%. At this concentration most metal cations were well separated with good resolution. This results supports the investigation by Yang et al. (62) on the simultaneous separation of ammonium and alkali, alkaline earth and transition metal ions in aqueous. They have found that with the absence of methanol in background electrolyte, Li, Ni and Zn are co-migration but after the addition of methanol they can get a separation of Li, Ni and Zn. However they observe this effect of methanol up to 30% at the

maximum amount for improving on the electrophoretic mobilities of the separation of cations. Other reported by Shi and Fritz (30) also was noted that the electrophoretic mobility decreases almost linearly as the percentage of methanol was added in the buffer solution. However they only experimented the methanol up to 20 % under their investigation. They obtained a good resolution by increasing the methanol concentration in the separation of eight metal cations using phthalic acid.

5.3.3.4. Reproducibility

A buffer composition of 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine at pH 3.5 was employed to study the reproducibility of metals from group I and group II and cadmium. The procedure used was followed the outlined in Section 4.5.1.3. for ANOVA calculation. The results obtained are given in Tables 5.12 - 5.14. In this study the standard mixtures containing 10 metals from group I, group II and transition metals were injected to observe the reproducibility of migration time, absorbance and peak area. These metals were Na, Ca, Mn, Co, Ni, Zn, Cr, Al, Cu and Pb.

5.3.3.4.1. Migration time (MT)

Full summarises reproducibility data for all ten metals is shown in Table 5.14.

Elements	Mean result, min. ± SD [a]	ANOVA *F* value[c]
Sodium	5.62 [b] ± 0.07	1.41
Calcium	6.15 [b] ± 0.08	1.80
Manganese	6.78 [b] ± 0.09	1.19
Cobalt	6.92 [b] ± 0.11	0.90
Nickel	7.06 [b] ± 0.07	1.41
Zinc	7.54 [b] ± 0.110	0.90
Chromium	7.98 [b] ± 0.12	1.32
Aluminum	8.93 [b] ± 0.14	1.26
Copper	9.93 [b] ± 0.17	0.01
Lead	11.16 [b] ± 0.19	0.01

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.14. Reproducibility data on migration time for metal cations at different batch samples using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine at pH 3.5.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.14 shows that the migration times were stable and able to provide consistent and reproducible data. This result obtained was agreed with the findings reported by Jones and Tarter (198) on the analysis of selected transition metal cations using 5 mmol/L oxalic acid, 3.75 mmol/L citric acid at pH 4.37. They observed the

reproducibility of retention time ranges from 0.03 to 0.05 of standard deviation. Therefore, results obtained under this work are consistent with their observation in similar field.

5.3.3.4.2. Absorbance

Full summarises reproducibility data for all ten metals is shown in Table 5.15.

Elements	Mean result, AU ($x10^{-3}$) ± SD [a]	ANOVA *F* value[c]
Sodium	2.62 [b] ± 0.06	1.16
Calcium	3.55 [b] ± 0.08	1.29
Manganese	5.53 [b] ± 0.10	1.60
Cobalt	7.34 [b] ± 0.18	0.97
Nickel	10.60 [b] ± 0.49	1.04
Zinc	4.81 [b] ± 0.09	1.11
Chromium	4.26 [b] ± 0.09	0.01
Aluminum	2.63 [b] ± 0.05	0.01
Copper	2.34 [b] ± 0.10	0.01
Lead	1.05 [b] ± 0.03	0.01

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.15. Reproducibility data on absorbance for metal cations at different batch samples using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine at pH 3.5.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ

significantly. Table 5.13 shows that the absorbance were stable and able to provide consistent and reproducible data for Na, Ca, Mn, Co, Ni, Zn, Cr, Al, Cu and Pb. This result indicated that absorbance for metal cations was suitable for quantitative analysis. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of Ni, Zn and Co with the employing of 5 mmol/L oxalic acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of absorbance in this work.

5.3.3.4.3. Peak area

Full summarises reproducibility data for all ten metals is shown in Table 5.16.

Elements	Mean result, peak area ($x10^6$) ± SD [a]	ANOVA *F* value[c]
Sodium	0.05 [b] ± 0.005	1.08
Calcium	0.07 [b] ± 0.015	1.08
Manganese	0.07 [b] ± 0.005	1.08
Cobalt	0.07 [b] ± 0.010	1.08
Nickel	0.07 [b] ± 0.004	1.02
Zinc	0.06 [b] ± 0.005	1.08
Chromium	0.09 [b] ± 0.005	0.01
Aluminum	0.11 [b] ± 0.020	0.06
Copper	0.09 [b] ± 0.029	0.01
Lead	0.02 [b] ± 0.008	0.01

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 5.16. Reproducibility data on peak area for metal cations at different batch samples using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine at pH 3.5.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. Table 5.16 shows that the peak area are stable for providing a consistent and reproducible data for Na, Ca, Mn, Co, Ni, Zn, Cr, Al, Cu and Pb. These results may be due to the acetic acid used for pH adjustment may influence the sample adsorption to capillary walls. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of Ni, Zn and Co with the employing

of 5 mmol/L oxalic acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of peak area in this work.

5.3.3.5. Linearity

Figs. 5.31 – 5.40 shows linearity for the group I and group II and transition metal cations. The procedure used for non-linearity of the graph was followed as outlined in Sectioned 4.5.1.4. There were six concentrations of multi-element standards used from 1 ppm to 40 ppm. Ten times at each concentration was injected and duplicates with the same set solution of each concentration were carried out sequentially. Lower concentration than 1 ppm were not tested. The average values of the results in linearity for Na ($y = 0.1167x + 0.2384$; and $R^2 = 0.9929$), Ca ($y = 0.1941x + 0.4323$; and $R^2 = 0.9904$), Mn ($y = 0.557x + 0.0849$; and $R^2 = 0.9952$), Co ($y = 0.3778x + 0.3831$; and $R^2 = 0.9968$), Ni ($y = 0.263x + 0.5037$; and $R^2 = 0.9918$), Zn ($y = 0.152x + 0.3146$; and $R^2 = 0.9915$), Cr ($y = 0.1587x + 0.3628$; and $R^2 = 0.991$), Al ($y = 0.1436x + 0.1585$; and $R^2 = 0.9976$), Cu ($y = 0.0931x + 0.214$; and $R^2 = 0.9905$) and Pb ($y = 0.0502x + 0.0836$; and $R^2 = 0.9936$) were acceptable and can be fitted a straight line for the above range.

It appears that the deviations of all metals are less than 5% at lower concentration than 40 ppm. However the non-linearity is observed for all metals at higher concentration than 40 ppm and the improvement to fit with a straight line may be required for quantitative analysis. The non-linearity observed above 40 ppm with deviations is about 6.8% for Na ($y = 0.1071x + 0.3345$; $R^2 = 0.9849$), deviations is about 6.6% for Ca ($y = 0.1788x + 0.5859$; $R^2 = 0.9841$), deviations is about 6.6% for Mn ($y = 0.5144x + 0.5115$; $R^2 =$

0.988), deviations is about 6.9% for Co ($y = 0.3471x + 0.6902$; $R^2 = 0.9878$), deviations is about 6.4% for Ni ($y = 0.2859x + 0.2744$; $R^2 = 0.9878$), deviations is about 6.2%Zn ($y = 0.1407x + 0.428$; $R^2 = 0.986$), deviations is about 5.1% for Cr ($y = 0.1488x + 0.4623$; $R^2 = 0.9885$), deviations is about 6.9% for Al ($y = 0.132x + 0.2752$; $R^2 = 0.9883$), deviations is about 5.1% for Cu ($y = 0.0873x + 0.2725$; $R^2 = 0.9881$) and deviations is about 6.1% for Pb ($y = 0.0466x + 0.1199$; $R^2 = 0.9879$). Some of the reason may due to the instrumental deviations from Beer's law ultimately limit quantitative analysis. At high analyte concentrations, the deviation usually takes the form of decreased slope of the calibration curve (as indicated above). The linear detection range is mainly limited by stray light reaching the detector. Ideally, all light should pass through the center of the capillary, not through the wall. There is an interdependent relationship between sensitivity and linear detection range. For a given design, increasing the light throughput, by increasing the slit size for example can improve detection limits by increasing the light level to photodiode and thereby decreasing the baseline noise. However, if the slit allows stray light through the capillary walls, the linear detection range will be compromised. If the slit is increase along the length of the capillary, resolution will be reduced. Depending on the analysis, slit size can be chosen to optimise either sensitivity, or resolution and linear detection range.

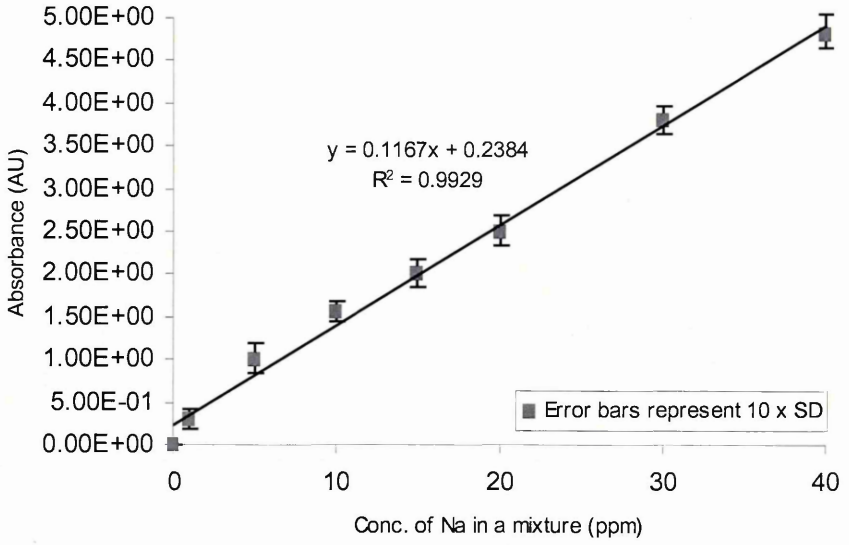


Fig. 5.31. Linearity range of Na based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

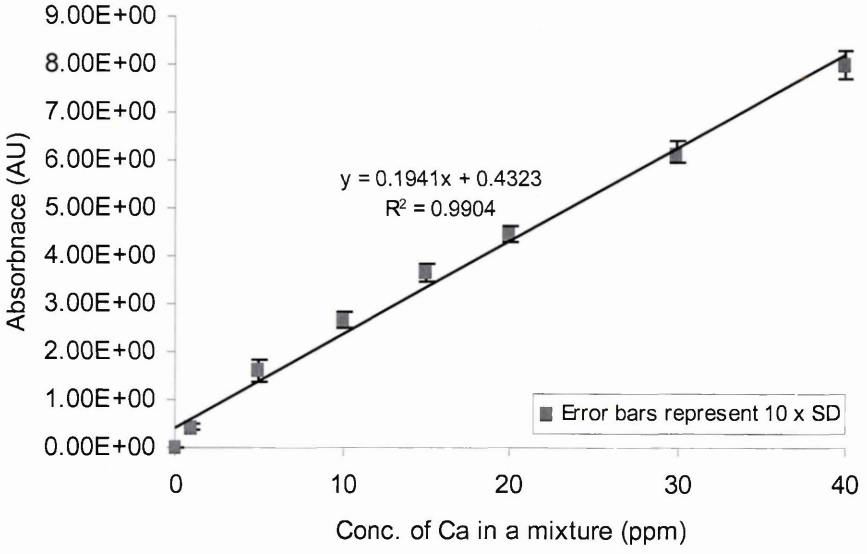


Fig. 5.32. Linearity range of Ca based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

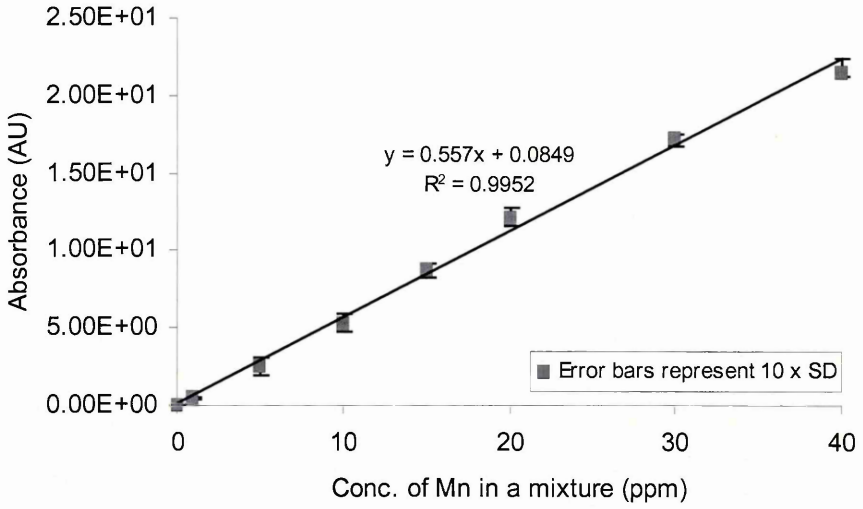


Fig. 5.33. Linearity range of Mn based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

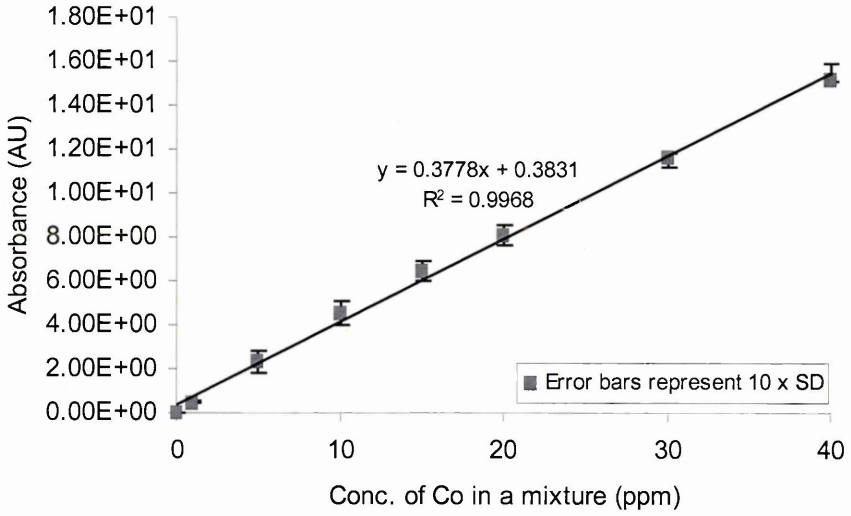


Fig. 5.34. Linearity range of Co based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

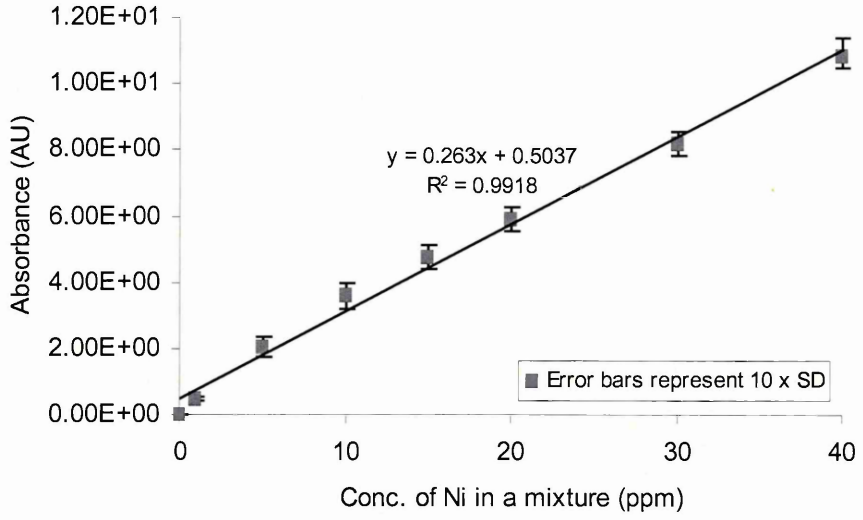


Fig. 5.35. Linearity range of Ni based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

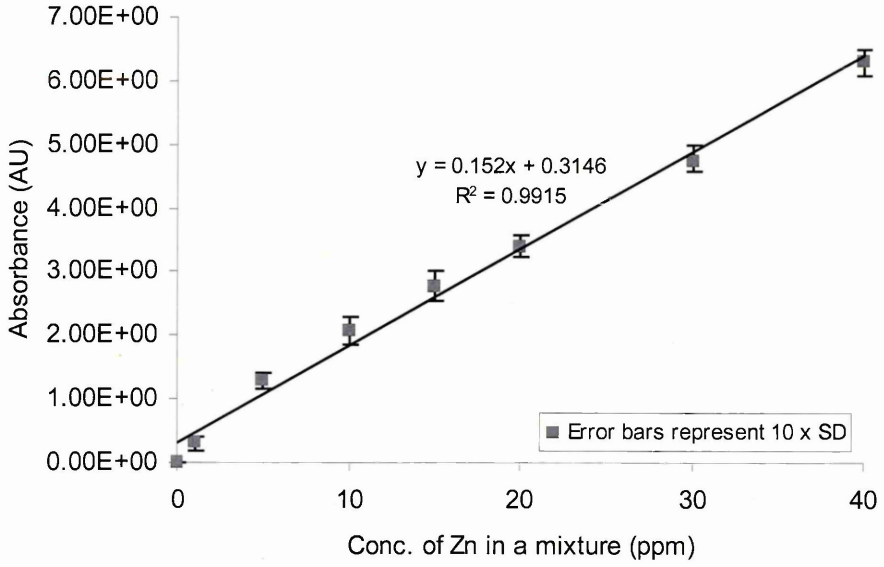


Fig. 5.36. Linearity range of Zn based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

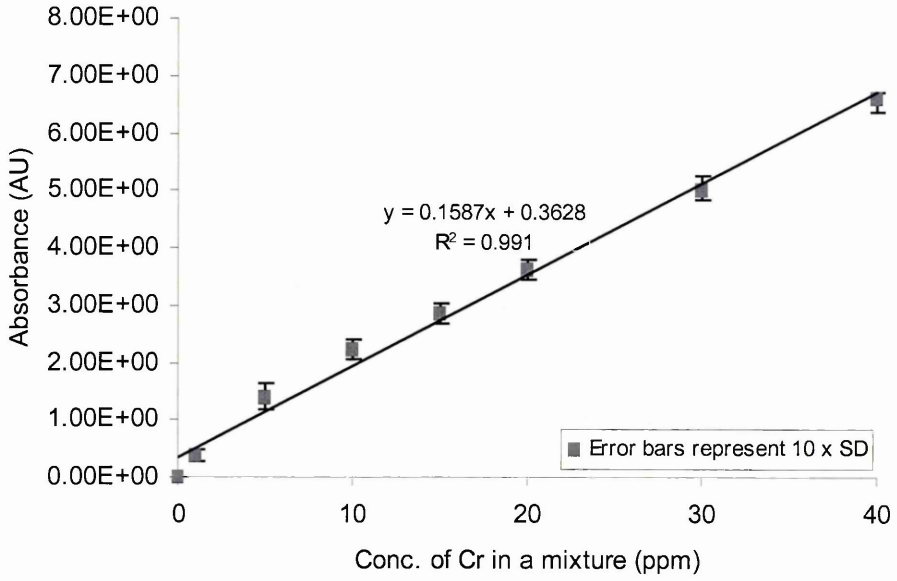


Fig. 5.37. Linearity range of Cr based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

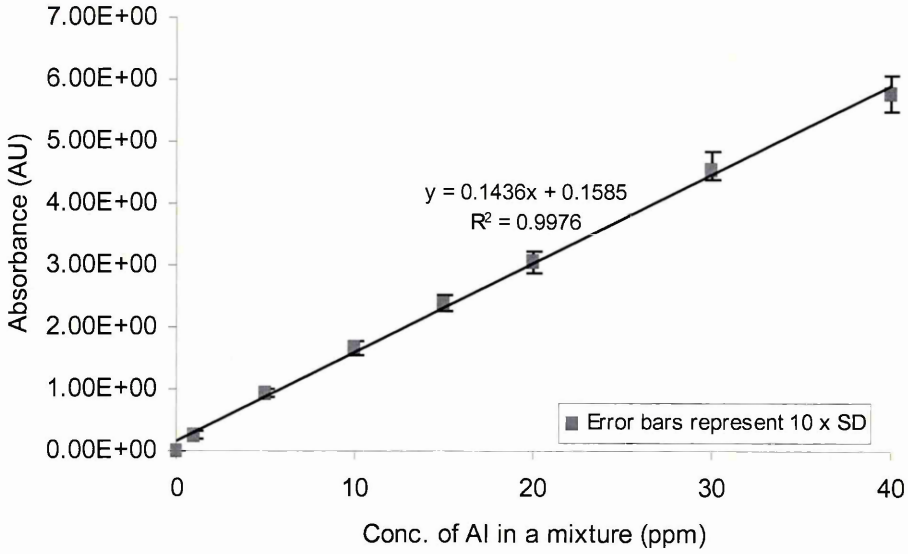


Fig. 5.38. Linearity range of Al based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

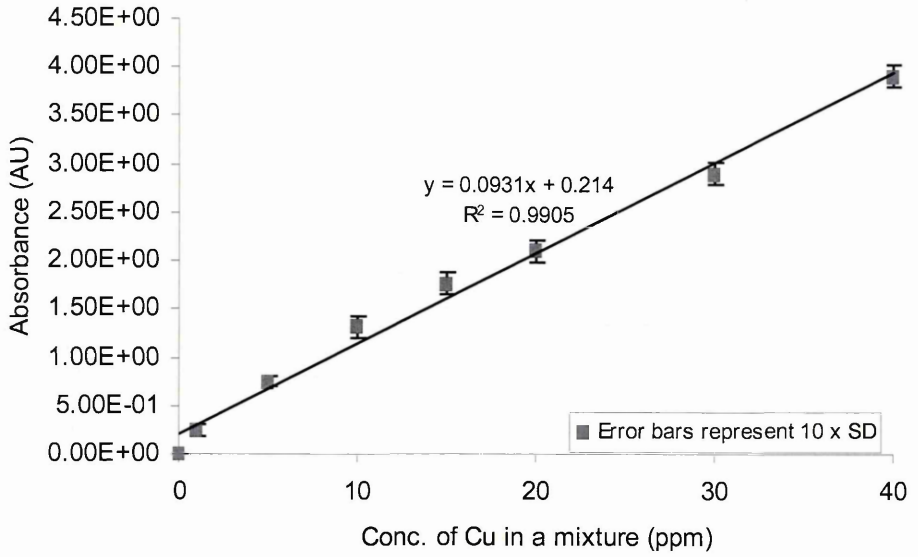


Fig. 5.39. Linearity range of Cu based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

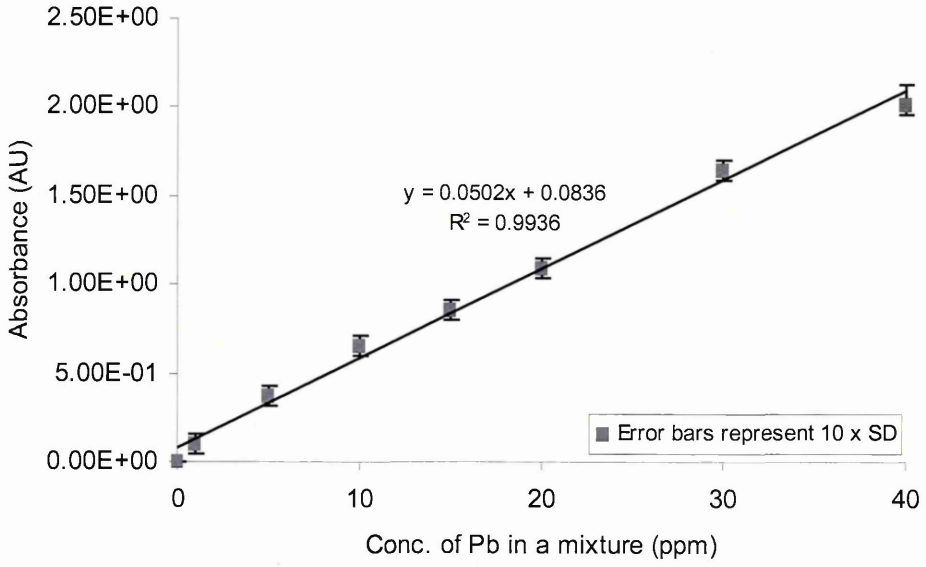


Fig. 5.40. Linearity range of Pb based on peak response (absorbance). The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5).

The overall linearity of ten metals from group I, group II and transition metals that are found to be up to 40 ppm in this work are not so good (the correlation coefficients, $R^2 <$ 0.99) compared to the previous work in similar field. Shi and Fritz (29) reported a good linear calibration curves were obtained for the metal cation studies in the 0.4 ppm to 10 ppm concentration range. They used the buffer solution of 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3 for the separation of eight metal cations include K, Na, Pb, Mn, Co, Ni, Zn and Cd. However they obtain a relatively poor correlation coefficient of linear regression for zinc. Meanwhile, Shi and Fritz (30) obtained a linear calibration curves for the metal cations from 0.1 to 10 ppm for the separation of Sr, Mg, Mn, Co and Zn. They used 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 0.6 mmol/L 18-crown-6, 10% methanol at pH 4.3. In another development, Yang et al. (63) reported the linear calibration curves up to 20 ppm was obtained for the separation of K, Na, Ca, Mg and Mn. They also found that at high concentrations peak distortion occurred owing to overloading, which caused insufficient selectivity of separation, so that the calibrations were no longer useful. At pH 4.5 the calibration line for Mn was linear only 6 ppm owing to the insufficient separation between Mn and Mg. Recently, N. Shakulashvili et al. (191) reported a good linear calibration curves from 0.75 to 80 ppm for the separation of sixteen metal cations using 10 mol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. Based on the above finding, this method is not suitable for quantitative analysis. Therefore, a further development need to be carried out to improve the correlation coefficient (R^2) of each metal cations for quantitative analysis.

5.3.3.6. Limit of detection (LOD)

Serial dilutions of a mixture standard solutions were made to determine the detection limits of the system. Minimum detectable concentrations for metal cations based on a peak signal (absorbance) to noise (3:1) are given in Table 5.17 . The buffer employed was 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 3.5.

Metal cations	Mean of detection limit[a] (ppm ± SD)
Sodium	0.94[a] ± 0.10
Calcium	0.76[a] ± 0.12
Manganese	0.12[a] ± 0.5
Cobalt	0.22[a] ± 0.06
Nickel	0.38[a] ± 0.4
Zinc	0.26[a] ± 0.06
Chromium	0.33[a] ± 0.07
Aluminum	0.92[a] ± 0.10
Copper	0.77[a] ± 0.10
Lead	0.84[a] ± 0.12

Notes: [a] = mean of 10 replicates

Table 5.17. Limit of detection for group I&II and transition metals.

Limits of detection are found to be in the sub ppm level but not as good compared to values obtained by Lee and Lin (57) in the analysis a mixture of nineteen metal ions (Li,

Na, K, Cs, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ag, Al and Pb) using 10 mmol/L pyridine, 12 mmol/L glycolic acid at pH 4.0. They obtained limit of detection in the range of 0.02 ppb for Na up to 0.208 ppm for Cr. Meanwhile, Yang et al. (62) obtained the limit of detection are as follows: 0.4 ppb for Li and Mg, 1 ppb for NH_4^+ and Ca, 2.5 ppb for Na, 10 ppb for K, 120 ppb for Sr, Mn, Cr and Zn, 500 ppb for Ba and Ni and 1000 ppb for Cu. The values for limit of detection are based on three times the baseline noise. They use 5 mmol/L imidazole, 6.5 mmol/L HIBA, 20% methanol and 1.33 mmol/L 18-crown-6 at pH 4.5. Shi and Fritz (29) also reported the limit of detection in the range 0.05 to 0.5 ppm for the separation of twenty seven alkali, alkaline earth, transition and rare earth metal cations. Light ions are in the low detection limit range, and heavy ions are in the high detection limit range. They employed 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. N. Shakulashvili et al. (191) obtained the limit of detection in the range 0.1 for potassium and 0.454 ppm for copper in a mixture of 16 metal ion which is separated using 10 mmol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. Although the limit of detection in this work is slight higher compared to the previous work but the method that has been developed can be used for quantitative analysis.

5.3.3.7. Effects of acetic acid on separation performance

Under this work only fourteen metals from group I, II and transition metals include Na, Ca, Mn, Co, Ni, Zn, Cr, Al, Cu, Pb and NH_4^+ were observed. Fourteen metals were separated in less than 7.5 minutes using 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine

and 50% methanol at pH 3.5. The successful separation of fourteen metal cations and NH_4^+ is shown in Fig. 5.30. The migration order follows the sequence Na, Ca, Mn, Co, Ni, Zn, Cr, Al, Cu and Pb. All peaks are completely baseline resolved except Co, Mn and Ni. The peaks become more symmetric with increasing concentration of methanol especially for all metals compared without the methanol added into the buffer solution. The buffer concentration has a great effect on the peak shape. If we compare the results with those achieved under the same conditions in previous studies, in spite of the use of a significantly longer capillary, the increases in buffer concentration case much more symmetrical peaks. These finding is better in term of number of analytes separated and overall run time compared to those observation by Shi and Fritz (29). They obtained eight metal cations including K, Na, Pb, Mn, Co, Ni, Zn and Cd in less than 6 minutes using 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3. They have a good peak shape and baseline resolved. In another development they have separated twelve metal cations using 2.5 mmol/L tartaric acid, 6 mmol/L *p*-toluidine, 20 % methanol at pH 4.8 in less than 8 min. Resolution is excellent with a steady, flat baseline. However an excellent separation of twenty seven metal cations has been achieved by Shi and Fritz (29) in a single run in less than 6 min. They used 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. Among those metal cations that were separated including K^+, Ba^{2+}, Sr^{2+}, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^+, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Cu^{2+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+}. The average deviation in peak height from one run to another was approximately ± 5% or smaller at the ion concentration used.

The plate numbers achieved for each metals are listed in Table 5.18.

Metal cations	Number of theoretical plates per meter (N/m)
Sodium	386,328
Calcium	354,201
Manganese	340,137
Cobalt	237,195
Nickel	609,667
Zinc	281,603
Chromium	265,047
Aluminum	243,852
Copper	204,494
Lead	206,776

Table 5.18. Peak efficiency for 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5) using CE-UV/Vis detection.

From Table 5.18, Ni has the highest number of theoretical plates (N), about 609,667 followed by Na, Ca, Mn, Zn, Cr, Al, Co, and Pb because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as acceptable to Cu has the lowest N value, about 204,494. This values obtained was excellent as compared to N value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the N value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of N between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. The result obtained shows that the peak efficiencies for each metal cations are suitable for quantitative analysis.

5.4. Conclusion

This investigation clearly demonstrated that the separation of metal cations from group I&II is possible without forming metal complexes via UV/Vis detection. It has been shown that partial complexation with an organic acid (e.g. oxalic acid or acetic acid) and a neutral compound (acac) are required to affect the separation of transition metals in a single run.

It was found that the incorporation of a complexing reagent was required in order to get a separation of metal cations from a mixture of group I, II and transition metals. Oxalic acid, acac and acetic acid were employed as the complexing reagent in conjunction with 4-methylbenzylamine. Indirect detection of metal cations in group I, II and transition metals was observed using UV/Vis detection.

The concentration, pH and viscosity of the buffer composition also will be affected the degree of separation observed. It was clearly demonstrated that organic modifier (methanol) was needed in the buffer composition in order to get a full separation of transition metals, but is limited to a certain percentage. For oxalic acid, the metal cations in group I and group II not much influenced by alteration of the pH of buffer in the range 3.5 to 4.5. Most transition metal groups are influenced by the modification of buffer pH and concentration due to formation of metal complexes with oxalic acid to enable a separation. In acetate buffer, the separation of transition metals was influenced by the alteration of buffer pH due to formation of metal complexes with acetic acid to enable a separation. The separation and resolution of metal cations were improved when the concentration was increased up to 50% methanol in the buffer composition.

The adjustment of pH, concentration and the percentage of methanol were investigated and optimum values determined. Separation of 6 metal cations was obtained using a buffer composition of 5mmol/L oxalic acid, 6.9 mmol/L 4-methylbenzylamine at pH of 4.0 in less than 9 min. The reproducibility of both migration time and peak absorbance are good ($F_{obs.} < F_{crit.}$). Limits of detection in the high ppb to low ppm range were shown but poor in linearity (correlation coefficients ranges 0.2 - 1 ppm with $R^2 > 0.96$).

The use of 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0 allowed the separation of 8 metals in less than 8.5 min. The reproducibility of migration times, peak absorbance and peak area were fairly good ($F_{obs.} < F_{crit.}$) at 10 ppm level. Limit of detection in the high ppb to low ppm range were shown but the linearity was poor (correlation coefficients ranges 1 - 25 ppm with $R^2 > 0.95$).

The use of 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 3.5 enabled a separation of 14 metals in less than 7 min. The migration time and peak absorbance were more reproducible when compared with peak area ($F_{obs.} < F_{crit.}$). Limits of detection in the high ppb to low ppm range were shown with good linearity (correlation coefficients ranges 1 - 40 ppm with $R^2 > 0.99$).

Acetic acid seemed to be the most suitable complexing reagent for the separation of metal cations compared with oxalic acid and acac. The use of 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol at pH 3.5 showed the most successful separation of metal cations in group I, II and transition metals in a very short period (less than 7 min.).

However none of the complexing reagents employed including oxalic acid, acac and acetic acid were able to demonstrate a separation of iron from other analyte components.

CHAPTER 6

THE INVESTIGATION OF METAL CATIONS USING A CONDUCTIVITY DETECTOR

6.1. Introduction

Until recently, most of the detection methods for the investigation of metal cations employed CE with a UV/Vis detector. The wide applications of UV/Vis detectors are due to excellent mass sensitivity and its universality as a detector for a wide range of analytes. However, the concentration sensitivity for such detectors is relatively poor as in CE the path length is effectively the inner diameter of the capillary column. Developments in capillary technology have enabled the production of capillaries with even smaller I.D. and while this will lead to increase efficiencies through better dissipation of Joule heat, the shorter path lengths will provide lower concentration sensitivity for photometric detection. The application of conductivity detection for the analysis of small ions may become more attractive because this method offers a simple alternative and can be employed for a wide range of analytes.

The first uses of a conductivity detector were reported by Hjerten (166), Virtanen (5), Mikkers (6) and Jorgenson (7) between 1967 and 1981. Huang et al. (38,167,168) carried out comprehensive experiments using conductivity detection for the investigation of metal cations. They showed that direct conductivity detection can be used as a universal and sensitive detector. They employed 30 mmol/L HIS (L-Histidine)/ MES (2-[N-morpholino]ethanesulphonic acid) and 2 mmol/L 18-crown-6 at

pH 6.1 in the separation of a number of metal and organic cations. This was achieved in less than 4.5 minutes. The method used was unable to separate transition metals and was limited to group I and II metals only. In 1993, Wu et al. (169) were also carried out an investigation using a conductivity detector for determination of alkali and alkaline earth metals. This investigation was also not successful in the determination of transition metals. In general, the applications of a conductivity detector particularly for the analysis of metal ions has, to-date had an emphasis on group I and II. Little work has been reported investigating the separation of transition metals.

In this study to investigate the use of a conductivity detector for the separation of metal ions lactic acid was employed as a complexing reagent with LiOH as a background electrolyte. This work looked at the effect of buffer pH, addition of organic modifier and changing the concentration of complexing reagent on the separation of metal ions. Reproducibility, limits of detection and linearity were also established.

6.2. Experimental procedures

6.2.1. Instrumentation

The CE instrument used was as described in section 3.2.1 in conjunction with a conductivity detector previously described in section 3.2.3. Voltages of 25 kV to 30 kV were applied.

6.2.2. Materials and reagents

All the reagents and materials used have been previously discussed (section 3.3). The procedures for the preparation of 10 ppm metal cation standards were as outlined in section 3.3.

6.2.3. Capillary preparation and cleaning

A special capillary (ConCap I) was employed as was mentioned in section 3.2.2 and this was used for each experiment. The conditioning and washing procedures were as described in section 3.4 and were carried out before starting each analysis. These procedures are important because some of the transition metals employed may precipitate in the capillary. If the capillary is not flushed properly, some metal salts may also precipitate in the detector cell and affect the background conductivity. Failure to wash may result in the solenoid valve, within the detector, not functioning properly and the background conductivity will increase. Reproducibility of the data will be poor. It is therefore important to flush with deionised water to ensure removal of all particles and/or salts from inside the conductivity detector.

6.2.4. Injection procedures

As mentioned in section 3.6.1, most of the samples were hydrodynamically injected into the column. At the beginning of analysis the capillary was flushed with deionised water for 2 minutes at an applied pressure of 2000 mBar prior to filling the capillary with buffer. The sample was then introduced using the hydrodynamic injection mode. The time of injection and/or pressure applied was at different levels depending on the sample concentration used.

6.2.5. Buffer preparation and pH adjustment

6.2.5.1. MES and L-Histidine

MES and L-Histidine were freshly prepared as required in accordance with procedures outlined section 3.5.2.1.

6.2.5.2. Lactic acid

Lactic acid was freshly prepared as required in accordance with procedures as outlined section 3.5.1.4.

6.2.5.3. Lithium hydroxide

LiOH was freshly prepared as required in accordance with procedures as outlined section 3.5.2.3.

6.2.5.4. Percentage of methanol

Methanol (10% by v/v) was freshly prepared as required in accordance with procedures as outlined section 3.3.

6.2.5.5. Sample preparation and pH adjustments

The preparation of samples in this investigation were in accordance with procedures as outlined section 3.6.2. The pH adjustment of solutions employed in these experiments followed procedures as mentioned in section 3.6.3 and 3.6.3.2.

6.2.6. Degassing and filtration procedure

The sample and buffers used in this work were treated according to procedures as described in section 3.6.4.

6.3. Results and discussion

6.3.1. Preliminary analysis using MES and L-Histidine

The combination of MES and L-Histidine is frequently employed for the separation of metal cations from group I and II by Huang et al. (38). Complexation of transition metals when using MES/L-Histidine as the buffer is problematic. However, the above procedure was followed in order to optimise the instrumentation using conductivity detection in this work.

Various sensitivity ranges were possible but the most successful sensitivity was obtained at 1000 nS. This enabled a good electropherogram of metal cations to be obtained. In the initial experiment, 30 mmol/L HIS/MES at pH 6.1 was employed to obtain a separation of Na, Ba, K, Ca, Mg and Mn in less than 5 minutes. The separation obtained is shown in Fig. 6.1. All metal cations in group I and group II were successfully separated. Transition metals, Ni, Mn, Cd, Pb, Fe, Cu, Zn, Al, Cr and Co were also injected together with the above metals but only Mn appeared using the above conditions. The separation obtained is agreement with the previous investigation by Huang et al. (38) using the same buffer composition and similar conditions for the analysis of metal cations. However, the resolution for some metal cations in this work not as good as shown in the previous work. This is due to the experimental conditions used not properly optimised accordingly to the original work. For instance, the absence of 18-crown-6 as the buffer composition could be affected the mobility rate and selectivity of all metal cations. The specific device is not available to control the temperature conditions when the experiment was carried out. However, the previous work is performed at the temperature of 35^0C for detection. This discrepancy of results was observed due to slightly changes of the analytical parameters e.g. temperature and viscosity that may affects the resolution and reproducibility of some

metal cations. The work carried out using the combination of HIS/MES is not suitable to obtain a separation of transition metals.

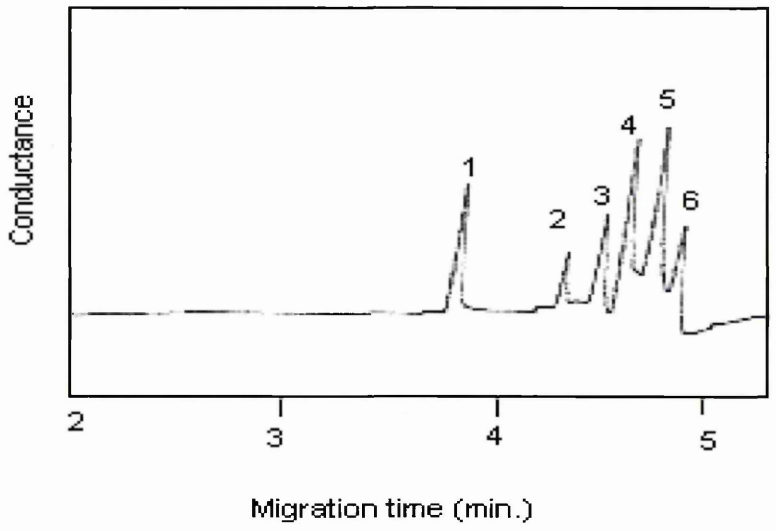


Fig. 6.1. CE analysis of metals from group I&II and transition metals with direct conductivity detection. Buffer: 30mmol/L MES/His, pH 6.1; 1=Na; 2=Ba; 3=K; 4=Ca; 5=Mg; 6=Mn. Capillary: ConCap I, 50μm I.D., length 60 cm; pressure injection, 30 mBar x 30 sec; potential: 30 kV; capillary and detector under ambient temperature.

6.3.2. Use of LiOH and lactic acid

The use of LiOH and lactic acid in CE has not been previously reported in the investigation of metal cations especially for the separation of transition metals. For this study, the separation of metal ions using a conductivity detector will be investigated and will employ LiOH and lactic acid. To date, little information regarding the separation of transition metals using conductivity detection is available. Lactic acid has been widely reported as a complexing reagent in many investigations (29,30,58-62,65,66) to separate metal ions (including alkali and alkaline earth and transition metals). An excellent separation of twenty seven metal cations has been achieved by Shi and Fritz (29) in a single run in less than 6 min using lactic acid as complexing reagent in their work. They employed 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. Among those metal cations that were

separated including K^+, Ba^{2+}, Sr^{2+}, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^+, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Cu^{2+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+} . However, LiOH is often employed as eluent buffer in ion chromatography (IC) for the separation of cations using a conductivity detector.

In the initial investigation, 10 mmol/L LiOH and 20 mmol/L lactic acid were employed at pH 4.3. This study was employed of 15 metal cations from groups I, II and transition metals were injected to observe the separation under the above conditions. The separation obtained is shown in Fig. 6.2. Both positive and negative peaks are observed. Some metals which elute as the negative peak are transition metals (Mn, Cd, Cr, Fe, Co, Ni, Zn and Cu). The metal cations from group I and II (K, Ba, Na, Ca and Mg) are seen as positive deflections. It is suggested that the observation of the negative peaks is due to the formation of complexes between the transition metals and lactic acid. This effectively lowers the conductance in the sample band compared to the background resulting in negative deflection.

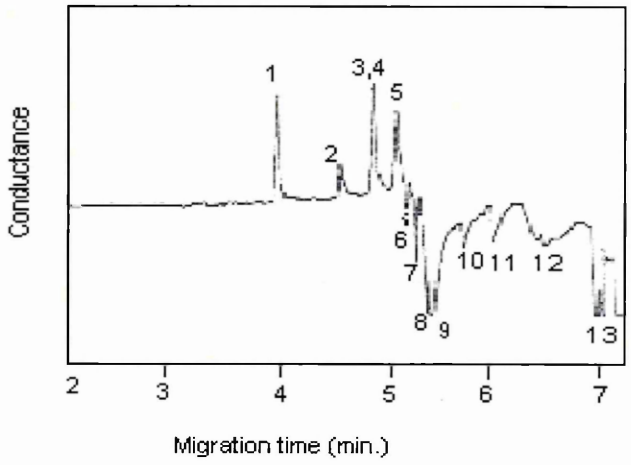


Fig. 6.2. Electrophoretic separation for preliminary analysis of thirteen metal cations using 10 mmol/L LiOH with 20 mmol/L lactic acid (pH 4.3). Applied voltage 30 kV; hydrodynamic injection for 30s; pressure applied of 20 mBar; 1=K; 2=Ba; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Cd; 8=Cr; 9=Fe; 10=Co; 11=Ni; 12=Zn; 13=Cu.

6.3.3. Effect of varying lactic acid concentration

As stated earlier the use of lactic acid as a complexing reagent in conductivity detection for the separation of metal cations is limited when compared to its application using UV/Vis detection. Experiments were carried out to study the effect of lactic acid on the separation of metal cations. The buffer employed was 10 mmol/L LiOH with various concentrations of lactic acid from 5 mmol/L to 30 mmol/L. The pH of the buffer was maintained at pH 4.3. The influence of complexing reagent concentration versus analyte migration times is shown in Fig. 6.3. In general, two effects of changing lactic acid concentration could be observed. First, the presence of lactic acid was required to affect a separation of transition metals and second, that this separation was concentration dependent. A concentration of at least 15 mmol/L was required to achieve resolution.

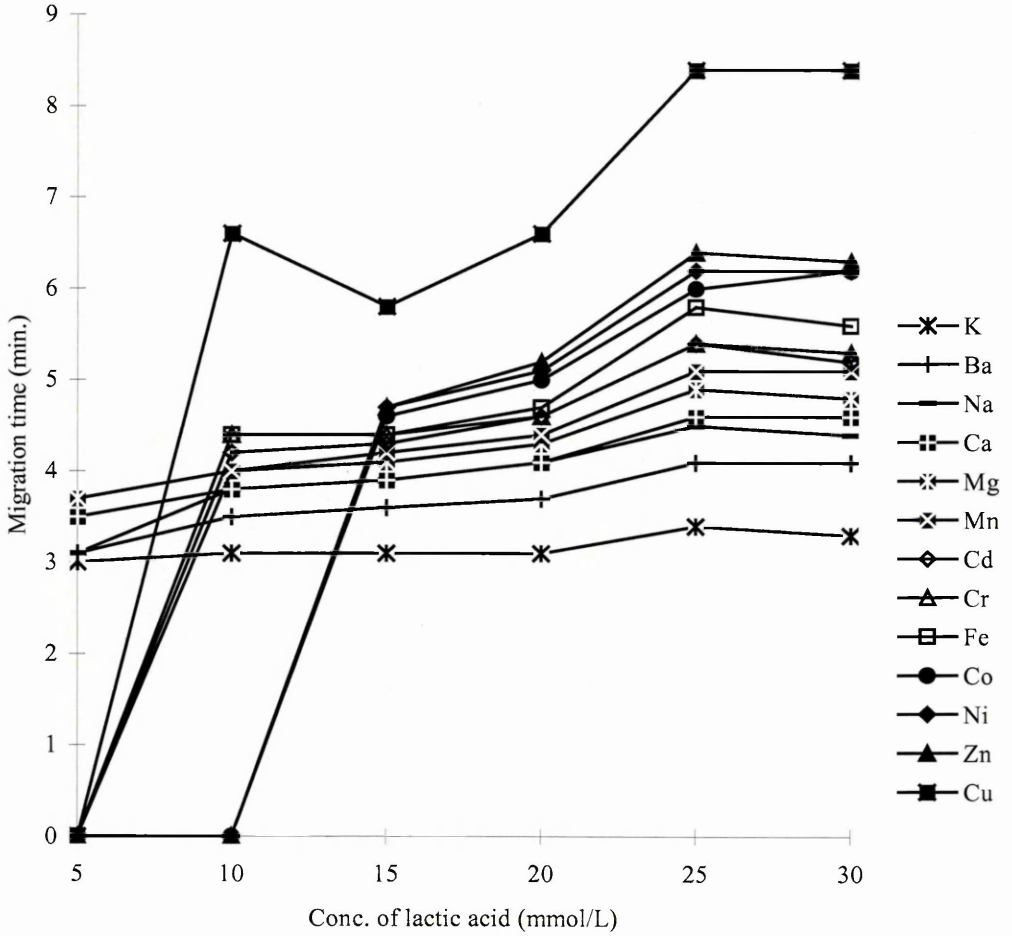


Fig. 6.3. Effect of changing the lactic acid concentration on migration times of metal cations. Buffer consists of 10 mmol/L LiOH, pH 4.3 and varying lactic acid concentrations.

The metal ions of group I and II can be separated without the presence of lactic acid. However, the migration times of transition metals are much more reliant on the concentration of the complexing reagent. Some of the transition metals could not be resolved at lower concentration as compared at higher concentration. At the lower concentration some metal e.g. chromium and lead were not observed and it is assumed that they co-eluted. They appear reported at the higher concentration. This is because the concentration of complexing reagent present will affects the formation of metal complex as mentioned in the previous work by Lin et al. (58) for the employing of

monoprotic acid as a complexing reagent in their investigation. They found that in the absence of a acetic acid, only five peaks (K, Ba, Ca, Li and Na-Mg) were found but Na and Mg could not be resolved. They found that by changing the concentration of acetic acid used in the buffer solution may affects the separation of the metal cations in group I and group II include K, Ba, Ca, Na, Mg and Li. However they do not investigate the separation of transition metals in their work for the above buffer conditions. A full separation of 13 metal cations could be obtained in less than 7 min in this work. This separation is clearly shown in Fig. 6.4. Co and Ni, however, were less will resolved. The optimum of lactic acid was found at 20 mmol/L. At this concentration most of metal cations in group I, II and transition metals were well separated with good resolution and reasonable analysis times. However, the noisy baseline was observed at other concentrations. It has demonstrated that the presence of varying lactic acid concentration as complexing reagent is very important for the separation of transition metals in conductivity detection.

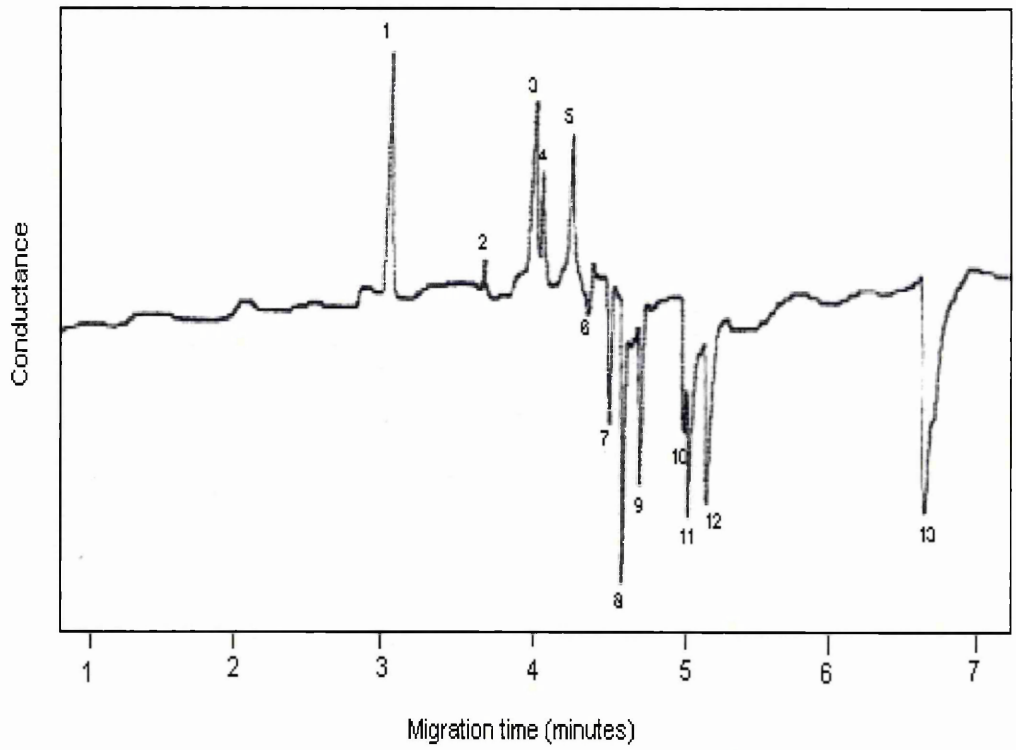


Fig. 6.4. Electrophoretic separation for preliminary analysis of thirteen metal cations standard using 10 mmol/L LiOH with 20 mmol/L lactic acid (pH 4.3). Applied voltage 30 kV; hydrodynamic injection for 30s; pressure applied of 20 mBar; 1=K; 2=Ba; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Cd; 8=Cr; 9=Fe; 10=Co; 11=Ni; 12=Zn; 13=Cu.

This work confirmed observations of Shi and Fritz (29) for employing lactate in the separation most metal cations from group I, group II and transition metals via UV/Vis detection. They found that the important conditions included the concentration s of lactate and UV visualisation reagent, and pH. A very good separation of the thirteen lanthanides was obtained. It was also found that excellent separations could be obtained under the same conditions for alkali metal ions, magnesium and the alkaline earths, and several divalent transition metal ions. All of these except copper (II) eluted before the lanthanides. An excellent separation of 27 metal ions was obtained in a single run that required only 6 min. In another experiment by Shi and Fritz (30) obtained an excellent separation of 16 metal ions with the employing of 11 mmol/L lactic acid, 2.6 mmol/L 18-crown-6, 7.5 mmol/L 4-methylbenzylamine, 8% methanol at pH 4.3. They

found that NH_4^+ and K cations also have virtually identical mobilities and are not complexed by lactic acid. Previous investigators also found that ammonium and potassium ions can be separated by CZE if a suitable crown ether is incorporated into the electrolyte by Fukushi and Hiiro (31) and Bachmann et al. (35). They observed the K^+ ion is selectively complexed and its mobility is reduced just enough to permit a good separation.

6.3.4. Effect of varying LiOH concentration

To study the effect of changing the LiOH concentration the buffer employed contained 20 mmol/L lactic acid with LiOH concentrations from 5 mmol/L to 20 mmol/L. The data obtained in this investigation are shown in Fig. 6.5.

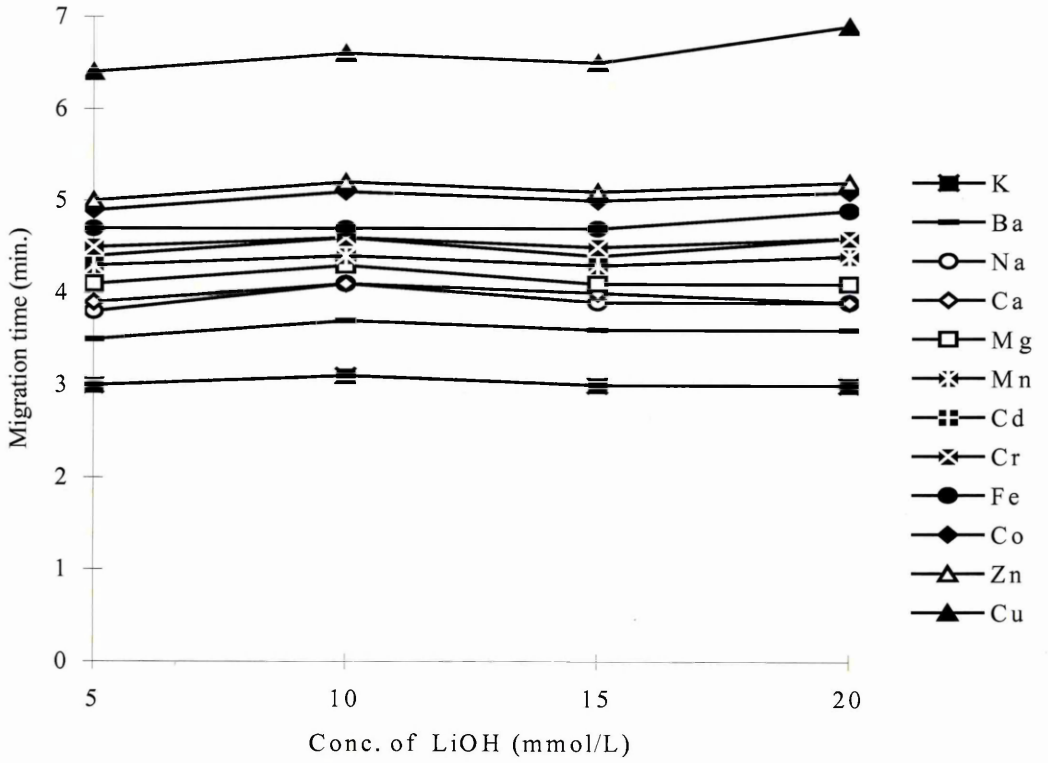


Fig. 6.5. Effect of changing the LiOH concentration on migration times of metal cations. Buffer consisted of 20 mmol/L lactic acid and varying concentration of LiOH at pH 4.3.

The migration times did not change much as the concentration of LiOH was increased. This indicates that the presence of LiOH in the buffer did not influence the formation of metal complexes between transition metals and lactic acid. Most metal cations in group I and II could be separated without LiOH in the buffer. However, in the absence of LiOH no separation of transition metals was observed. A separation obtained is represented in Fig. 6.6. Most of the 13 metal cations from group I, group II and transition metals were well separated except for Co and Ni, in less than 6.5 min. The poor resolution for Co and Ni suggest that complexation with lactic acid is insufficient to separate them under these conditions. The optimum concentration of LiOH was found at 15 mmol/L. At this concentration both metal cations from group I, II and transition metals were well resolved with a reasonable migration times.

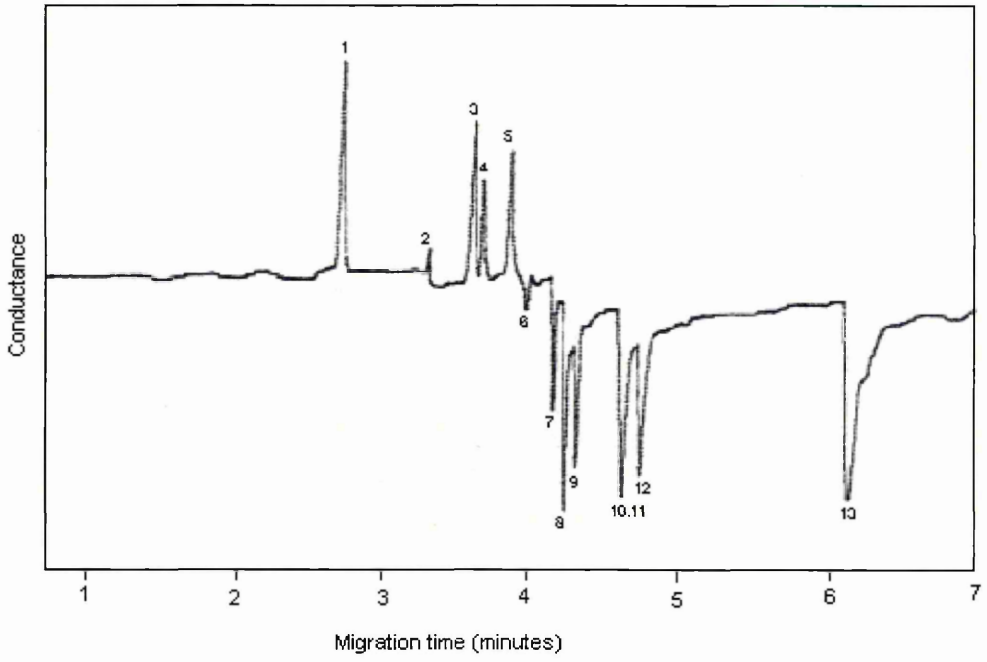


Fig. 6.6. Electrophoretic separation for thirteen metal cations using 15 mmol/L LiOH with 20 mmol/L lactic acid (pH 4.3). Applied voltage 30 kV; hydrodynamic injection for 30s; pressure applied of 20 mBar; 1=K; 2=Ba; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Cd; 8=Cr; 9=Fe; 10=Co; 11=Ni; 12=Zn; 13=Cu.

This result agreed with the findings reported by Shi and Fritz (30) in the separation of K, Ba, Sr, Ca, Mg, Na, Al, Cu, Li and VO using 8 mmol/L nicotinamide at pH 3.2. They found that Ca and Sr co-migrate and Mg and Na co-migrate in first experiment. The separation was repeated under the same conditions but with 0.6 mmol/L 18-crown-6 also added to the electrolyte. The crown ether lengthened the migration times of Sr and Ba so that separation of Ca and Sr was obtained. The separation of Al^{3+} was tried under the same conditions as used for the previous separation but with lactic acid added top the electrolyte. No Al^{3+} peak was found, suggesting that kinetics of forming and dissociating the Al^{3+} -lactate complex may be slow. The crown ether in the electrolyte probably does not complex Al^{3+}. In the later development, by adjusting slightly the

concentrations of nicotinamide and crown ether, and by adding methanol to the electrolyte, it was possible to completely resolve a mixture containing Mg^{2+}, Al^{3+}, the alkaline earths and the first three alkali metal ions.

6.3.5 Effect of pH

The pH of the buffer in CE separations is a very important parameter. It will determine the magnitude of the electroosmotic flow and the electrophoretic mobilities of metal ion complexes may be affected. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH at varying values of pH between pH 3.5 to pH 5.0. The migration times observed can be seen in Fig. 6.7.

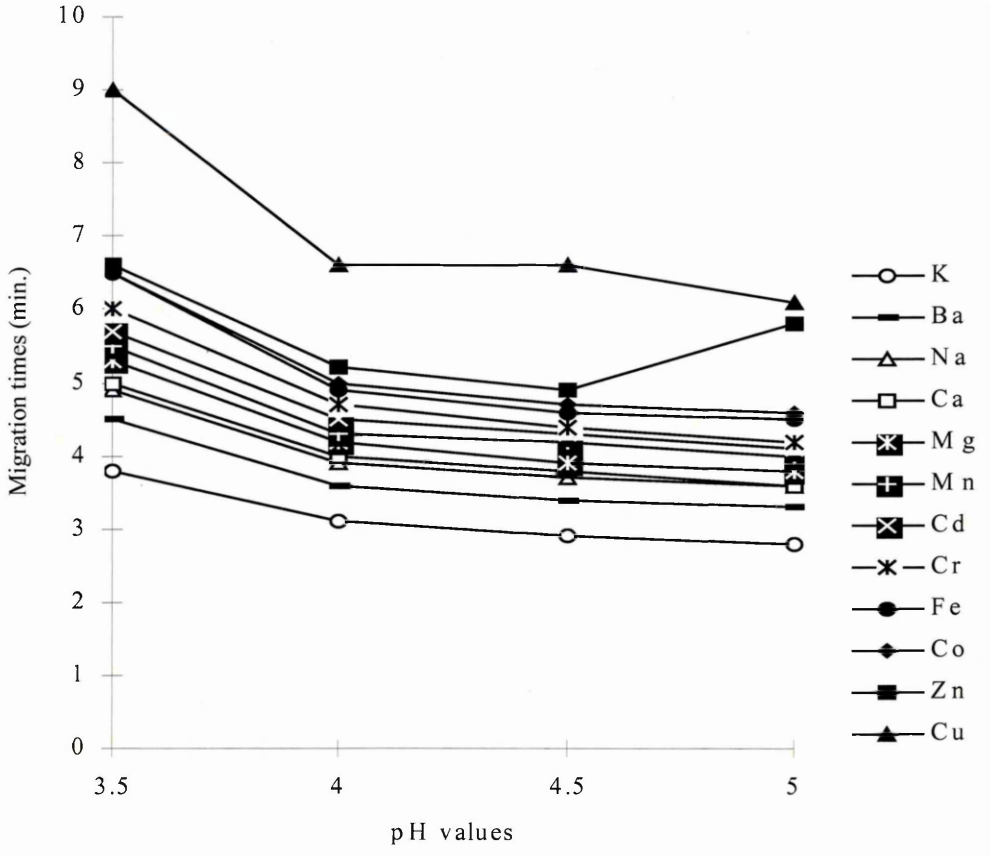


Fig. 6.7. Effect of pH buffer on migration times of metal cations. Buffer consists of 20 mmol/L lactic acid, 15 mmol/L LiOH and varying pH

The degree of complexation between metal ions and lactic acid is pH dependent as it is a weak acid. The pK_a of lactic acid is 3.86 thus small changes in pH in this region may have significant affect on the type and stabilities of complexes formed. Migration times increase as the pH is reduced. At lower pH the electroosmotic flow falls as a result of the reduction of negative charge at the capillary wall.

The separation of metal cations can be obtained at between pH 4.0 to pH 4.5. As an example an electropherogram is shown in Fig. 6.8. The buffer employed was 15 mmol/L LiOH, 20 mmol/L lactic acid at pH 4.3 and enabled a separation of 13 metal cation in less than 7 minutes. All the metal cations were well separated except Co which still co-eluted with Ni. Most of the metal cations in groups I, II and transition metals can be separated at this pH with a good resolution. In the previous, Weston et al. (22) was observed a similar trend for the effect of pH on the migration time of eleven alkali, alkaline earth and transition metals of the altering the pH. The combination of 6.5 mmol/L HIBA at pH 4.4 was employed in their investigation. They found that as the pH is lowered, the migration time is increase, due to decrease in the EOF. Likewise, as the pH increased, the migration times decease, due to increase in the EOF. In another experiment, Weston et al. (23) also found that potassium and ammonium co-elute at pH 6.15 owing to their identical equivalent ionic conductivity. By altering the pH of the electrolyte, the ionization of alkali metals and their mobilities will be essentially unaffected. However, as the pH of electrolyte is increased, the ammonium becomes progressively less protonated (pK_b of NH_4^+ = 4.75) and its apparent mobility decreases. At pH 8.5, the apparent mobilities of K and NH_4^+ become sufficiently different to permit an effective separation. As can be seen from this work, it was found that the optimum buffer conditions consisted of 20 mmol/L lactic

acid and 15 mmol/L LiOH for the separation most of the metal cations was found at pH 4.3.

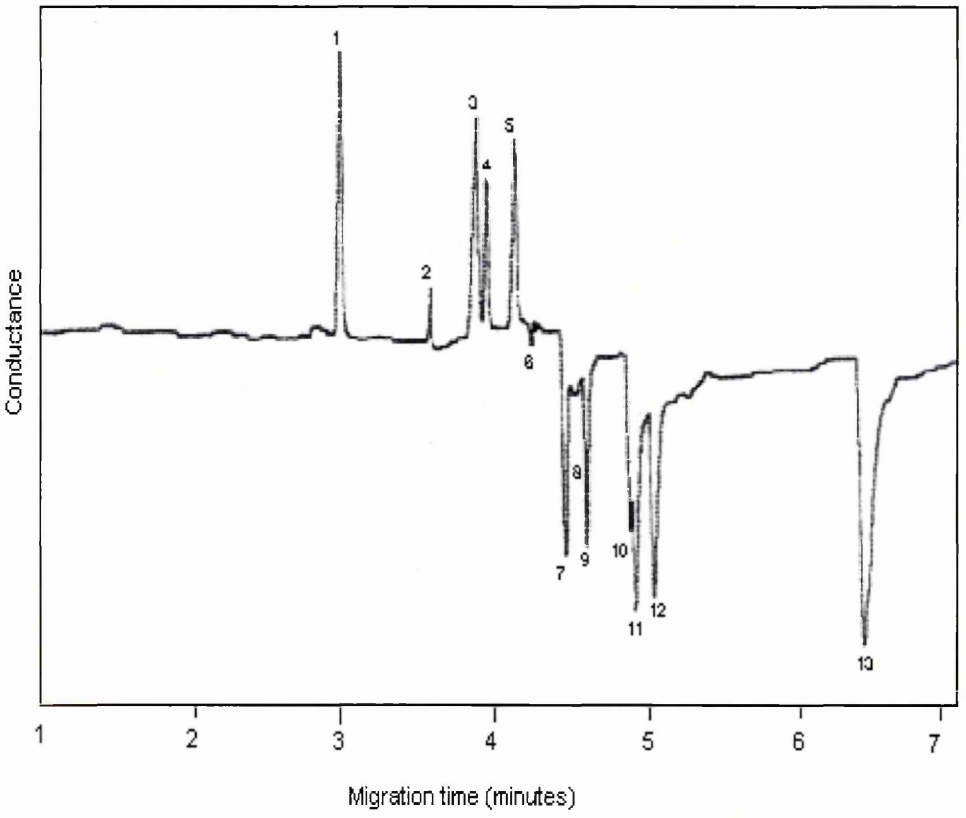


Fig 6.8. Electrophoretic separation for preliminary analysis of thirteen metal cations using 15 mmol/L LiOH with 20 mmol/L lactic acid (pH 4.3). Applied voltage 30 kV; hydrodynamic injection for 30s; pressure applied of 20 mBar; 1=K; 2=Ba; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Cd; 8=Cr; 9=Fe; 10=Co; 11=Ni; 12=Zn; 13=Cu.

6.3.6. Effect of methanol

The addition of methanol to the buffer is often employed to improve the separation efficiency and resolution of analytes. In a number of applications (41-43,163), methanol was used to obtain a good separation, and also to improve the baseline, for metal cation separations. In this work, percentages of methanol from 5% up to 20% were used to study its affect on the separation of metal cations. In these experiments 15 mmol/L LiOH and 20 mmol/L lactic acid at pH 4.0 was used. Methanol then was

added at different concentration while maintaining the concentration and pH of the buffer. The separation of metal cations and the affect on migration times were studied in a mixture of metal cations. Fig. 6.9 shows the effect of increasing methanol concentration on migration times.

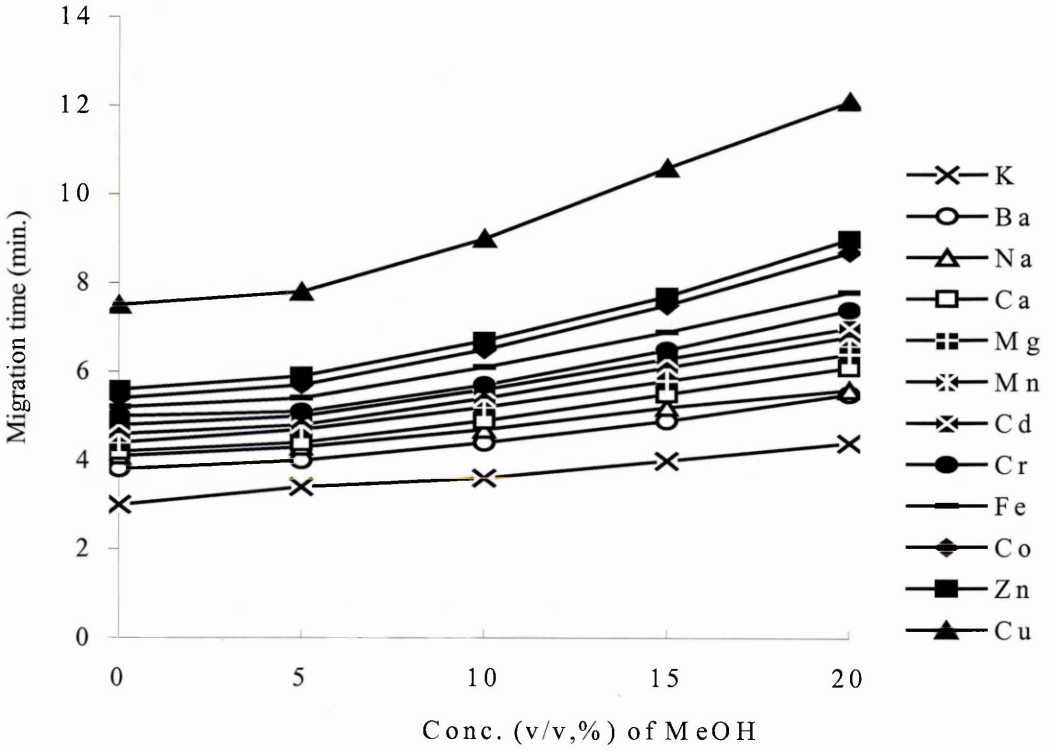


Fig. 6.9. Effect of methanol addition on migration times of metal cations. Buffer consists of 20 mmol/L lactic acid, 15 mmol/L LiOH, pH 4.0 and varying amounts of methanol.

Migration times are increased but improvements in resolution may be seen. The effect of methanol in the buffer composition can be seen in Fig 6.10. The baseline of the electropherograms were also improved with the presence of methanol in the buffer. Employing a buffer of 15 mmol/L LiOH, 20 mmol/L lactic acid, 10% methanol at pH 4.3 enabled the separation of 13 metal cations in less than 8.5 min. This conditions was

obtained a good separation of most metal cations in group I, II and transition metals. Other methanol concentrations were not suitable due to the noisy baseline observed. This results supports the investigation by Yang et al. (62) on the simultaneous separation of ammonium and alkali, alkaline earth and transition metal ions in aqueous. They have found that with the absence of methanol in background electrolyte, Li, Ni and Zn are co-migration but after the addition of methanol they can get a separation of Li, Ni and Zn. However they observe this effect of methanol up to 30% at the maximum amount for improving on the electrophoretic mobilities of the separation of cations. Other reported by Shi and Fritz (30) also was noted that the electrophoretic mobility decreases almost linearly as the percentage of methanol was added in the buffer solution. However they only experimented the methanol up to 20 % under their investigation. They obtained a good resolution by increasing the methanol concentration in the separation of eight metal cations using phthalic acid. It has shown that the optimum concentration of methanol in the buffer consists of 20 mmol/L lactic acid and 15 mmol/L LiOH (pH 4.3) was found at 10% .

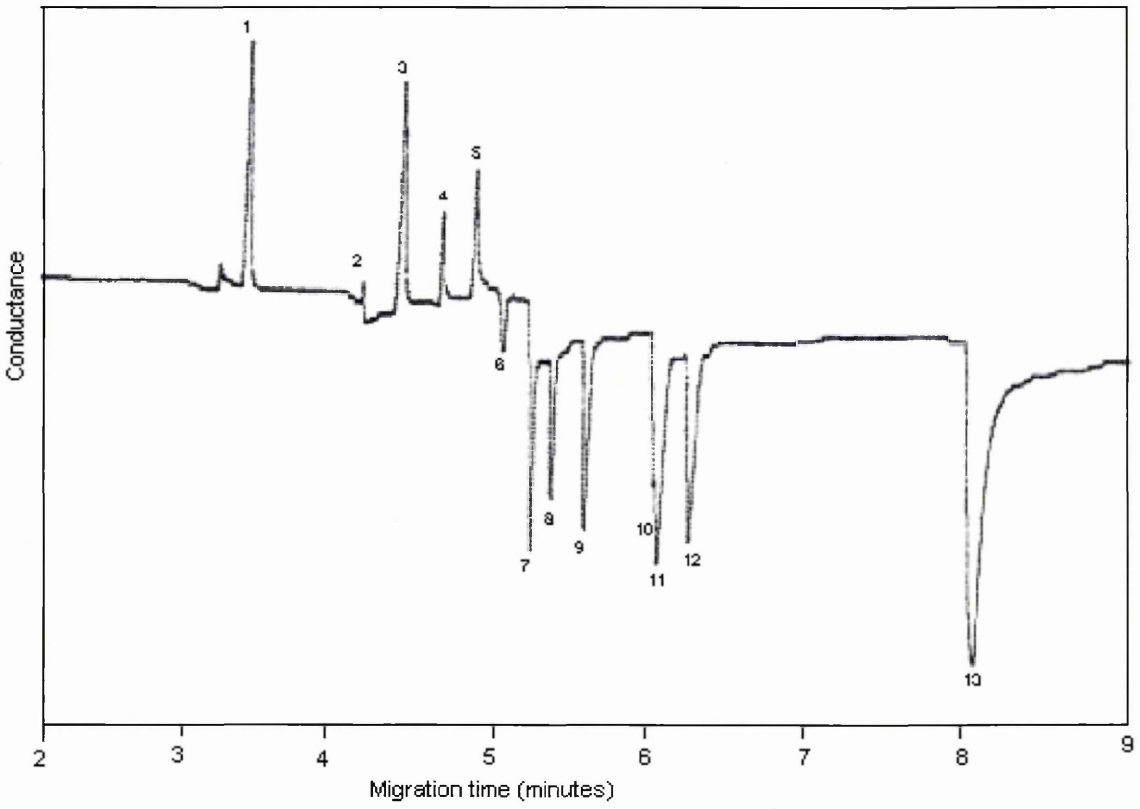


Fig. 6.10. Electrophoretic separation for thirteen metal cations standard using 15 mmol/L LiOH, 20 mmol/L lactic acid and 10% methanol (pH 4.3). Applied voltage 30 kV; hydrodynamic injection for 30s; pressure applied was 20 mBar; 1=K; 2=Ba; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Cd; 8=Cr; 9=Fe; 10=Co; 11=Ni; 12=Zn; 13=Cu.

6.3.7. Reproducibility

A buffer composition of 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3 was employed to study the reproducibility of metals from group I and group II and cadmium. The procedure used was followed the outlined in Section 4.5.1.3. for ANOVA calculation. The results obtained are given in Tables 6.1 – 6.3. In this study the standard mixtures containing 6 metals from group I, group II and transition metals were injected to observe the reproducibility of migration time, absorbance and peak area. These metals were include K, Na, Ca, Mg, Mn and Fe.

6.3.7.1. Migration time (MT)

Full summarises reproducibility data for all six metals is shown in Table 6.1.

Elements	Mean result, min. ± SD [a]	ANOVA *F* value[c]
Potassium	3.82 [b] ± 0.01	0.90
Sodium	5.08 [b] ± 0.03	0.92
Calcium	5.32 [b] ± 0.03	0.01
Magnesium	5.67 [b] ± 0.05	1.80
Manganese	5. 77[b] ± 0.05	1.80
Iron	6.17 [b] ± 0.05	1.80

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 6.1. Reproducibility data on migration time for metal cations at different batch samples using 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not differ significantly. The data obtained in Table 6.1 shows that the migration times were stable and capable of providing consistent and reproducible data. These values obtained for reproducibility on migration time is consistent with the results of Weston et al. (23) in their investigation on the analysis of K, Ca, Na and Mg using the buffer solution of UV Cat-2, tropolone, HIBA at pH 3. They have reported the reproducibility in less 2% of relative standard deviation (RSD) compared to less than 1.7% of RSD in this work. However the results obtained in this work were not as good as the results obtained by Weston et al. (64) on the analysis of K, Na, Ca and Mg using the buffer

solution of 5 mmol/L, UV Cat-1 and 6.5 mmol/L HIBA at neutral pH. They have reported RSD on migration time was 0.34 for K, 0.37 for Na, 0.37 for Ca and 0.83 for Mg. Meanwhile Lin et al. (58) reported the RSD for migration time was 0.8 for K, 0.59 for Ca, 0.58 for Na and 0.47 for Mg. They have employed 3 mmol/l succinic acid with 5 mmol/L imidazole. Other reported by Riviello and Harrold (71) on RSD for migration time was 0.28 for potassium, 0.25 for sodium, 0.26 for calcium and 0.25 for magnesium.

6.3.7.2. Conductivity

Full summarises reproducibility data for all six metals is shown in Table 6.2.

Elements	Mean result, (mV x10^{-3}) ± SD [a]	ANOVA *F* value[c]
Potassium	6.80 [b] ± 0.09	1.10
Sodium	6.54 [b] ± 0.12	1.50
Calcium	3.81 [b] ± 0.14	0.90
Magnesium	3.34 [b] ± 0.13	0.91
Manganese	1.03 [b] ± 0.03	1.08
Iron	4.50 [b] ± 0.23	1.26

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 6.2. Reproducibility data on conductivity for metal cations at different batch samples using 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not

differ significantly. As can be seen the data in Table 6.2, the conductivity obtained were stable and capable of providing consistent and reproducible data. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of nickel, zinc and cobalt with the employing of 5 mmol/L oxalic acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of conductivity in this work.

6.3.7.3. Peak area

Full summarises reproducibility data for all six metals is shown in Table 6.3.

Elements	Mean result, peak area ($x10^6$) ± 1 SD [a]	ANOVA *F* value[c]
Potassium	2.80 [b] ± 0.10	1.08
Sodium	3.41 [b] ± 0.08	2.09
Calcium	1.41 [b] ± 0.06	1.26
Magnesium	2.42 [b] ± 0.10	1.24
Manganese	2.82 [b] ± 0.14	0.90
Iron	2.73 [b] ± 0.47	0.93

[a] = Corrected for mean of 3 batch samples.
[b] = Mean of 10 replicates.
[c] = Single-factor ANOVA, $F_{crit.} = 2.393$, $p = 0.05$.

Table 6.3. Reproducibility data on peak area for metal cations at different batch samples using 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

Since the calculated value of *F* from series observations is less than the critical value of *F*, therefore the null hypothesis is retained. Meaning that the sample means do not

differ significantly. The data obtained from Tables 6.3 shows that the peak areas were stable, providing consistent and reproducible data for K, Na, Ca, Mg, Mn and Fe. The data obtained also supports the findings reported by Jones and Tarter (198) on the precision method for the determination of Ni, Zn and Co with the employing of 5 mmol/L oxalic acid and 3.75 mmol/L citric acid at pH 4.37. They reported the standard deviations are ranging from 0.01 to 0.22. However they observed the detection response based on peak height/cm instead of peak area in this work.

6.3.8. Linearity range

Figs. 6.11 – 6.16 show linearities for the group I and group II and transition metal cations. The procedure used for linearity of the graph was followed as outlined in Sectioned 4.5.1.4. There were six concentration of multi-element standards used from 0 ppm to 100 ppm for the analysis using CE with conductivity detection. Ten times at each concentration was injected with and duplicates with the same set solution of each concentration were carried out sequentially. The average values of the results in linearity for K ($y = 8E\text{-}05x + 0.0001$; and $R^2 = 0.9989$), Na ($y = 8E\text{-}05x + 0.0003$; and $R^2 = 0.9947$), Ca ($y = 5E\text{-}05x + 0.0002$; and $R^2 = 0.998$), Mg ($y = 6E\text{-}05x + 0.0002$; and $R^2 = 0.9955$), Mn ($y = 2E\text{-}05x + 6E\text{-}05$; and $R^2 = 0.9974$) and Fe ($y = 09E\text{-}05x + 8E\text{-}05$; and $9^2 = 0.9985$) were acceptable and can be fitted a straight line for the above range.

It appears that the deviations for all metals are less than 5% at lower concentration than 100 ppm except Fe. The average values of the result in linearity shows the deviations is about 6% at concentration higher than 80 ppm for Fe ($y = 7.7E\text{-}05x + 2E\text{-}04$; and $R^2 = 0.9874$). The non-linear calibration curve for iron may be due to several reasons in

the use of indirect conductivity detection method for the analysis of transition metals, e.g. the use of low ionic strength, poorly ionised buffer system to produce a low conductivity background. If good separation is desired, the concentration of background electrolyte must be high relative to the concentration of the sample. However this condition (high electrolyte concentration) results in a decrease in sensitivity due to elevated background conductivity. Conductivity detection is generally applicable to the identification of charged molecules and complexing as conductivity is a common property related to ionic mobilities.

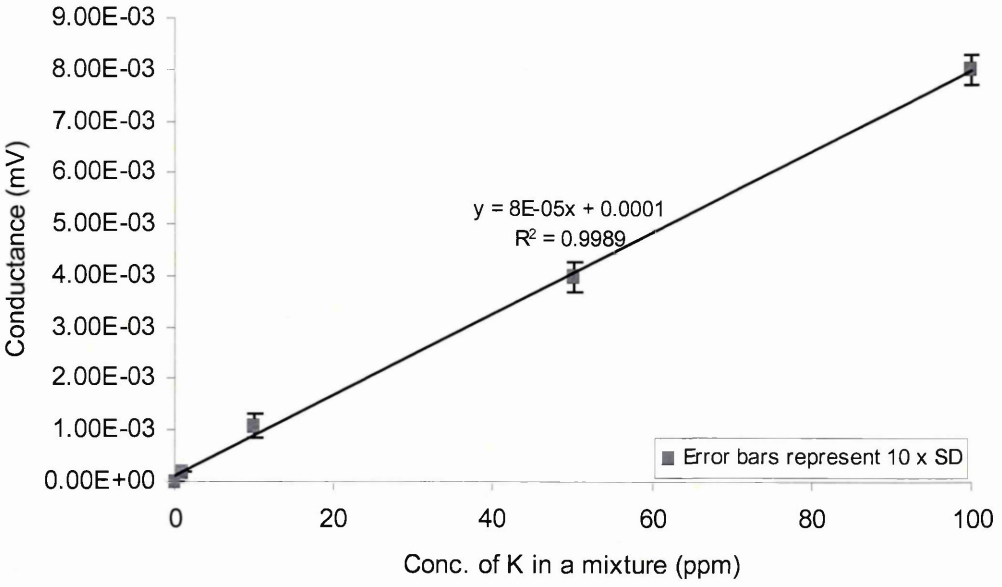


Fig. 6.11. Linearity range of K. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

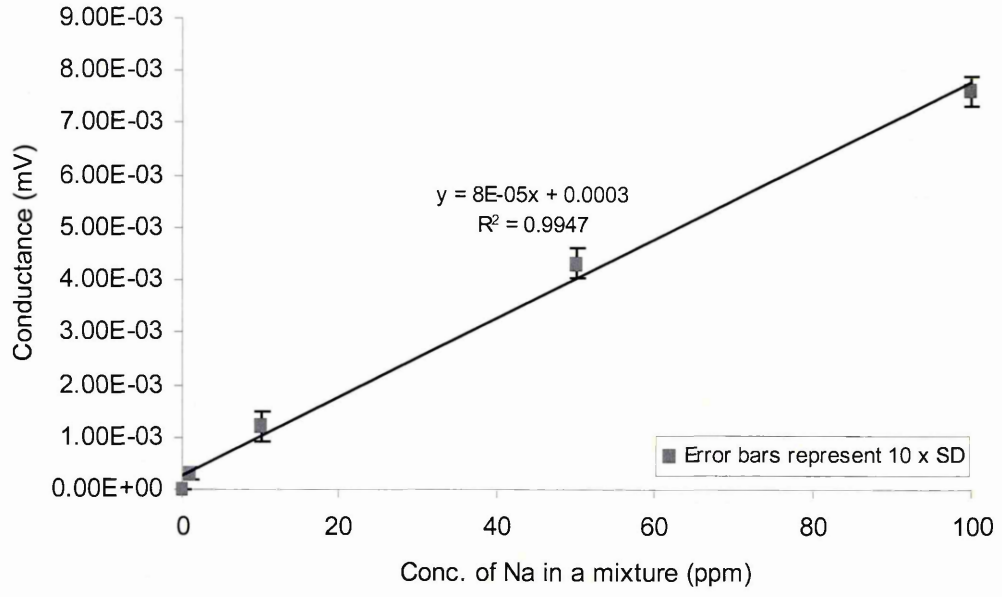


Fig. 6.12. Linearity range of Na. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

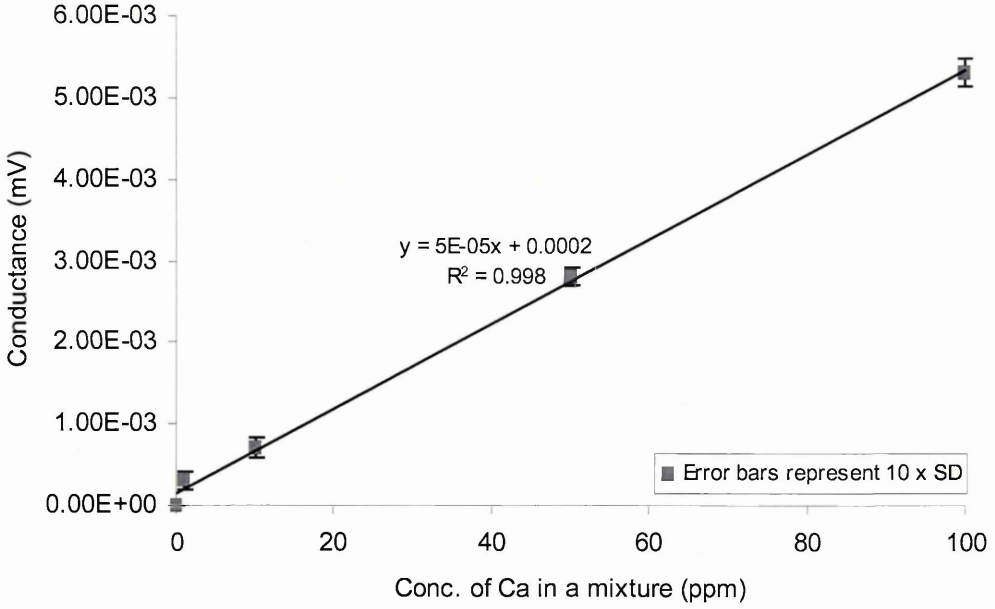


Fig. 6.13. Linearity range of Ca. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

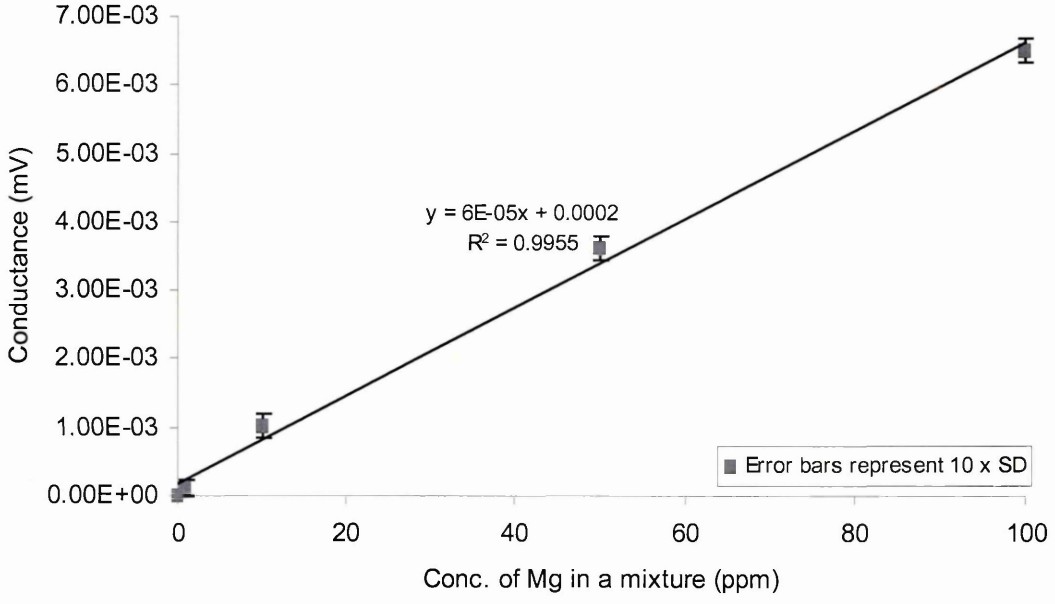


Fig. 6.14. Linearity range of Mg. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

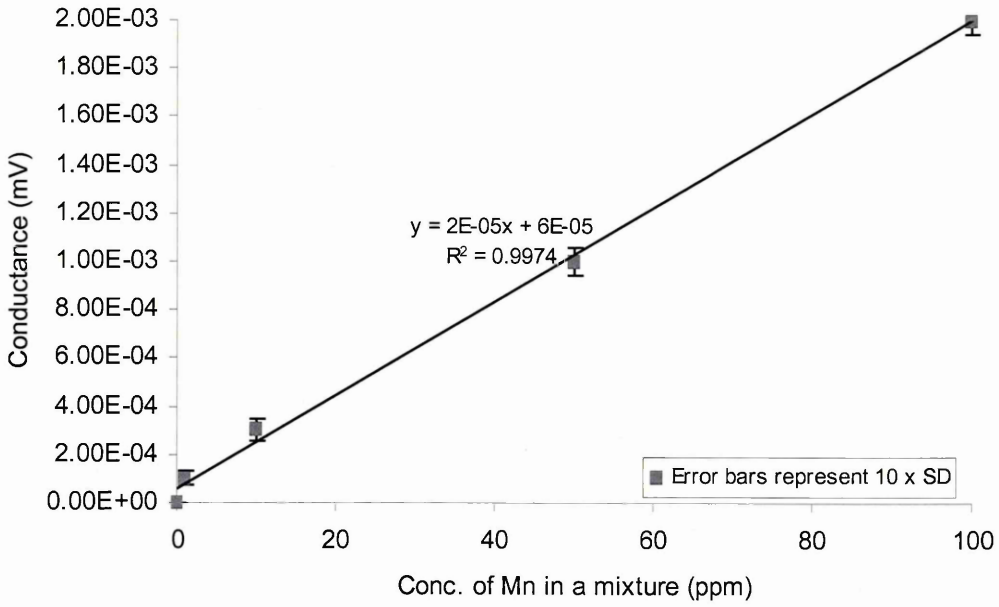


Fig. 6.15. Linearity range of Mn. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

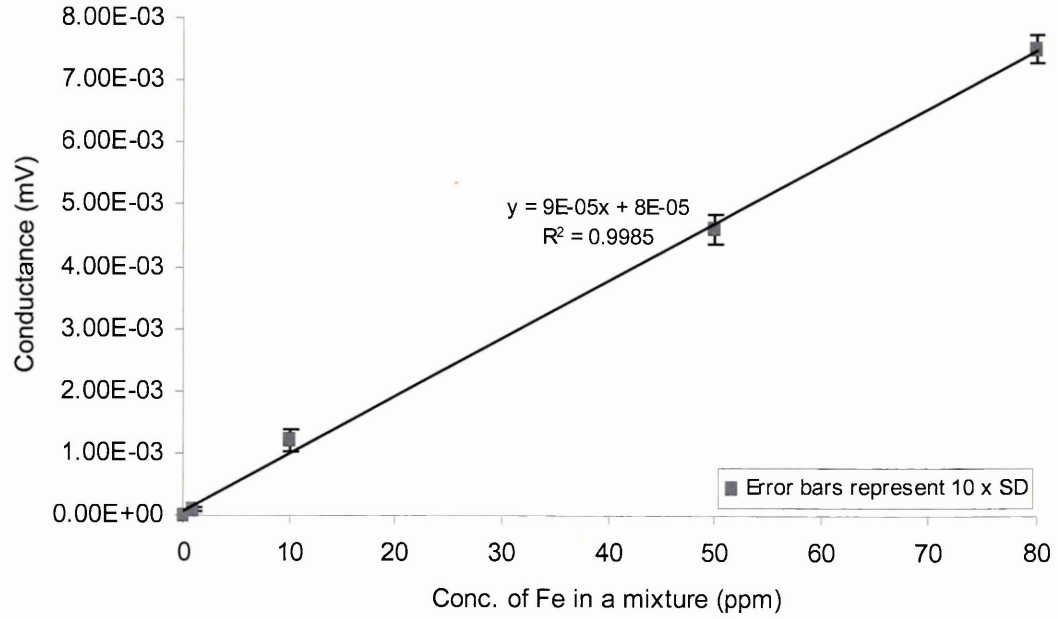


Fig. 6.16. Linearity range of Fe. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

The overall linearity of ten metals from group I, group II and transition metals that are found to be up to 100 ppm in this work are excellent (the correlation coefficients, R^2 < 0.99) compared to the previous work as discussed below. Shi and Fritz (29) reported a good linear calibration curves were obtained for the metal cation studies in the 0.4 ppm to 10 ppm concentration range. They used the buffer solution of 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3 for the separation of eight metal cations include K, Na, Pb, Mn, Co, Ni, Zn and Cd. However they obtain a relatively poor correlation coefficient of linear regression for Zn. Meanwhile, Shi and Fritz (30) obtained a linear calibration curves for the metal cations from 0.1 to 10 ppm for the separation of Sr, Mg, Mn, Co and Zn. They used 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 0.6 mmol/L 18-crown-6, 10% methanol at pH 4.3. In another development, Yang et al. (63) reported the linear calibration curves up to 20 ppm was obtained for the separation of K, Na, Ca, Mg and Mn. They also found that at high concentrations peak distortion occurred owing to overloading, which caused insufficient selectivity of separation, so that the calibrations were no longer useful. At pH 4.5 the calibration line for manganese was linear only 6 ppm owing to the insufficient separation between manganese and magnesium. Recently, N. Shakulashvili et al. (191) reported a good linear calibration curves from 0.75 to 80 ppm for the separation of sixteen metal cations using 10 mol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. In general, the excellent linearity observed for all metal cations can be fitted a straight line for quantitative analysis.

6.3.9. Limits of detection

The method by which detection limits were determined was by making serial dilutions of a standard solution. Minimum detectable concentrations are based on a peak height

signal to noise of (3:1) and are given in Table 6.4. The buffer employed was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.1.

Metal cations	Mean of detection limit[a] (ppm ± SD)
Potassium	0.4[a] ± 0.08
Sodium	1.6[a] ± 0.05
Calcium	0.6[a] ± 0.04
Magnesium	1.2[a] ± 0.05
Manganese	2.9[a] ± 0.06
Iron	1.3[a] ± 0.04

Notes: [a] = mean of 10 replicates

Table 6.4. Limits of detection for metals in group I&II and transition metals.

Limits of detection are found to be in the sub ppm level and these values compared favourably with that seen by Haber et al. (67) using indirect conductivity detection method in similar field. They have separated NH_4^+ and sixteen metal cations including Cs, K, Ba, Sr, Ca, Cr, Na, Mg, Mn, Cd, Li, Fe, Co, Zn, Ni and Pb in less than 7 minutes using 0.1 mmol/L acetic acid, 1 mmol/L oxalic acid at pH 2.84. They obtained limit of detection in the range of 0.665 ppb for Cs, NH_4^+ and K is unresolved, 0.687 ppm Ba, 0.438 ppm for Sr, 0.221 ppm for Ca, 0.520 ppb for Cr, 0.127 for Na, 0.134 for Mg, 0.549 for Mn, 1.1 ppm for Cd, 0.035 ppm for Li, 0.558 ppm for Fe, 0.589 ppm for Co, 0.654 ppm for Zn, 0.587 ppm Ni and 2.1 ppm for Pb. However a good limit of detection are shown in several literatures using UV/Vis detection method as compared to conductivity detection. Lee and Lin (57) in the analysis a mixture of nineteen metal

ions (Li, Na, K, Cs, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ag, Al and Pb) using 10 mmol/L pyridine, 12 mmol/L glycolic acid at pH 4.0. They obtained limit of detection in the range of 0.02 ppb for Na up to 0.208 ppm for Cr. Meanwhile, Yang et al. (62) obtained the limit of detection are as follows: 0.4 ppb for Li and Mg, 1 ppb for NH_4^+ and Ca, 2.5 ppb for Na, 10 ppb for K, 120 ppb for Sr, Mn, Cr and Zn, 500 ppb for Ba and Ni and 1000 ppb for Cu. The values for limit of detection are based on three times the baseline noise. They use 5 mmol/L imidazole, 6.5 mmol/L HIBA, 20% methanol and 1.33 mmol/L 18-crown-6 at pH 4.5. Shi and Fritz (29) also reported the limit of detection in the range 0.05 to 0.5 ppm for the separation of twenty seven alkali, alkaline earth, transition and rare earth metal cations. Light ions are in the low detection limit range, and heavy ions are in the high detection limit range. They employed 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. N. Shakulashvili et al. (191) obtained the limit of detection in the range 0.1 for K and 0.454 ppm for Cu in a mixture of 16 metal ion which is separated using 10 mmol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. Although the limit of detection in this work is slightly poor compared to the previous work but further development can be done for quantitative analysis.

6.3.10. Effects of lactic acid and LiOH on separation performance

Thirteen metals from group I, II and transition metals include K, Ba, Na, Ca, Mg, Mn, Cd, Cr, Fe, Co, Ni, Zn and Cu were observed. Thirteen metals were separated in less than 7 minutes using was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3. The successful separation of thirteen metal cations is shown in Fig. 6.8. The migration order follows the sequence K, Ba, Na, Ca, Mg, Mn, Cd, Cr, Fe, Co, Ni, Zn and Cu. All peaks are completely baseline resolved. The peaks become more

symmetric with increasing concentration. The buffer concentration has a great effect on the peak shape. If we compare the results with those achieved under the same conditions in previous studies, in spite of the use of a significantly longer capillary, the increases in buffer concentration case much more symmetrical peaks. These finding is better in term of number of analytes separated and baseline resolved even excellent separation compared to those observation by Haber et al. (67) using indirect conductivity detection method in similar field. They have separated NH_4^+ and sixteen metal cations including Cs, K, Ba, Sr, Ca, Cr, Na, Mg, Mn, Cd, Li, Fe, Co, Zn, Ni and Pb in less than 7 minutes using 0.1 mmol/L acetic acid, 1 mmol/L oxalic acid at pH 2.84. Resolution is not properly optimized. Most metals observed not are baseline resolved including Ba, Sr, Ca, Cr, Na and Mg. Mn and Cd are co-eluted together. Meanwhile, Shi and Fritz (29) obtained eight metal cations including K, Na, Pb, Mn, Co, Ni, Zn and Cd in less than 6 minutes using 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3. They have a good peak shape and baseline resolved. In another development they have separated twelve metal cations using 2.5 mmol/L tartaric acid, 6 mmol/L *p*-toluidine, 20 % methanol at pH 4.8 in less than 8 min. Resolution is excellent with a steady, flat baseline. However an excellent separation of twenty seven metal cations has been achieved by Shi and Fritz (29) in a single run in less than 6 min. They used 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. Among those metal cations that were separated including K^+, Ba^{2+}, Sr^{2+}, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^+, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Cu^{2+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+}. The average deviation in peak height from one run to another was approximately ± 5% or smaller at the ion concentration used.

Plate numbers achieved for selected metal actions are listed in Table 6.5.

Metal cations	Number of theoretical plates per meter (N/m)
Potassium	349,837
Sodium	618,681
Calcium	678,520
Magnesium	770,735
Manganese	311,782
Iron	356,508

Table 6.5. Peak efficiency for 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3 using CE-Conductivity detection.

From Table 6.5, Mg has the highest number of theoretical plates (N), about 770,735 followed by Ca, Na, Fe and K because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as acceptable to Mn has the lowest N value, about 311,782. This values obtained was excellent as compared to N value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the N value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile,

Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of N between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. The result obtained shows that the peak efficiencies for each metal cations are suitable for qualitative analysis.

6.4 Conclusion

It has been shown that capillary electrophoretic separations of metal cations using a conductivity detector is feasible. The low conductivity buffer MES/L-His allowed the separation of group I & II metals, however, separation of transition metals did not prove possible with this buffer.

As with UV/Vis detection it was found that incorporation of a complexing reagent was required in order to obtain sufficient resolution of transition metals. Lactic acid was employed as the complexing reagent in conjunction with lithium hydroxide. Direct detection of group I&II metals was observed with effective indirect detection of transition metals.

The concentration of lactic acid in the buffer influences the degree of separation observed. This is not surprising as the degree of complexation occurring will be dependent on the concentration of the complexing reagent. However, metals group I&II show little dependence on the amount of lactic acid presence. This would be expected as these metals have a low tendency to complex.

It was found that the presence of lithium hydroxide was needed to obtain separation of the transition metals but varying the concentration did not appear to be too influential. Both adjustment of pH and the addition of an organic modifier (methanol) were investigated and optimum values determined. The separation of 13 metal cations was achieved using a buffer composition of 15 mmol/L LiOH, 20 mmol/L lactic acid with 10% methanol at pH of 4.3. Migration times were shown to be reproducible with good standard deviation ($F_{obs.} < F_{crit.}$). Peak areas and peak absorbance were not reproducible. Limits of detection in the low ppm range were shown with good linearity (correlation coefficients ranges 1 - 100 ppm except for Fe with $R^2 > 0.99$).

The combination of lactic acid and LiOH using conductivity detection was relatively good as compared with the previous works in similar field for qualitative determination of metal cations. A further development should be investigated in order to obtain a good results which is acceptable for quantitative works.

CHAPTER 7

ANALYSIS OF ACID MINE DISCHARGES

7.1. Introduction

Work reported in the previous chapters has established the suitability of CE methods for the determination of metal cations. An investigation to determine the level of metal cations present in acid mine discharges was undertaken to evaluate the applicability of CE to a real problem.

Capillary Electrophoresis is characterised by high efficiencies and the extensive possibilities of varying the analytical conditions. The separation process is performed on the basis of the different electrophoretic mobilities, which depend on the structures and radii of the ions. Even very similar species with nearly the same electrophoretic mobilities can be separated by this method and preservation of the original bonding forms is often possible by choosing suitable buffer systems. Leachates that are washed out from old mine workings contain a number of metal species. These may eventually enter the surrounding water table and thus can pose environmental problems. The determination of the identity of such metals is therefore important.

7.2. Experimental procedures

7.2.1. Instrumentation

The CE instrument used was as described in section 3.2.1. Both UV/Vis and conductivity detection were employed (section 3.2). The voltages used were between 25 kV to 30 kV.

7.2.2. Materials and reagents

The chemical and reagents employed in this investigation were described in section 5.2.2.

7.2.3. Capillary preparation and cleaning

The fused-silica capillary used and cleaning procedures for UV/Vis detection can be found in section 5.2.3. Procedures for capillary use and cleaning in conductivity detection are according to procedures outlined section 6.2.3.

7.2.4. Injection procedures

The procedures of sample loading were described in section 5.2.4 for UV/Vis detection. The procedures for indirect conductivity detection were discussed in section 6.2.4.

7.2.5. Buffer preparation and pH adjustment

Details of procedures for buffer preparation were followed in accordance to section 5.2.5 for UV/Vis detection. Meanwhile the preparation of the buffer for indirect conductivity detection was discussed in section 6.2.5.

7.2.5.1. A crown ether for UV/Vis detection

A crown ether (100 mmol/L 18-crown-6) was prepared by weighing 2.6432 g of material and making up to 100 mL with water in a volumetric flask. Meanwhile, 2.6 mmol/L 18-crown-6 was prepared by taken 2.6 mL of 18-crown-6 from the above stock solution and diluting with buffer in a 100 mL volumetric flask.

7.2.6. Degassing and filtration procedure

The procedures described in section 3.6.4 were followed.

7.3. Result and discussion

7.3.1. Separation of metal cations by CE - UV/Vis detection method

The aim of this study was to employ the techniques developed, using lactic acid as a complexing reagent, for the separation of metals present in acid mine discharges. This method was followed because none of the previous complexing reagents employed i.e oxalic acid, acetic acid and acac were able to resolve manganese and iron (Chapter 5). It was decided to repeat the methods using lactic acid as a complexing reagent that previously used by Shi and Fritz (29) for the separation of metal cations. It has shown an excellent separation examples for alkali, alkaline earths, transition and rare-earth metals ions by CE. They enabled a separation of 27 metal cations including Mn but Fe was not tested in their investigation.

In this work, lactic acid was used to get a separation of K, Na, Ca, Mg, Fe and Mn for the determination of metal cations in acid mine discharges. Metals from group I, group II and the transition series, were investigated. Lactic acid in the presence of 4-methylbenzylamine as the UV absorber was used. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was employed to determine the metals present in acid mine discharges prior to samples being analysed by CE. The metal cations expected to be found in acid mine discharges were K, Na, Ca, Mg, Mn and Fe.

7.3.1.1. Lactic acid as a complexing reagents

The use of lactic acid and 4-methylbenzylamine to enable a separation of 27 alkali, alkaline earth, transition metals and rare earth metals was described by Shi and Fritz (29) as an excellent separation of metal cations using CE in 1994. The separation was achieved in less than 6 minutes using 15 mmol/L lactic acid, 8 mmol/L 4-

methylbenzylamine, 5% methanol at pH 4.25. Metal cations separated were K^+, Ba^{2+}, Sr^{2+}, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^+, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Cu^{2+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+}. The same buffer conditions was repeated here to study whether it was possible to separate Fe from Mn presents in acid mine discharges. In a preliminary analysis a composition of 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine and 5% methanol at pH 4.25 was employed to observe a separation of K, Ba, Na, Ca, Mg, Mn, Cd, Fe, Co, Pb, Ni, Zn and Cu. The separation obtained is shown in Fig. 7.1.

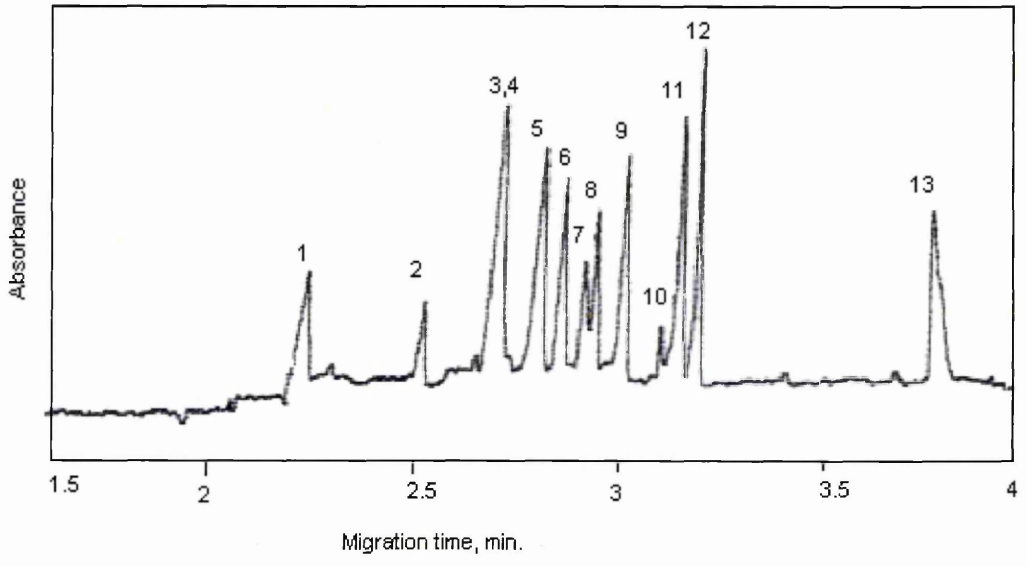


Fig. 7.1. Electrophoretic separation of thirteen metal cations using 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine and 5% methanol (pH 4.25). Applied voltage 30 kV; Hydrodynamic injection for 30s; pressure applied was 20 mBar; UV/Vis detector; 1=K; 2=Ba; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Cd; 8=Fe; 9=Co; 10=Pb; 11=Ni; 12=Zn; 13=Cu.

A separation of Mn and Fe was achieved, however, Na was found to co-elute with Ca. Meanwhile Cd and Fe peaks are not baseline resolved. The same buffer conditions were employed to obtain a separation of standard mixtures containing K, Na, Ca, Mg,

Mn and Fe that available in acid mine discharges . The separation is shown in Fig. 7.2.

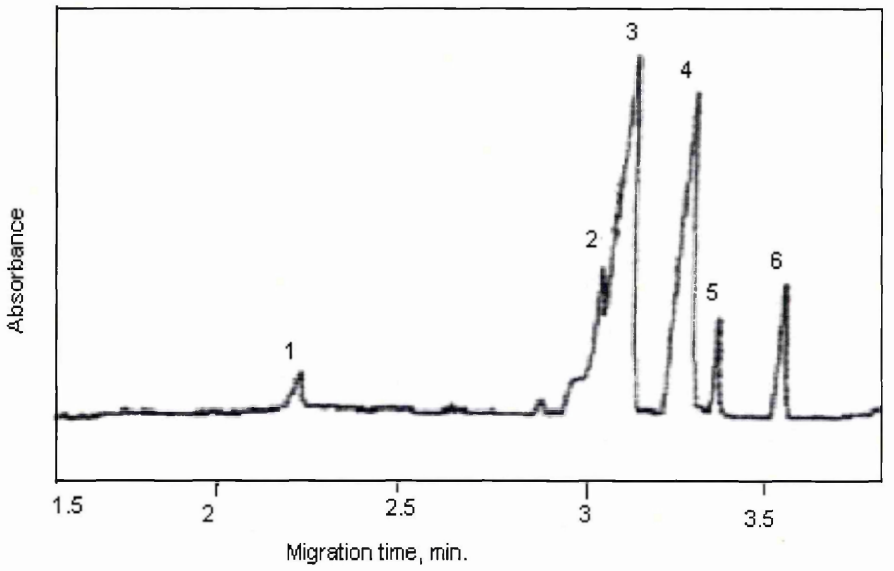


Fig. 7.2. Electrophoretic separation of six metal cations in acid mine discharges using 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine and 5% methanol (pH 4.25). Applied voltage 30 kV; Hydrodynamic injection for 30s; pressure applied was 20 mBar; UV/Vis detector; 1=K; 2=Na; 3=Ca; 4=Mg; 5=Mn; 6=Fe.

As can be seen in Fig. 7.2, full resolution of Na from Ca was not possible. In order to improve the separation of certain metal cations, 18-crown-6 has been employed e.g in 1994 Shi and Fritz (30) used 18-crown-6 to obtain a separation of 16 metal cations in less than 6 minutes. They obtained a good separation for most metal cations include Na, Ca, Mn and Fe. The same buffer conditions was repeated to observe a separation of Na from Ca and Fe from Mn. A buffer composition of 11 mmol/L lactic acid, 7.5 mmol/L 4-methylbenzylamine, 2.6 mmol/L 18-crown-6, 8% methanol (pH 4.3) was employed for the separation of a mixture 13 metal cations as given in Fig. 7.3.

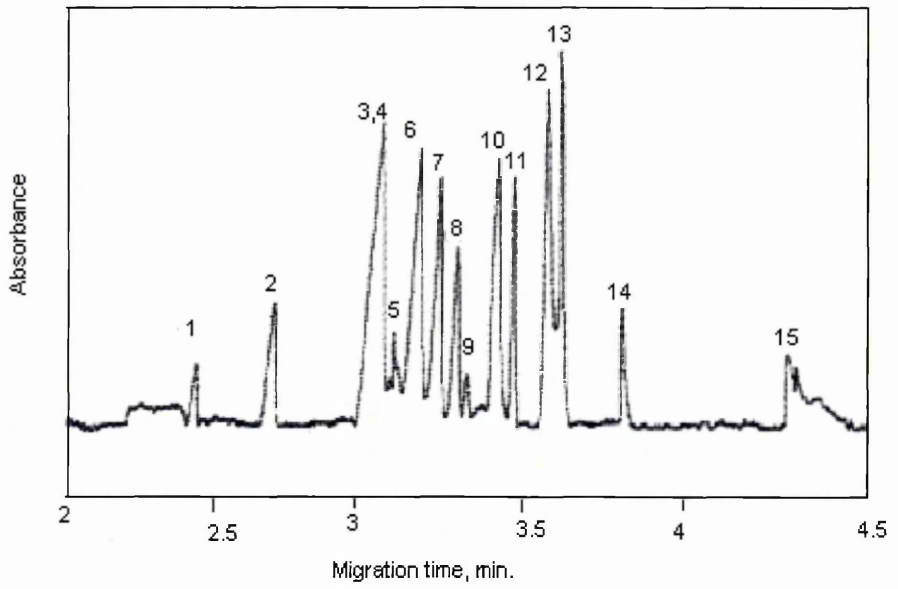


Fig. 7.3. Electrophoretic separation of thirteen metal cations using 11 mmol/L lactic acid, 7.5 mmol/L 4-methylbenzylamine, 2.6 mmol/L 18-crown-6 and 8% methanol (pH 4.3). Applied voltage 30 kV; Hydrodynamic injection with 30s; pressure applied was 20 mBar; UV/Vis detector; 1=NH_4^+; 2=K; 3=Na; 4=Ca; 5=unknown; 6=Mg; 7=Mn; 8=Cd; 9=Fe; 10=Co; 11=Ba; 12=Ni; 13=Zn; 14=Pb; 15=Cu.

The electropherogram obtained clearly shows the migration order is different when comparing with the previous data (Fig. 7.1) where lactic acid alone was employed. Under these conditions Mn was successfully separated from Fe but Na was still not separated from Ca. It was observed that Ni and Zn was not baseline resolved. Meanwhile Cu (peak 15) that migrated more slowly showed peak trailing and has broader peak width. Presumably the Cu ions formed complexes with lactic acid and 18-crown-6 that have higher stability constants.

In an attempt to separate Na from Ca the previous buffer conditions as employed by Shi and Fritz (29) in the first method was altered. The concentration of 4-methylbenzylamine was increased to 10 mmol/L instead of 8 mmol/L, other buffer conditions were fixed. This method still unable to separate Na from Ca. The concentration of methanol was changed to 10% instead of 5%, meanwhile 15 mmol/L

lactic acid and 10 mmol/L 4-methylbenzylamine were maintained at pH 4.25. Under these conditions, the separation of 13 metal cations in less than 6 minutes is shown in Fig. 7.4 and Na is now resolved from Ca but the peak shape for Cu is very broad and appeared in 6 min.

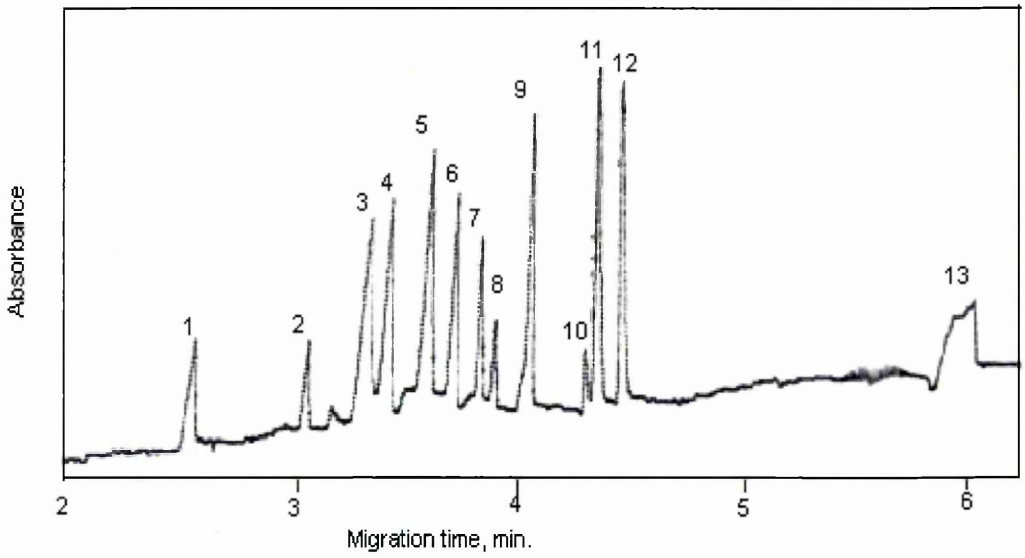


Fig. 7.4. Electrophoretic separation of thirteen metal cations using 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol (pH 4.25). Applied voltage 30 kV; Hydrodynamic injection with 30s; pressure applied was 20 mBar; UV/Vis detector; 1=K; 2=Ba; 3=Na; 4=Ca; 5=Mg; 6=Mn; 7=Cd; 8=Fe; 9=Co; 10=Pb; 11=Ni; 12=Zn; 13=Cu.

This finding is consistent with the results of Yang et al. (62) in their investigation on the simultaneous separation of ammonium and alkali, alkaline earth and transition metal ions in aqueous-organic media by CE. They have observed the effect of methanol in the separation of Li, Ni and Zn using a different concentration of HIBA (0 to 7 mmol/L) as a complexing reagent. It was found that HIBA provides an efficient selectivity of the separation for most of the cations studied, but the separation between Li, Ni and Zn is insufficient even at 6.5 mmol/L HIBA. A further increase in HIBA concentration was found to produce an interference peak, which can interfere with the other cations. They found that the cause is not clear. Therefore, the amount of HIBA in

the buffer composition was not increased further. However, by adding 10% (v/v) methanol to the electrolyte buffer, the mobilities of Ni and Zn are greatly decreased, so that they are separated from Li. It was noted that the presence of methanol in the buffer composition may improves the separation of metal cations by decreasing the electrophoretic mobility.

The new buffer conditions that have been developed were employed to separate the metal cations in acid mine discharges. None of the previous complexing reagents in this study enabled the complete separation of the 6 metals cations expected present in acid mine discharges. In order to confirm the trend of separation, the sample of acid mine discharges containing 6 metal cations was injected to observe the separation using 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine and 10% methanol at pH 4.25. The separation obtained is shown in Fig. 7.5.

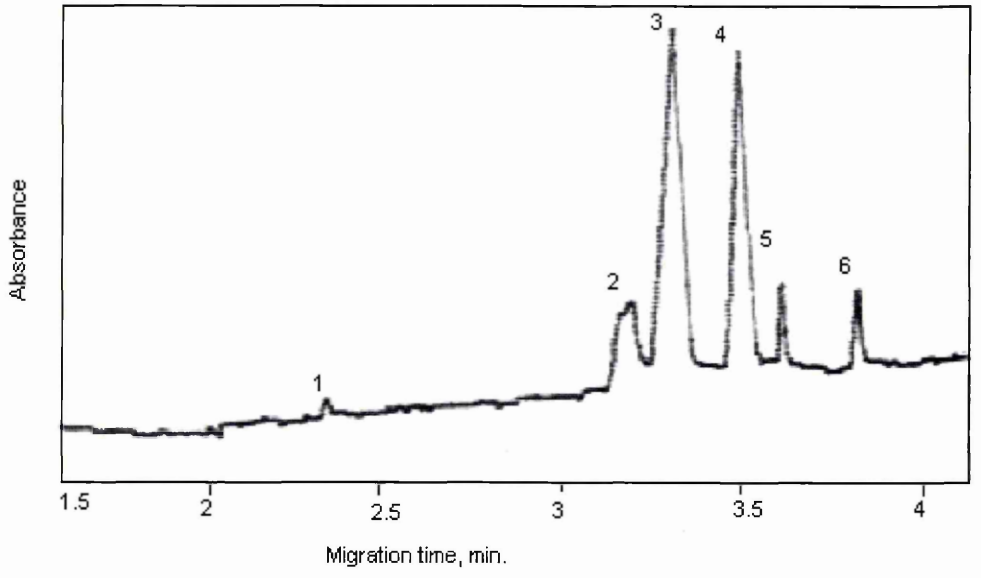


Fig. 7.5. Electrophoretic separation contain six metal cations using 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol (pH 4.25) in acid mine discharges. Applied voltage 30 kV; Hydrodynamic injection for 30s; pressure applied was 20 mBar; UV/Vis detector; 1=K; 2=Na; 3=Ca; 4=Mg; 5=Mn; 6=Fe.

These shows that the metals expected to be present in acid mine discharges are well separated. Therefore, this buffer conditions were employed for the separation and determination of metal cations in acid mine discharges.

7.3.2. Separation of metal cations by CE - Conductivity detection method

The CE separation of group I and II metal cations may be achieved by using a low conductivity buffer such as MES and L-Histidine and this has been demonstrated in Chapter 6. This buffer is only suitable for the investigation of metals in group I, group II as reported by Huang et al. (38). However, in order to obtain the separation of a wider range of metals, including transition metals, a different approach must be used. Most of the previous works using CE-Conductivity have not successfully separated the transition metals and they assumed was problematic. It has been demonstrated that the use of a complexing reagent, lactic acid, employed in conjunction with LiOH enables the separation of both group I, II and transition metals when using CE in conjunction with conductivity detection. Therefore, this buffer conditions will be used for the separation and determination of metals in acid mine discharges.

7.3.2.1. Earlier investigations using LiOH and Lactic acid

The separation of metal cations using conductivity detections have been noted in the previous works by Hjerten (166), Virtanen (5), Mikkers (6) and Jorgenson (7) between 1967 and 1981. In fact, Huang et al. (38,167,168) carried out comprehensive experiments using conductivity detection for the investigation of metal cations. However the use of this method for routine analysis is problematic. Most work using CE with conductivity detection was emphasis on group I and group II metals due to complication in sensitivity signal for transition metals as compared to other techniques

e.g. UV/Vis detection. More recently, the separation of metal cations in group I, group II and transition metals has been demonstrated by Haber et al. (67) using conductivity detection. They used a low concentration of acetic acid in conjunction with oxalic acid to obtain a separation of 16 metal cations in less then 7 min. The combination of LiOH and lactic acid as the buffer resulted in the successful separation of metal cations in acid mine discharges (Chapter 6). These metals included were K, Na, Ca, Mg, Mn and Fe i.e. these metals found in acid mine discharges. Analysis of acid mine discharges employed a buffer of 20 mmol/L lactic acid, 15 mmol/L LiOH and 10% methanol at pH 4.3. In this example, the concentration for each metal cations were employed at 10 ppm to observe the signal response using the method that have been developed for CE- conductivity detection. An example of the separation obtained for the metal cation standard mixture is shown in Fig. 7.6.

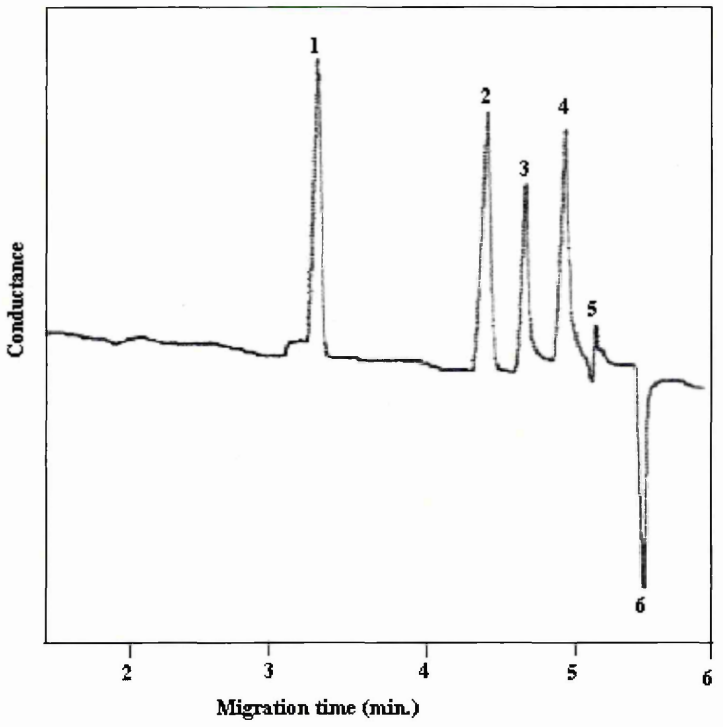


Fig. 7.6. Electrophoretic separation of metal cation standard mixture employing 20mmol/L lactic acid, 15mmol/L LiOH, 10% methanol (pH 4.3). Applied voltage 30 kV; Hydrodynamic injection for 20s; pressure applied of 20 mBar; Conductivity detector; 1=K; 2=Na; 3=Ca; 4=Mg; 5=Mn; 6=Fe.

Based on the above electropherogram, all metals provide a good peak separation and acceptable sensitivity except for Mn. Good signal to noise was observed for all metals except manganese with a small peak height. A similar trend for Mn was observed in the previous work (Chapter 6) which is poor in signal response (1.03×10^{-3} mV) and low in number of theoretical plate count (311,782) compared to other metals. All metals were separated in less than 5.5 min. It demonstrated that the quantitative analysis for Mn not so sensitive at low concentration as compared to other metals present in acid mine discharges. However, peak response shows a good performance at

higher concentration and may be used for quantitative analysis as shown in further investigation of acid mine discharges (clause 7.3.3.1.5.).

7.3.3. Quantitative analysis of acid mine discharges

7.3.3.1 Calibration curve

The external calibration techniques was applied for determinations of metal cations in acid mine discharges. Five standards at different concentration of K, Na, Ca, Mg and Mn and Fe ranges (0–20 ppm for UV/Vis detection) and (0-25 ppm for conductivity detection except Fe) were prepared respectively. These ranges were selected because good signal response for all metals that presence in acid mine discharges. Sensitivity is defined as the slope of the calibration curve (detector signal versus sample concentration). A steeper slope indicates better sensitivity. Ten consecutive injections were made at each point and the peak absorbance averaged for UV/Vis detection. Meanwhile, the average of peak conductance was used to plot a calibration graph in conductivity detection. Standards used for calibration graph were freshly prepared for each analysis. The graph is plotted after subtracting the blank value to give a curve passing through zero. A linear relationship was demonstrated for each metal cations (Figs. 7.7 - 7.19) respectively. The procedure used for linearity of the graph was followed as outlined in Sectioned 4.5.1.4. If the concentration of samples were obtained above the range, the appropriate dilution were made to make sure the signal fell within the range of the standard mixture. The equations from the calibration graphs were used to calculate the actual amount of acid mine discharges after consideration of the dilution factor.

The concentrations range of standards used depended on the concentrations of metal cations that would be found in the acid mine discharges i.e. Na, K, Ca, Mg, Mn and Fe. Therefore, it is very important to carry out studies on realistic concentrations and then progress to make several dilutions of the sample acid mine discharges.

7.3.3.1.1. Determination of potassium

Plots of K concentration against the response for both UV/Vis absorbance and conductivity detection are shown in Figs. 7.7 - 7.8. The linearity plot for K can be fitted a straight line, therefore the regression and equation were used for calculation of K ($y = 0.0003x + 0.0002$; and $R^2 = 0.992$) as seen in Fig. 7.7.

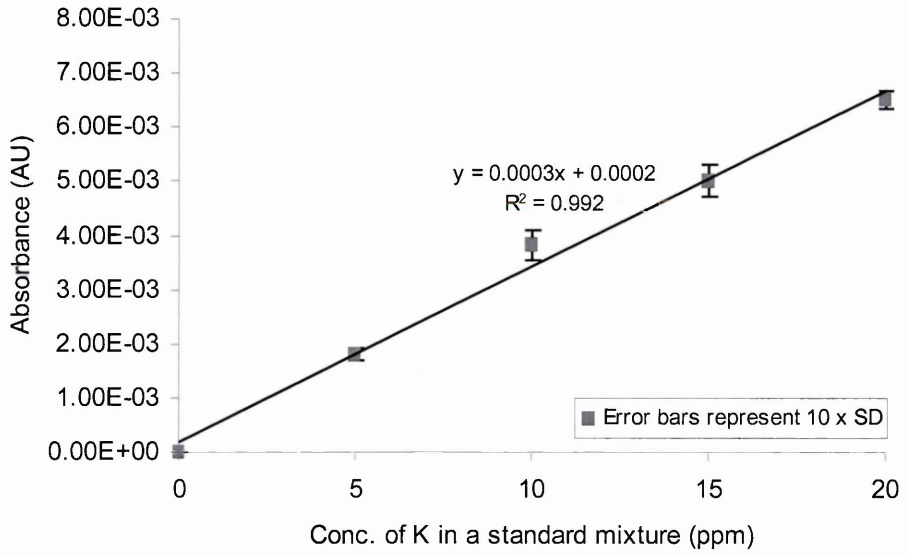


Fig. 7.7. Calibration graph for K in a mixture standard using CE-UV/Vis detection. The buffer used was 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25.

It was found that the none linear relationship of K occurred at low concentration (less than 0.5 ppm) using UV/Vis detection. This result was in agreement with Shi et al. (30), the linearity of absorbance against concentration was very poor at the range 1 to

10 ppm. In order to increase the sensitivity and limit of detection, Li (I) was employed as internal standard in their investigation. However they obtained a good linear relationship after the employing of peak area against concentration of standard. An improvement of 100-fold was observed (0.1 to 10 ppm).

A plot of K concentration against the response in peak area for conductivity detection (Fig. 7.8) shows a good linear relationship ($y = 0.0001x + 9E\text{-}06$; and $R^2 = 0.9976$).

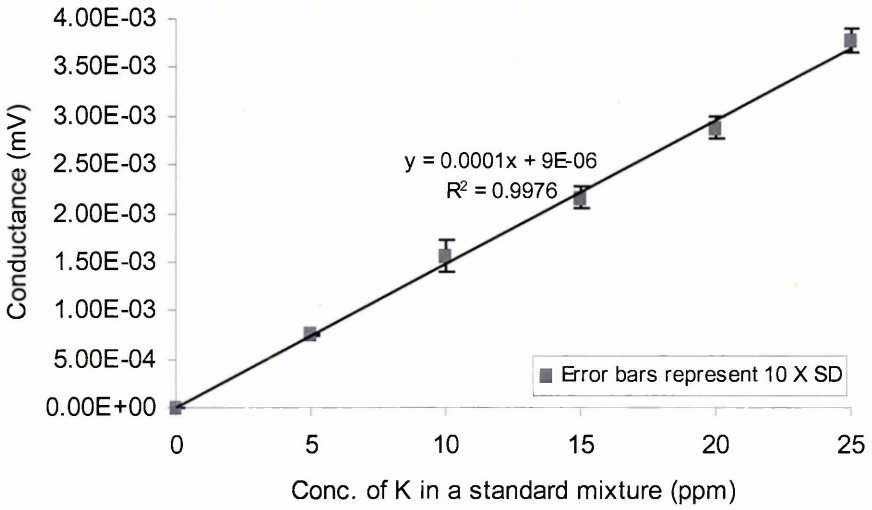


Fig. 7.8. Calibration graph for K in a mixture standard using CE-Conductivity detection. The buffer used was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

As can be seen in Fig. 7.7, the calibration slope of K is better when using CE-UV/Vis as compared to CE-Conductivity detection. It demonstrated that the signal response for UV/Vis detection is very sensitive at 20 ppm. However, the linear relationship of K for CE-Conductivity is better than CE-UV/Vis method and can be fitted a straight line up to 25 ppm.

7.3.3.1.2. Determination of sodium

Plots of Na concentration against absorbance for both UV/Vis and conductivity detection are shown in Figs. 7.9 - 7.10. A good linear relationship ($y = 0.0003x + 0.0002$; and $R^2 = 0.9925$) for Na is seen in Fig 7.9. These results are based on the average of three electropherograms for Na standard mixture.

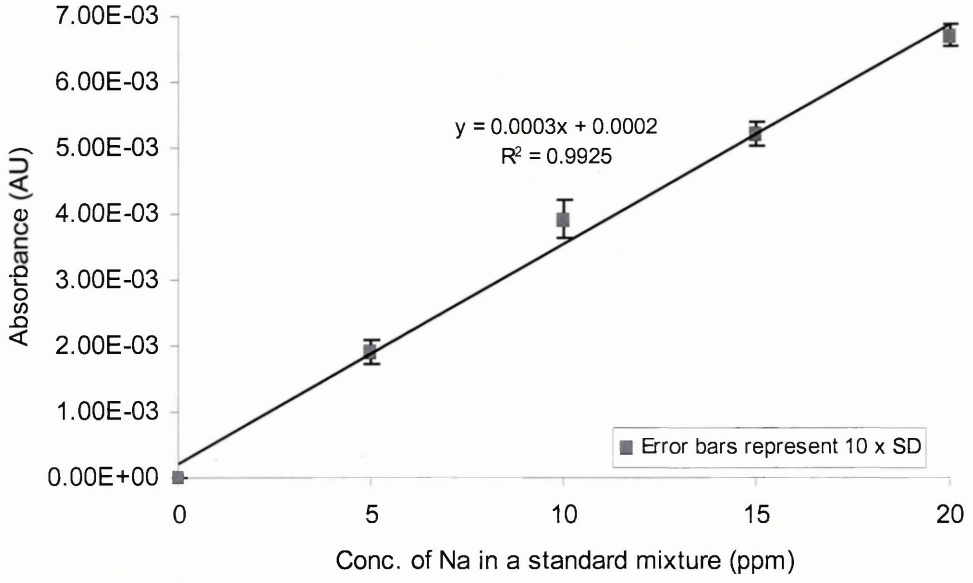


Fig. 7.9. Calibration graph for Na in a mixture standard using CE-UV/Vis detection. The buffer used was 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25.

A plot of Na concentration against the response of peak area for conductivity detection (Fig. 7.10) shows a good linear relationship ($y = 0.0002x - 3E\text{-}05$; and $R^2 = 0.9948$).

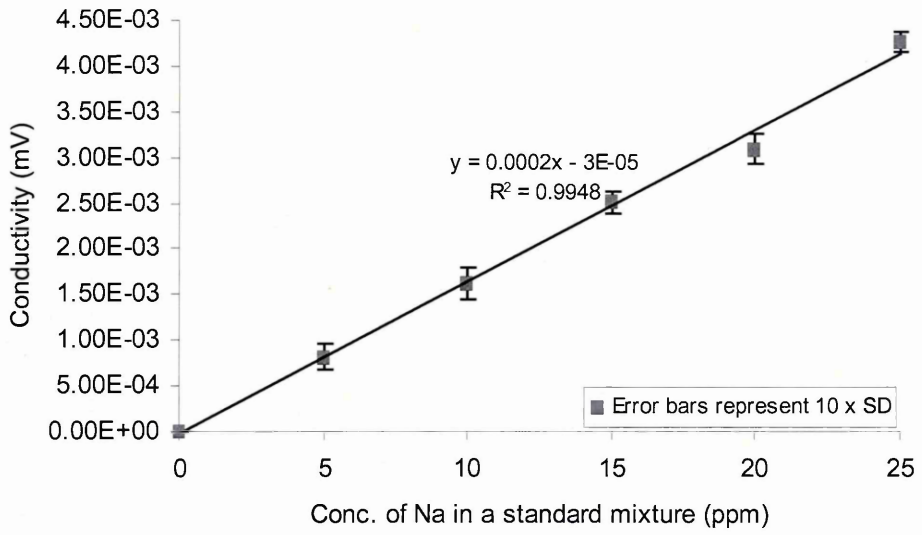


Fig. 7.10. Calibration graph for Na in a mixture standard using CE-Conductivity detection. The buffer used was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

As can be seen in Fig. 7.9, the calibration slope of Na is better when using CE-UV/Vis as compared to CE-Conductivity detection. It demonstrated that the signal response for UV/Vis detection is very sensitive at 20 ppm. However, the linear relationship of Na for CE-Conductivity is better than CE-UV/Vis method and can be fitted a straight line up to 20 ppm.

7.3.3.1.3. Determination of calcium

A plot of Ca concentration against the absorbance for both UV/Vis and conductivity detection in Figs. 7.11 - 7.12. A good linear relationship ($y = 0.0001x + 3E\text{-}05$; and $R^2 = 0.9952$) is seen for Ca in Fig. 7.11. These results are based on the average of three electropherograms for Ca standard.

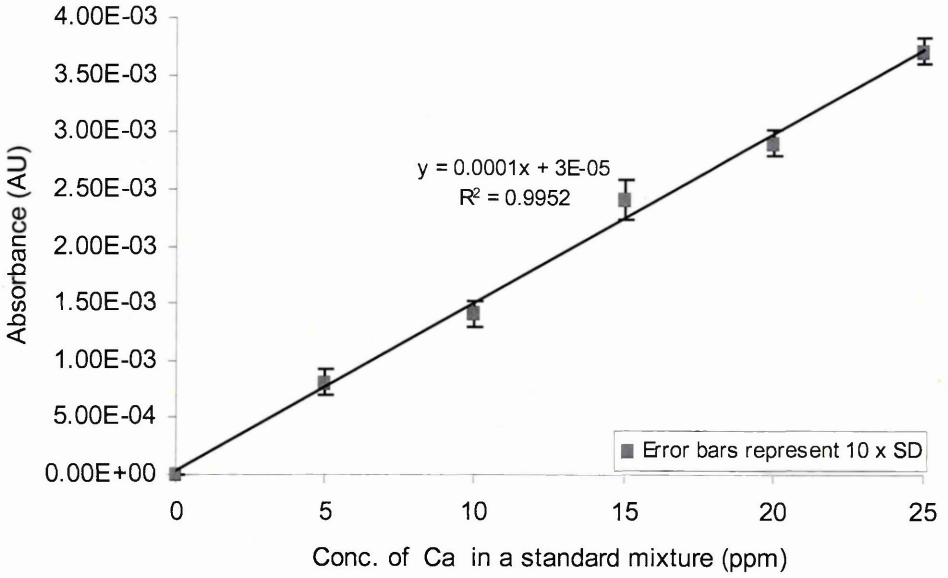


Fig. 7.11. Calibration graph for Ca in a mixture standard using CE-UV/Vis detection. The buffer used was 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25.

A plot of Ca concentration against the response of peak area for conductivity detection (Fig. 7.12) shows a good linear relationship (y = 8E-05x + 2E-05; and R^2 = 0.9982).

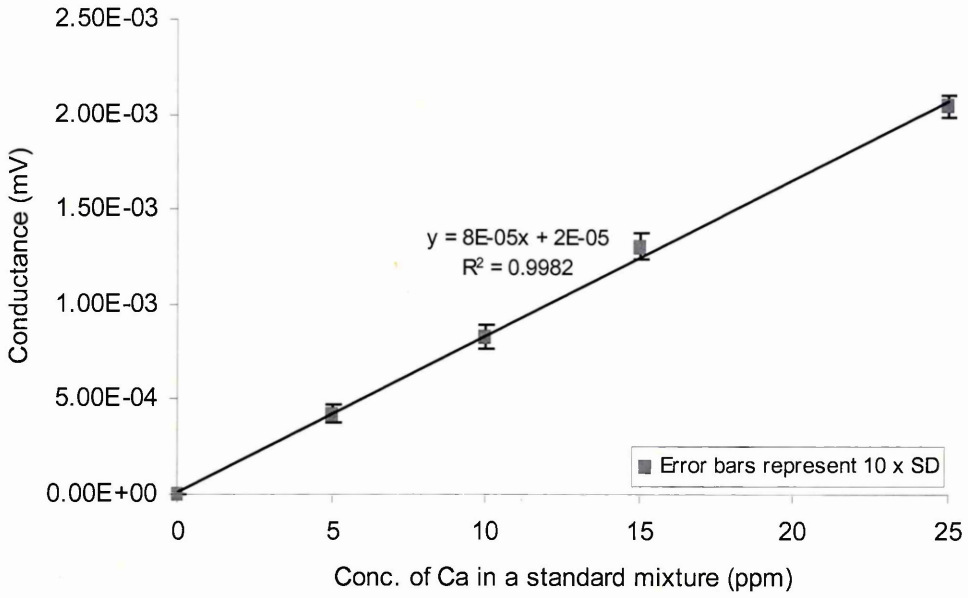


Fig. 7.12. Calibration graph for Ca in a mixture standard using CE-Conductivity detection. The buffer used was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

As can be seen in Fig. 7.11, the calibration slope of Ca is better when using CE-UV/Vis as compared to CE-Conductivity detection. It demonstrated that the signal response for UV/Vis detection is very sensitive at 20 ppm. However, the linear relationship of Ca for CE-Conductivity is better than CE-UV/Vis method and can be fitted a straight line up to 25 ppm.

7.3.3.1.4. Determination of magnesium

Plots of Mg concentration against absorbance and conductivity are shown in Figs. 7.13 - 7.14. The calibration curve for Mg can be fitted to a straight line with good linear relationship ($y = 0.0002x + 9E\text{-}05$; and $R^2 = 0.9958$) in Fig. 7.13. These results are based on the average of three electropherograms for magnesium standard mixture.

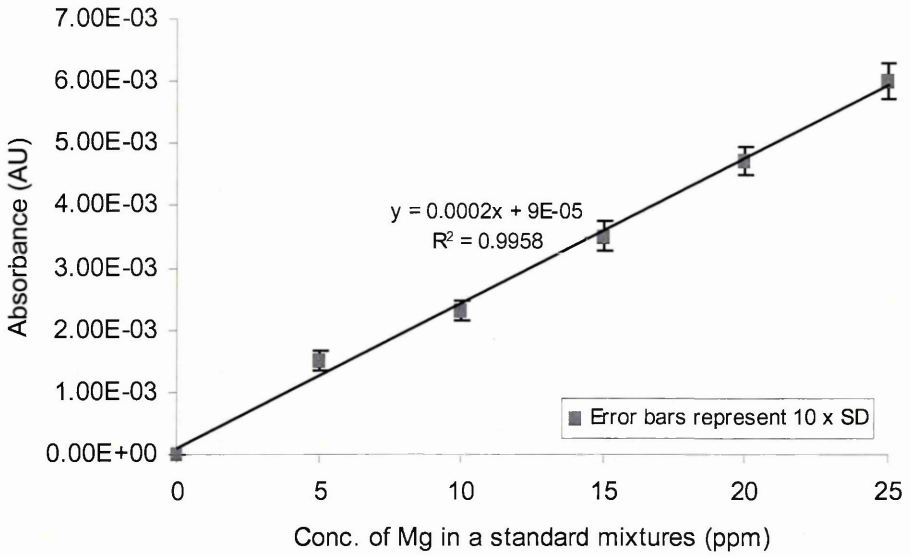


Fig. 7.13. Calibration graph for Mg in a mixture standard using CE-UV/Vis detection. The buffer used was 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25.

A plot of magnesium concentration against the response of peak area for conductivity detection (Fig. 7.14) can be fitted with a straight line and shows a good linear relationship ($y = 0.0001x - 4E\text{-}06$; and $R^2 = 0.9958$).

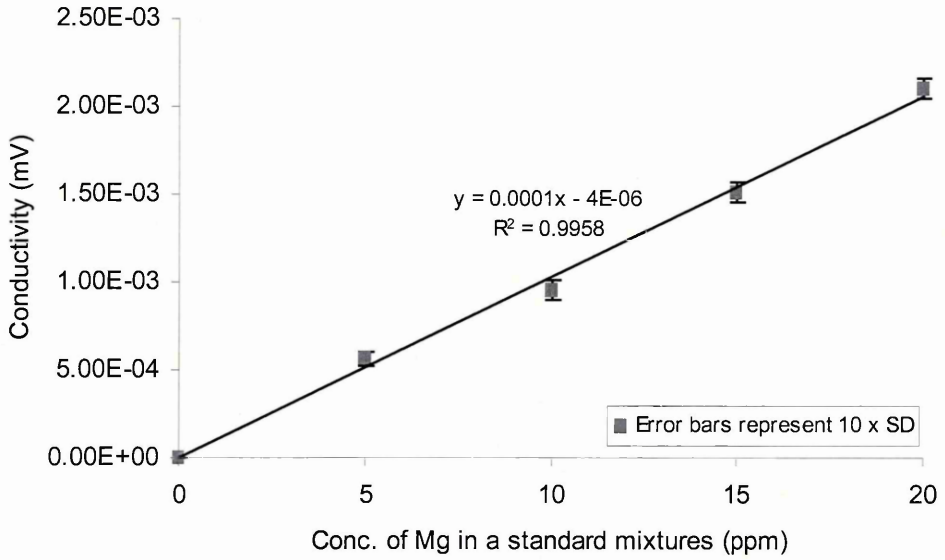


Fig. 7.14. Calibration graph for Mg in a mixture standard using CE-Conductivity detection. The buffer used was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

As can be seen in Fig. 7.13, the calibration slope of Mg is better when using CE-UV/Vis as compared to CE-Conductivity detection. It demonstrated that the signal response for UV/Vis detection is very sensitive at 25 ppm. However, the linear relationship of Mg for CE-Conductivity is better than CE-UV/Vis method and can be fitted a straight line up to 20 ppm.

7.3.3.1.5. Determination of manganese

Plots of Mn concentration against CE-UV/Vis absorbance and CE-conductivity are shown in Figs. 7.15 – 7.16. Fig. 7.15 shows that Mn can be fitted a straight line with a good linear relationship ($y = 0.0002x + 0.0001$; and $R^2 = 0.9976$). These results are based on the average of three electropherograms for Mn standard.

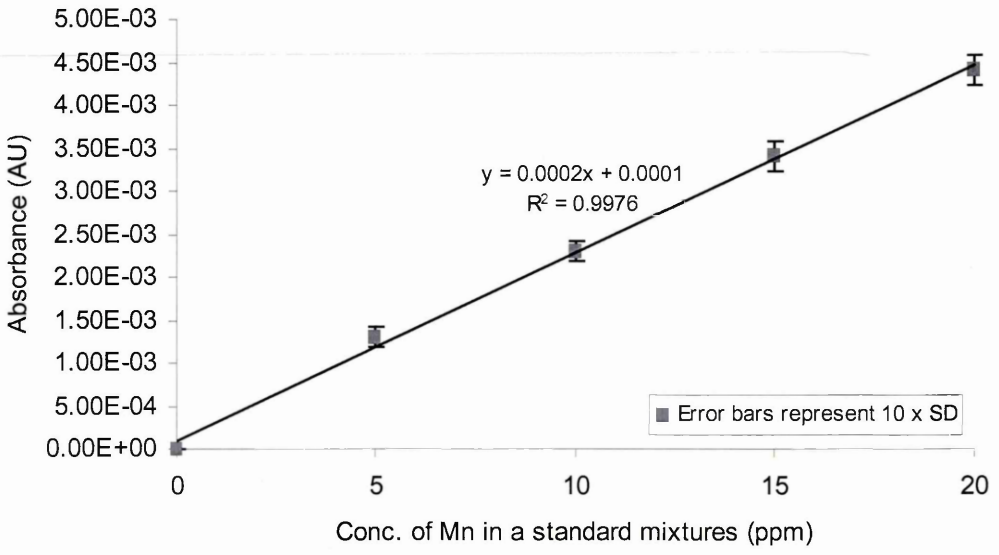


Fig. 7.15. Calibration graph for Mn in a mixture standard using CE-UV/Vis detection. The buffer used was 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25.

A plot of Mn concentration against the response of peak area for conductivity detection (Fig. 7.16) shows that can be fitted with a straight line with a good linear relationship ($y = 0.0002x + 0.0001$; and $R^2 = 0.991$). The separation of Mn using conductivity detection method was very poor at lower concentration due to incomplete separation. The proper investigation and optimisation at low concentration may be can improve the separation of manganese.

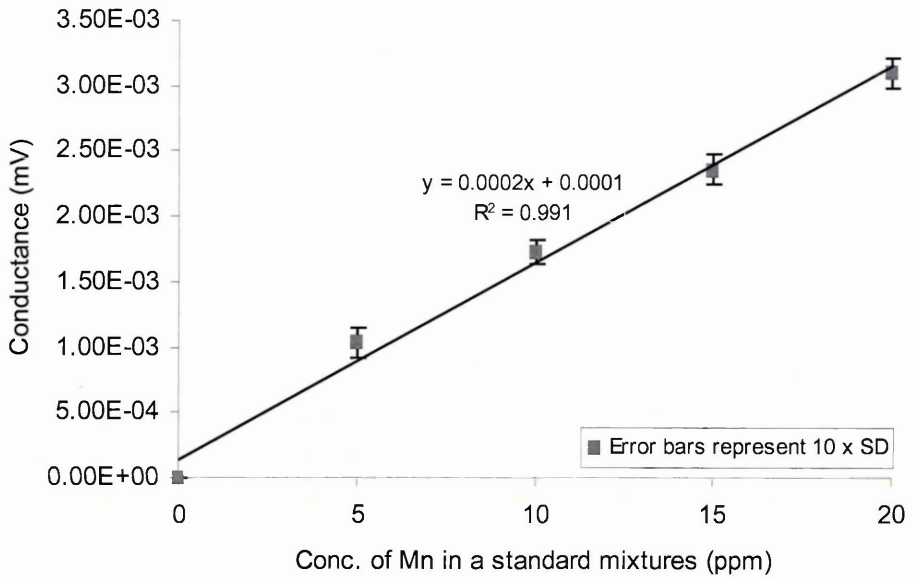


Fig. 7.16. Calibration graph for Mn in a mixture standard using CE-Conductivity detection. The buffer used was 20 mmol/L lactic acid, 15 mmol/L LiOH, 10% methanol at pH 4.3.

As can be seen in Fig. 7.15, the calibration slope of Mn is better when using CE-UV/Vis as compared to CE-Conductivity detection. It demonstrated that the signal response for UV/Vis detection is very sensitive at 20 ppm. However, the linear relationship of Mn for CE-Conductivity is better than CE-UV/Vis method and can be fitted a straight line up to 20 ppm.

7.3.3.1.6. Determination of iron

Plots of Fe concentration against CE-UV/Vis absorbance and CE-conductivity detection are shown in Figs. 7.17 – 7.18. From Fig. 7.17, a straight line is fitted with a good linear relationship ($y = 0.0004x + 0.0003$; and $R^2 = 0.991$). These results are based on the average of three electropherograms of iron standard mixture.

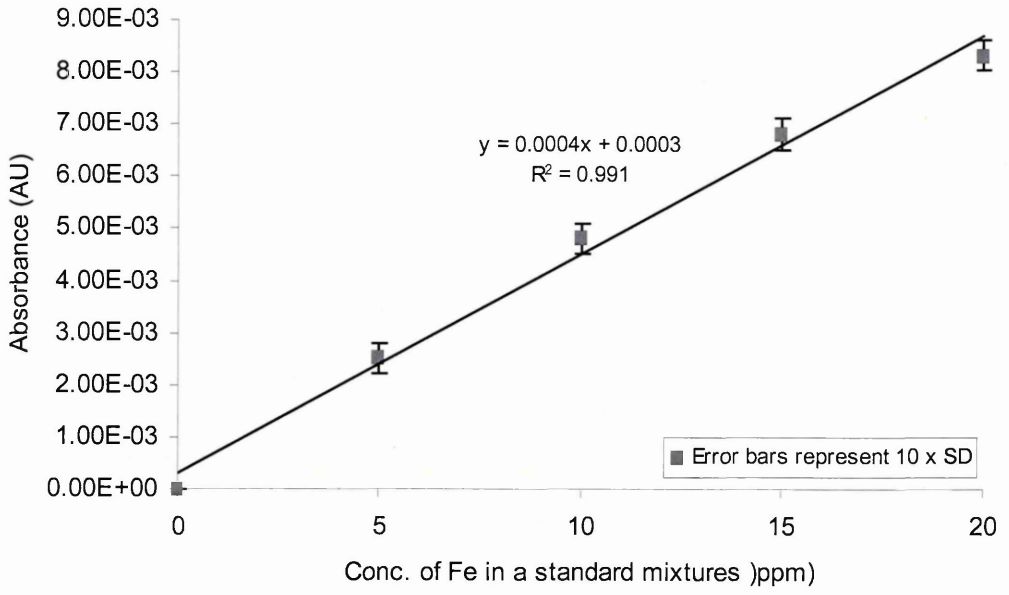


Fig. 7.17. Calibration graph of Fe in mixture standard using CE-UV/Vis detection. The buffer used was 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25.

A plot of Fe concentration against the response of peak area for conductivity detection (Fig. 7.18) shows that a straight line can be fitted with good linear relationship ($y = 0.0002x + 0.0001$; and $R^2 = 0.9969$).

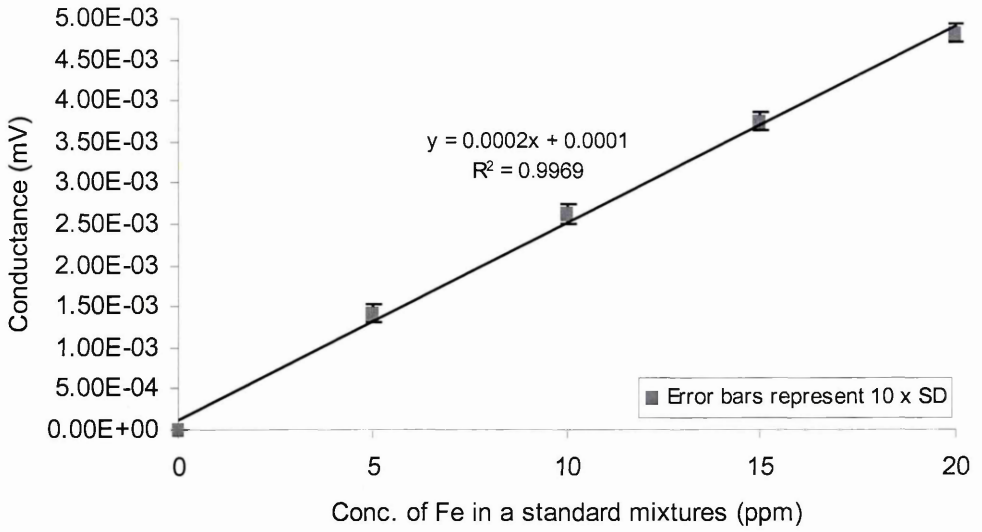


Fig. 7.18. Calibration graph of Fe in a mixture standard using CE-Conductivity detection. The buffer used was 20 mmol/L lactic acid, 15 mol/L LiOH, 10% methanol at pH 4.3.

As can be seen in Fig. 7.17, the calibration slope of Fe is better when using CE-UV/Vis as compared to CE-Conductivity detection. It demonstrated that the signal response for UV/Vis detection is very sensitive at 20 ppm. The linear relationship of Fe for CE-UV/Vis is better than CE-Conductivity method and can be fitted a straight line up to 20 ppm.

Based on the above calibration graph plotted shows that the linear relationship are good for all metals. The selected range for each metals is good enough to quantify the concentration presence in acid mine discharges. In CE-UV/Vis detection, the calibration graph for K, Na, and Fe are very sensitive relatively as compared to Mg (0 – 25 ppm) and Mn but Ca is less sensitive at range between 0 to 20 ppm. However, the calibration graph in CE-Conductivity detection for all metals are less sensitive at range between 0 to 20 ppm. Further analysis may be employed to improve the sensitivity for both detection techniques using different approaches. Low concentration

sensitivity in absorbance-based detectors in CE may be due to a number of factors. First, from Lambert-Beer law (see Equation 7.1) the absorbance is proportional to path length and the path length in CE for the detection window is very short. This path length relies on the internal diameter of the capillary column (usually between 50 - 100 μm). Moring et al. (110) employed a *z-cell* with a 3 mm capillary section of path length and obtained a tenfold increase in sensitivity. In another development Tsuda et al. (111) used *rectangular* capillaries of 1000 μm path length with an increase of about 20-fold in sensitivity. Later Wang et al. (112) used a *multireflection cell* to enable an increase of 40-fold in peak height. The path length also can be increased using a *bubble cell* as demonstrated by Heiger (202) when he obtained a 3-fold increase in peak height.

Another option to enhance sensitivity in sample concentration is by Isotachophoresis (ITP). It is performed by sandwiching a sample between a leading and terminating, or trailing, buffer and by applying an electric field in the constant current mode. This method is unable to obtain the separation of anions and cations in a single run. The method employed can enhance sensitivity by up to 3 orders of magnitude (usually between 100-1000 enhancement). This method was carried out by Stegehius et al. (123) to obtain a sensitivity enhancement of the order of 100 times in the separation of fluorescein isothiocyanate-amino acids by ITP-CE. Meanwhile, ITP conditions were employed for the investigation of anions by Haber et al. (67). They obtained the detection limits of 100 ppt using a conductivity detector.

7.3.3.2. Results of analysis by CE-UV/Vis, CE-Conductivity and ICP-OES detection

Representative of five batch samples of acid mine discharges were mixed for determination using CE-UV/Vis, CE-Conductivity and ICP-OES detection. The same batch samples were also analysed by ICP-OES to obtain a confirmation of the metal levels present in acid mine discharges. However the analysis work using ICP-OES was carried out by other researcher. The results of analysis are statistically evaluated and are displayed in Table 7.1 - 7.2.

Elements	Mean result, ppm ± SD [a]		$\lvert t \rvert$ observed[c]
	UV/Vis	ICP-OES	
Potassium	ND	13 [b] ± 0.7	NA
Sodium	1046 [b] ± 21	995.6 [b] ± 62.7	1.71
Calcium	380.2 [b] ± 9.3	363.4 [b] ± 14.0	2.24
Magnesium	322.2 [b] ± 54.4	290.2 [b] ± 20.4	1.23
Manganese	23.6 [b] ± 7.5	24.8 [b] ± 10.0	0.21
Iron	5.4 [b] ± 2.1	1.92 [b] ± 0.86	3.47

[a] = Corrected for mean of 5 batch samples.
[b] = Mean of 10 replicates.
[c] = t-test, $t_{crit.} = 2.31$, $p = 0.05$.
ND = None detected.
NA = Not applicable.

Table 7.1. Results for the analysis of acid mine discharges by CE-UV/Vis and ICP-OES detection.

It was demonstrated that From Table 7.1, the observed value of $\lvert t \rvert$ is less than the critical value ($t_{crit.}$) so the null hypothesis is retained for Na, Ca, Mg and Mn. In this

work, the level of K is not detectable and the observed value of $|t|$ for K is not calculated. This can conclude that the means of the results given by the two methods are equal. However, the observed value of $|t|$ for Fe is more than the critical value ($t_{crit.}$) so the null hypothesis is rejected.

Elements	Mean result, ppm ± SD [a]		$\|t\|$ observed[c]
	Conductivity	ICP-OES	
Potassium	12.6 [b] ± 2.1	13 [b] ± 0.7	0.41
Sodium	933.6 [b] ± 19.0	995.6 [b] ± 62.7	2.12
Calcium	300.8 [b] ± 66.8	363.4 [b] ± 14.0	2.19
Magnesium	262.6 [b] ± 32.2	290.2 [b] ± 20.4	1.23
Manganese	27.8 [b] ± 11.1	24.8 [b] ± 10.0	0.45
Iron	ND	1.92 [b] ± 0.86	NA

[a] = Corrected for mean of 5 batch samples.
[b] = Mean of 10 replicates.
[c] = t-test, $t_{crit.} = 2.31$, $p = 0.05$.
ND = None detected.
NA = Not applicable.

Table 7.2. Results for the analysis of acid mine discharges by CE-Conductivity and ICP-OES detection.

From Table 7.2, the observed value of $|t|$ is less than the critical value ($t_{crit.}$) so the null hypothesis is retained for K, Na, Ca, Mg and Mn. This can conclude that the means of the results given by the two methods are equal. In this work, the level of Fe is not detectable and the observed value of $|t|$ for Fe is not calculated. In general, there are some discrepancies of the results comparing CE-UV/Vis with CE-conductivity detection techniques. All metals detected using CE-UV/Vis were found to

be higher compared to ICP-OES. Meanwhile the metals level using CE-conductivity detection was found to be lower than ICP-OES. The main reason for CE-conductivity detection is due to signal has been saturated when the presence of high concentration of sodium in the samples. Therefore, the result is reported lower than actual level of particular metals in acid mine discharged. In CE-UV/Vis detection the factor of carry over may influenced for high level of certain metals during the plotting of the calibration curve. The employing of internal standard may improved the precision and accuracy of the result for this investigation.

As can be seen from Table 7.1, six metals from group I, group II and transition metals include K, Na, Ca, Mg, Mn and Fe were analysed in acid mine discharges. All metals were separated in less than 4 minute using 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25 using CE-UV/Vis detection. The migration order follows the sequence K, Na, Ca, Mg, Mn and Fe. All peaks displayed for Na, Ca and Mg were co-migrate and not completely baseline resolved except for Mn and Fe. The K peak (Table 7.1) could not be determined due to a very poor separation in the presence of large amount of metal cations i.e. Na, Ca and Mg in acid mine discharges. It was a trend in CE where ion peaks become very broad at higher concentrations. The separation obtained is shown in Fig. 7.19.

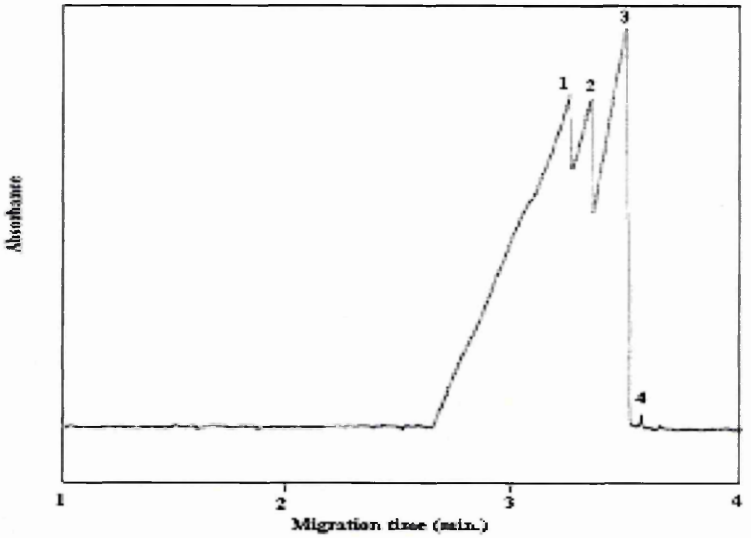


Fig. 7.19. Electrophoretic separation of 4 metal cations in acid mine discharges (5x dilution) when using 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol (pH 4.25). Applied voltage 30 kV; Hydrodynamic injection with 30s; pressure applied of 20 mBar; UV/Vis detector; 1=Na; 2=Ca; 3=Mg; 4=Mn.

These finding is better in term of number of analytes separated and overall run time compared to those observation by Shi and Fritz (29). They obtained eight metal cations including K, Na, Pb, Mn, Co, Ni, Zn and Cd in less than 6 minutes using 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3. They have a good peak shape and baseline resolved. In another development they have separated twelve metal cations using 2.5 mmol/L tartaric acid, 6 mmol/L *p*-toluidine, 20 % methanol at pH 4.8 in less than 8 min. Resolution is excellent with a steady, flat baseline. However an excellent separation of twenty seven metal cations has been achieved by Shi and Fritz (29) in a single run in less than 6 min. They used 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. Among those metal cations that were separated including K^{+}, Ba^{2+}, Sr^{2+}, Na^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^{+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Cu^{2+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and

Lu^{3+} . The average deviation in peak height from one run to another was approximately ± 5% or smaller at the ion concentration used.

The K peak still could not be separated from Na even after 50 times dilution was made. The level of K also is lower compared to Na, Ca and Mg. The same problem was encountered in all five batch samples. It was confirmed the same problem that found by Shi et al. (30). They were unabled to separate 1 ppm of Mg, Ca, Sr and Ba with the present of 1000 ppm Na. The separation was only successful when different concentration of methanol (8%, 16%, 32%) and 18-crown-6 were added to the buffer composition. Under this investigation, the reduction of voltage also can improve the separation of metals at lower concentration. Based on the above conditions, K was not detected by UV/Vis detection. However the result obtained for conductivity detection was good as compared to ICP-OES. The large differences for determination of Ca level in acid mine discharges were observed between CE-UV/Vis, CE-conductivity and ICP-OES detection methods. The problem for CE-UV/Vis detection was expected due to the formation complex between Ca and lactic acid was not properly optimised during the method developments. The same samples used for ICP-OES was re-tested for CE-UV/Vis without adjusted the pH, and this sample could be in acidic condition during the sample preservation. This conditions could be influenced the high Ca level when using UV/Vis detection method. However the calcium level between conductivity detection and ICP-OES were comparable. The level of manganese (Table 7.1 - 7.2) in acid mine discharges were comparable between CE-UV/Vis, CE-conductivity and ICP-OES detection methods. It was indicated that the present of low manganese concentration not much influenced by the high concentration of other metals from group I and group II. Meanwhile the Fe level was slightly higher using CE-UV/Vis detection compared to ICP-OES. This observation is supported by the

observed value of $|t|$ for Fe is more than the critical value ($t_{crit.}$) so the null hypothesis is rejected (Table 7.1). Therefore, the difference between the two result for Fe is not significant at the 5% level and the null hypothesis is rejected. This could be due to the interference of signal to noise at low concentration of Fe content. For absorptive detectors the absorbance of a solute is dependent on path length, b, concentration, C, and molar absorptivity, ε , as defined by Beer's Law.

$$A = bC\ \varepsilon \qquad (7.1)$$

The short path length is the factor that mainly limits sensitivity in CE. Due to curvature of the capillary, the actual path length in the capillary is less than the inner diameter since only a fraction of the light passes directly through the centre. The actual path length can be determined by filling the capillary with a solute of known concentration and molar absorptivity. High sensitivity can often be realised by use of low-UV detection wavelengths. Peptides and carbohydrates, for example, have no strong chromophores but can be adequately detected at 200 nm or below. Detection of these low wavelengths necessitates the use of minimally absorbing running buffers since high background absorbance increases baseline noise and decreases signal. Number of theoretical plates (N) achieved for each metals are listed in Table 7.3.

Metal cations	Number of theoretical plates per meter (N/m)
Potassium	N/A
Sodium	585,567
Calcium	621,471
Magnesium	692,204
Manganese	774,365
Iron	114,719

Table 7.3. Peak efficiency for the analysis of acid mine discharges by CE-UV/Vis detection.

From Table 7.3, Mn has the highest number of theoretical plates (N), about 774,365 followed by Mg, Ca and Na because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as compared to Fe has the lowest N value, about 114,719. This values obtained was excellent as compared to N value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the N value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of N between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. The result obtained shows that the peak efficiencies for each metal cations are suitable for quantitative analysis.

As can be seen from Table 7.2, five metals were separated in less than 6 minutes using 20mmol/L lactic acid, 15mmol/L LiOH, 10% methanol at pH 4.3 using CE-Conductivity detection. The migration order follows the K, Na, Ca, Mg, Mn and Fe. All peaks are completely baseline resolved except Fe. These finding is better in term of number of analytes separated and baseline resolved even excellent separation compared to those observation by Haber et al. (67) using indirect conductivity detection method in similar field. They have separated NH_4^+ and sixteen metal cations including Cs, K, Ba, Sr, Ca, Cr, Na, Mg, Mn, Cd, Li, Fe, Co, Zn, Ni and Pb in less than 7 minutes using 0.1 mmol/L acetic acid, 1 mmol/L oxalic acid at pH 2.84. Resolution is not properly optimized. Most metals observed not are baseline resolved including Ba, Sr, Ca, Cr, Na and Mg. Mn and Cd are co-eluted together. Meanwhile, Shi and Fritz (29) obtained eight metal cations including K, Na, Pb, Mn, Co, Ni, Zn and Cd in less than 6 minutes using 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3. They have a good peak shape and baseline resolved. In another development they have separated twelve metal cations using 2.5 mmol/L tartaric acid, 6 mmol/L *p*-toluidine, 20 % methanol at pH 4.8 in less than 8 min. Resolution is excellent with a steady, flat baseline. However an excellent separation of twenty seven metal cations has been achieved by Shi and Fritz (29) in a single run in less than 6 min. They used 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine, 5% methanol at pH 4.25. Among those metal cations that were separated including K^+, Ba^{2+}, Sr^{2+}, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Li^+, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Cu^{2+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+}. The average deviation in peak height from one run to another was approximately ± 5% or smaller at the ion concentration used.

The results obtained for Na in acid mine discharges using the CE-UV/Vis and CE-conductivity detection were not as good as ICP-OES due to several reasons during the analysis. The results obtained by UV/Vis was poor due to the limited path length when involved high concentration of analytes. Several dilutions from the original sample could be made to get proper analytical consideration and could be influenced the reproducibility of result for sodium in acid mine discharges. The problem for conductivity detection was due to the ConCap I fused-silica capillary had a limited lifetime of approximately 3 weeks. The depreciation of the capillary was observed by the increased in the electroosmotic flow marker peak mobility increasing and by the baseline deteriorating until a stable baseline could no longer be achieved. Many different methods such as NaOH, KOH and HNO_3 have been tried to rejuvenate the capillary but all were unsuccessful. This problem may be affected by the saturation of conductivity signal and the level of sodium was expected lower than the actual concentration. The differences level between CE-UV/Vis, CE-conductivity detection and ICP-OES for magnesium (Table 7.1 - 7.2) may have a same reason as mentioned in the previous investigations. It was expected the formation complex for Mg with lactic acid could be incompletely resolved when they separated under the UV/Vis detection. This factor may be influenced the reproducibility of Mg level. However the Mg level for conductivity detection was expected lower than ICP-OES because of the low performance of conductivity cell and the problem with ConCap I fused silica capillary as mentioned earlier. From Table 7.1, the Fe peak was not detected using the CE-Conductivity detection. It may be due to low sensitivity for Fe as compared to CE-UV/Vis and ICP-OES. The separation obtained is given in Fig. 7.20.

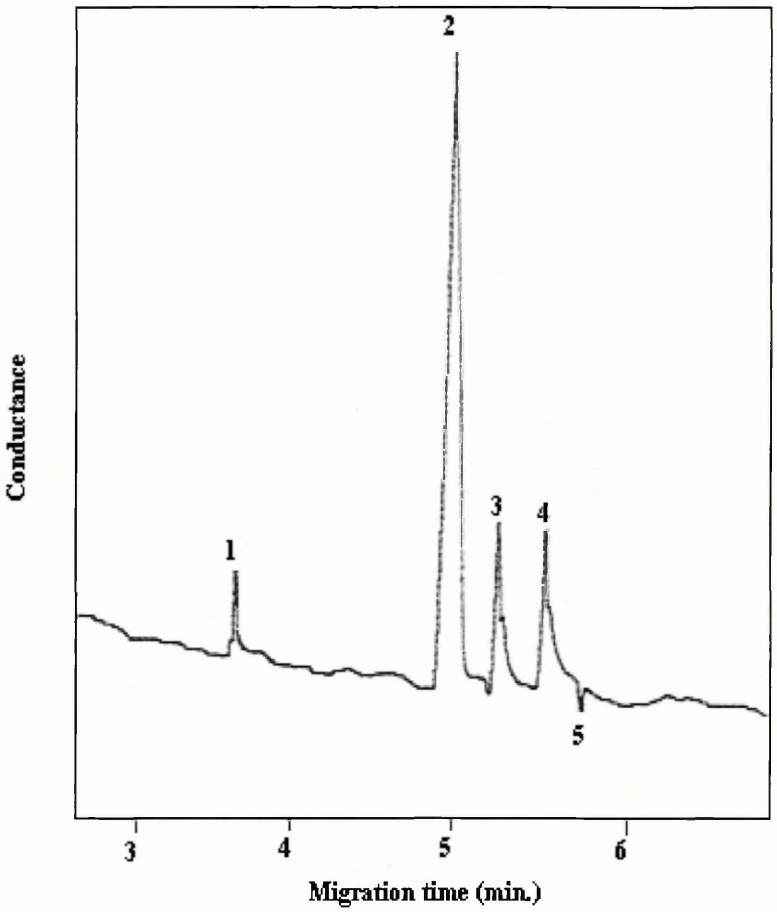


Fig. 7.20. Electrophoretic separation of 5 metal cations present in acid mine discharges (50x dilution) when using 20mmol/L lactic acid, 15mmol/L LiOH, 10% methanol (pH 4.3). Applied voltage 30 kV; Hydrodynamic injection with 20s; pressure applied of 20 mBar; conductivity detector; 1=K; 2=Na; 3=Ca; 4=Mg; 5=Mn.

Number of theoretical plates (N) achieved for each metals are listed in Table 7.4.

Metal cations	Number of theoretical plates per meter (N/m)
Potassium	101,909
Sodium	91,250
Calcium	101,607
Magnesium	97,616
Manganese	83,163
Iron	N/A

Table 7.4. Peak efficiency for the analysis of acid mine discharges by CE-Conductivity detection.

From Table 7.4, K has the highest number of theoretical plates *(N)*, about 101,909 followed by Ca, Mg and Na because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as compared to Mn has the lowest *N* value, about 83,163. This values obtained was not excellent as compared to *N* value about 300,00 for Na in the previous work established by Yang et al. (63). In another development, Simunicova et al. (68) obtained the *N* value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions and K has the highest *N* value about 380,400. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Therefore, the method that have been developed for CE-Conductivity detection need a further development to improves the peak efficiency for quantitative analysis.

7.4. Conclusion

It has been shown that partial complexation plays a useful role in the separation of some metals, e.g. iron. Lactic acid in conjunction with LiOH was found to be a useful combination for the separation of metal cations using conductivity detection. This method was suitable for the investigation of metal cations in acid mine discharges.

The use of lactic acid as a complexing reagent to separate iron from manganese for both CE-UV/Vis and CE-conductivity detection was investigated. The combination of 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25 was employed to get a separation of 13 metal cations in less than 6 min using CE-UV/Vis detection. The presence of 18-crown-6 with lactic acid changed the migration order of metal cations in group I and group II and transition metals. The above buffer conditions were employed to get a full separation, including iron, from manganese in acid mine discharges in less than 4 min. In conductivity detection, the separation of 6 metal cations include iron was obtained by the use of 20 mmol/L lactic acid, 15 mmol/L LiOH and 10% methanol at pH 4.3. The separation was completed in less than 6 min.

As can be seen from comparison results between CE-UV/Vis detection and CE-Conductivity detection also clearly proved that the above methods are able to used for the quantitative and qualitative analysis. However further investigation should be carried for CE-Conductivity detection before quantitative work. Several approaches may be used include internal standard calibration to improves the detection methods.

CHAPTER 8

CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

8.1. Reported developments in CE separations since the work was completed

Much of the literature reviewed in this section will focus on the development of capillary electrophoresis methods for the separation of metal cations since the work reported in this thesis, which was completed in 1999.

At present most of the successful separations of metal cations have involved the use of complexation with macrocyclic ligands. The complex formation technique uses chemical derivatisation procedures in which the metal cations are converted into anionic complexes and separated together with the common inorganic anions. There are two main approaches for accomplishing the derivatization of metal cations with a particular ligand: on-capillary complexation and pre-capillary complexation. These two techniques already discussed in Chapter 2 of the thesis. The separation and detection of metal ions in the form of precapillary-formed metal complexes has become an accepted practice in capillary zone electrophoresis (171,172). An overview of the latest advances in CZE as applied to metal ion analysis (173) claims that pre-separation derivatization procedures still hold great promise for multi-element separations. Furthermore, selecting metal-ligand complexes that govern speciation of a metal in environmental and biological systems is becoming an important area of CZE research (174). Whilst a number of modelling approaches and mathematical treatments involving partial on-

capillary complex-formation have been reported (175), the migration behavior of metal complexes still lacks quantitative interpretation. As an example, metal complexes of this type are limited to transition metal chelates formed by bidentate neutral ligands and their derivatives (176). For example, coordination of acetate ions to $[Cu(bpy)_2]^{2+}$ and $[Cu(phen)_2]^{2+}$ was proven to occur in acetate buffer electrolytes. Other approaches involved include on-capillary complexation, where 2,6-pyridinedicarboxylic acid (PDC) (177) and ion-pair interactions, in particular the effect of certain ion pairing additives, change the migration behavior of metal complexes of 4-(2-pyridylazo)resorcinol (PAR) (178). In another applications, the use of Alizarine complexon demonstrated a poor regression derived for metal chelates (179). Some of the metal complexes are less stable, being prone to dissociation during the separation, but may be stabilized using a combination of on-capillary and pre-capillary complexation in which the complex is formed prior to injection but a small amount of ligand is also added to the carrier electrolyte [10]. A more recent study by Kuban et al. (180) has demonstrated the separation of 19 common anions and metal-EDTA chelates in about 6 min. using chromate electrolyte. However, this chromophore is more suited for the analysis of highly mobile inorganic anions. The peak shapes of less mobile metal chelates are broad and poorly shaped. In general, the main advantage of indirect UV detection is universal application and thus it is very useful for the multi-elemental determination of major sample constituents. Detection limits attained with indirect UV detection, however, are often only at ppm levels (unless on-capillary pre-concentration techniques are applied), i.e. about 1-2 orders of magnitude higher than when direct UV detection is used. On-capillary sample stacking is one of the simplest ways to improve detectability in CE of ionic analytes (181). Fung and Lau (182) have developed a new analytical method based on CZE using the highly sensitive 1,10-Phenanthroline

reagent for direct UV detection of Zn, Mn, Cu, Co, Cd, and Fe at ppb concentrations in water samples after pre-column formation of positively charged chelates with 1,10-Phenanthroline. However the ligand purity plays an important role in separations of cations (183). The ligand and the other reagents should be free of metal ions and other substances that could possibly form more stable complexes with analytes.

The approaches using partial complexation technique usually involves the addition of a weak complexing ligand to the carrier electrolyte with subsequent partial complexation of sample cations within the capillary. UV-absorbers from aromatic bases such as histamine, 4-aminopyridine, imidazole, creatinine, 4-methylbenzylamine and 4-methylaminophenol were used to obtain the metal separation by indirect UV/Vis detection techniques. Timerbaev et al. (184) employed cationic chromophores in acidic media (pH <6) due to their deprotonation or low solubility at high pH. In another investigation, Boyce (185) used organic solvents such as methanol in the buffer solution to improve the separation selectivity of metal cations. A more recent study by Juang and Wu (186) has demonstrated the separation of alkali and alkaline earth metals in 5 mmol/L 4-aminopyridine, 5%v/v methanol with indirect UV detection. The pH was adjusted to be 4.3 by adding 1mol/L glycolic acid. Some physico-chemical parameters were optimised to achieved the quantitation of alkali and alkaline earth metals cations by Cahours et al. (187). The present of Na, K, Mg and Ca were determined in seawater and formation water using 4-methylbenzylamine as buffer and α-hydroxyisobutyric acid as a complexing reagent by Tangen et al. (188). New approaches for the simultaneous separation of inorganic anions and metal cations are described by Padarauskas et al. (189) and Kuban et al. (190). Shakulashvili et al. (191) employed immidizole, 4-methylbenzylamine, 4-aminopyridine as UV-absorbing

compound and α-hydroxyisobutiric acid (HIBA) as complexing reagent (pH 4.5) to get simultaneous determination of alkali, alkaline earth and transition metal ions by indirect UV detection. In this investigation the separation of 16 metal cations were achieved with detection limits between 92 ppb for Ca and 454 ppb for Cu with hydrostatic injection. Tarja et al. (192) demonstrated that cations were separated at pH 3.6 after formation of anions using pyridine, glycolic acid and 18-crown-6-ether solution in environmental water samples via indirect-UV detection. In these analyses, NH_4^+ migrated first followed by K, Ca, Na and Mg at concentration levels of 0.5-250 ppm.

Few reports concerning method development for metal cations analysed by conductivity detection since this work have been published. The reason may be the same problems encountered in the analysis of metal cations using conductivity detection during this investigation. High frequency (contactless conductivity) detection has been suggested for analysis of Rb, K, Na, Ca, Mg, Mn, Cd and Li cations, with a detection limit of about 1.10^{-4} mol/L (193). In this investigation, a buffer electrolyte of morpholinoethane sulfonic acid (MES) and histidine was employed to obtain the above separation. More recently, a capacitively coupled contactless conductivity detector (called a C4D) for CE was introduced by Zemann et al. (193,194). The detector consists of two metal tube electrodes which are placed around the outer polyimide coating of the capillary with a 1-mm detection gap between the electrodes. When a high audio or low oscillation frequency between 40 and 100 kHz is applied to one of the electrodes, a signal is produced when an analyte zone with a different conductivity passes through the retention gap. An amplifier and rectifier is connected to the other electrode and the signal is further processed. A thin piece of copper foil is placed perpendicularly between the two electrodes to prevent a capacitive transition between

that would bypass the detection gap and increase the background noise level. Good separations of both anions and cations were obtained with the C4D electrode with limits of detection in the low ppb concentration range. Linear calibration curves were obtained over four orders of magnitude from – 0.1 to 1000 ppm. This investigation reported separation of eight metal cations include Rb, NH_4^+, K, Ca, Na, Mg, Mn and Li with a crown ether added to achieve resolution of the ammonium and potassium peaks. Organic ions with lower conductivities could be detected by indirect conductivity.

8.2. Conclusion

Initial work centred on the use of indirect UV/Vis detection and a method was developed that gave reasonable results using mixtures of standard solutions. An alternative method of detection for metal cations via indirect conductivity measurements was then used. In this relation, optimisation of the background electrolyte was required to enable the simultaneous separation of the metal cations especially transition metals. The presence of a complexing reagent was found to be necessary and the type and concentration of such species were investigated. The effect of other factors such as pH, ionic strength and viscosity were studied. An electrolyte system that allowed the separation and subsequent detection by UV/Vis and conductivity was developed. We undertook to examine the practicality of employing CE to investigate the presence of metal cations in acid mine discharges. The application of CE to separation of certain metal cations e.g. K, Na, Ca, Mg, Fe, Mn presence in acid mine discharges was demonstrated. It has been shown that capillary electrophoretic separations of metal cations in group I, group II and transition metals were feasible using a UV/Vis detector. Separation of metal cations in group I and II was observed without forming metal complexes using UV/Vis detection. However,

electrophoretic mobilities of many metal cations are similar and this led to a number of strategies for optimising separation selectivity. Partial complexation of metal ions has been reported (195) to be the most straightforward of these approaches. Among those complexing reagents employed to date, HIBA (22-24,26,28-30,54-64) and lactic acid (29,30,58-62,65,66) or citric acid (23,58) followed by tartaric (29,58,65,68) and oxalic acids (58,67) appear to offer the most satisfactory separations. Most of these complexing reagents enable a complete separation to be obtained in a very short time and with high selectivity. The fastest separation so far reported was of 27 metal ions (including alkali, alkaline earths, transition and lanthanide metals) in only 6 min. This was achieved by employing 15 mmol/L lactic acid, 8 mmol/L 4-methylbenzylamine and 5% methanol at pH 4.25 (65). In a later development, Chen and Cassidy (28) obtained a good separation of 26 metal ions in 10 minutes employing 4.2 mmol/L HIBA, 6 mmol/L *N,N*-dimethylbenzylamine, 0.2 mmol/L Triton X-100 at pH 5.0. Most of the metals separated under this investigation included alkali, alkaline earth, transition metals and lanthanides. Other complexing reagents used have been phthalic acid (29,58) or acetic acid (26,58,67,70) in the separation of alkali and alkaline earth and transition metals. For this work, partial complexation with an organic acid (e.g. oxalic acid, acetic acid and lactic acid) and neutral compound (acac) with the present of other organic modifier in the electrolyte was required for the separation of transition metals. The employing of acetic acid, oxalic acid and acac allow the separation of group I and group II metal cations and transition metals in a single run, however, separation of iron did not prove possible with this buffer. Most work with oxalic acid and acetic acid as complexing reagents by Lee et al. (58) separated alkali and alkaline earth metal ions using indirect UV detection. They employed imidazole as a UV-absorber. The use of acac was reported by Saitoh et al. (92) in micellar electrokinetic

chromatography techniques for the separation of Cr (III), Co (III), Ru (III) and Pt (II) (or Pa (II). The effects of oxalic acid, acac and acetic acid on the separation of both metal cations in group I and group II and transition metals were demonstrated and observed in this investigation. It shows that the incorporation of these complexing reagents in conjunction with 4-methylbenzylamine will increase the differences in electrophoretic mobilities of transition metals.

Development of separations for metal cations using CE methods were achieved and successfully demonstrated in-conjunction with UV/Vis and conductivity detection method. A new analytical procedure of conductivity detection using CE with the buffer conditions of 20 mol/L lactic acid, 15 mmol/L LiOH, 10% MeOH at pH 4.3 was developed. These metals (K, Ba, Na, Ca, Mg, Mn, Cd, Cr, Fe, Co, Ni, Zn and Cu) were separated in less than 8.5 min. The use of lactic acid and LiOH with conductivity detection has been shown to be successful for metal separations and feasible for the investigation of acid mine discharges. However, further development need to be carried out to improve the precision and accuracy of results in quantitative analysis. Good sensitivity and satisfactory working ranges, baseline separation, peak efficiencies for K, Na, Ca, Mg, Mn and Fe in acid mine discharged were observed. The combination of lactic acid and LiOH using conductivity detection was relatively good compared with the previous work for qualitative analysis of metal cations. This finding was an agreement with Haber et al. (67) who determined alkali, alkaline-earth and some organic amines using a combination of L-Histidine/MES and 18-Crown-6 to separate 17 cations in less than 4.5 min. via direct conductivity detection. Haber et al. (67) also demonstrated the separation of alkali, alkaline earth and transition metals using indirect conductivity detection. They used a low concentration of acetic acid in conjunction with oxalic acid to obtain a separation of 16 metal cations in less than 7 min.

Meanwhile, the employing of 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 10% methanol at pH 4.25 for the separation of all metal cations present in acid mine discharges by CE-UV/Vis detection was excellent. The results obtained for K, Na, Ca, Mg and Mn were acceptable for determination of metal cations as compared to ICP-OES ($F_{obs} < F_{crit.}$) except for Fe. The buffer conditions employed is relatively good in term of sensitivity, satisfactory working ranges, baseline separation, peak efficiencies for K, Na, Ca, Mg, Mn and Fe that presence in acid mine discharges.

Chapter 4

The separation of metal cations in mineral water by CE is quite straight forward compared to ion chromatograph (IC). Comparison of the method or results obtained were subjected to statistical analysis of variance (ANOVA) to determine variability between batch samples. In CE, reproducibility was demonstrated ($F_{obs} < F_{crit.}$) for each metal cations at 95% confidence level. Good reproducibility for migration times (SD 0.05-0.09, n = 10; F_{obs}, 0.94-1.11 < $F_{crit.}$, 3.93), absorbance (SD 0.02-0.10, n=10; F_{obs}, 0.84-2.56 < $F_{crit.}$, 3.93), limit of detection was good and sensitive between 0.09 to 0.15 ppm, satisfactory working ranges (1-100 ppm, R^2 = 0.9956 to 0.998) and baseline separation were obtained for K, Na, Ca and Mg. Efficiencies for CE were excellent (204,723-343,153) theoretical plates for Na, K, Mg and Ca as compared to IC. Good calibration curve were plotted for determination of mineral water with working ranges (1-20 ppm, , R^2 = 0.9901 to 0.993). All metal cations were separated in less than 6.5 minutes using 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0 using CE-UV/Vis detection. The migration order follows the sequence K, Na, Ca and Mg. All peaks are completely baseline resolved. The peaks

become more symmetric with increasing concentration. These values obtained for the overall migration time is consistent with the results of Weston et al. (23) in the analysis of group I and group II metal cations for commercial cough syrup using the buffer solution of 5mmol/L UV-Cat1, 6.5 mmol/L HIBA at pH 4.2. They reported the overall migration time in less 5 min to get a separation of K, Ca, Na and Mg compared to less than 6.5 min in the analysis of a Volvic mineral water. They obtained a good peak shape with baseline resolution between the majority of the peaks. Another excellent result was obtained by Weston et al. (64) on the analysis of a standard solution of K, Na, Ca and Mg using 5 mmol/L UV Cat-2 and tropolone at pH 4. They reported the overall migration time in less than 3 min to get a separation of K, Ba, Sr, Ca, Na, Mg and Li. The mobility of UV Cat-2 is similar to that Na, resulting in an almost symmetrical peak shape for Na. The metals migrating faster than Na show a little degree of fronting, while the metals migrating slower than sodium show a little tailing. Under these conditions of more closely matched mobilities, the concentration of the analytes may even approach the concentration of the background electrolyte without electromigration dispersion producing a significant effect. Meanwhile Lin et al. (58) reported the migration time in less than 2 min for K, Ba, Ca, Na, Mg and Li. They used acetic, glycolic, lactic, HIBA, oxalic malonic, malic, tartaric, succinic and citric acid as a complexing reagent for the separation six alkali and alkaline erath metal cations. They found that, if the ions that co-migrate closer to the carrier imidazole have better *N* values that ranging from 16, 000/m to 760, 000/m. Thus the fronting K and the trailing Li both have poorer *N* values than the other ions and are affected less by the type of acid present. The *N* values for Ca and Mg varied most significantly; they are particularly low in oxalic acid and citric acid, in which the stability constants of the complexing reagent with these two ions is high. In citric acid, Na has the best *N*, but all

other divalent ions have the poorest *N*. In glycolic acid, Mg has the highest *N* of all, reaching 760, 000/m.

In IC, reproducibility was demonstrated ($F_{obs} < F_{crit.}$) for each metal cations at 95% confidence level. Good reproducibility for retention times (SD 0.01-0.04, n = 10; F_{obs}, 0.28-1.08 < $F_{crit.}$, 3.93), conductivity (SD 0.02 for all metals, n=10; F_{obs}, 0.61-1.72 < $F_{crit.}$, 3.93), limit of detection at sub-ppm and less sensitive between 0.10 to 0.30 ppm, satisfactory working ranges (1-80 ppm, $R^2 = 0.9953$ to 0.9978) and baseline separation were obtained for K, Na, Ca and Mg. Efficiencies for CE were excellent (144,786-299,301) theoretical plates for Na, K, Mg and Ca. All metals were separated in less than 11 minutes using 60 mmol/L HCl/6 mmol/L DAP.HCl with conductivity detection. As can be seen from the chromatogram obtained shows that by using the high buffer concentration gave a faster separation compared to the lower concentration (at 40 mmol/L HCl/2 mmol/L DAP.HCl peak came out after 38 minutes. The peaks become more symmetric with increasing concentration. This result is in agreement with the finding reported by Thienpont et al. (199) that the eluent concentration used will affects the separation of metal cations. Faster separation elution could have been achieved by increasing the strength of the mobile phase. They employed 2 mmol/L DAP.HCl and 40 mmol/L HCl to get a separation of calcium and magnesium in less than 9.4 min with IC system via conductivity detection. The retention times is this work considerably lower than with a conventional column combination using the same eluents (approximately 19 min for calcium and 16 min for magnesium) another experiment by manufacturer (201). This observation supports the results obtained by Morawski et al. (196) for the analysis of cationic nutrients in foods by IC system. They used 0.1 mmol/L EDTA and 3 mmol/L nitric acid with

conductivity detection to analyse cations from various sample matrices such as pretzels (salted), parsley (dried), bread crumbs, parmesan cheese and peanut butter. All metals cations including Na, K, Mg and Ca were eluted after 20 min. The resolution for each metals is no so good and baseline resolved). This finding also demonstrated that the IC method used in this work is suitable for quantitative analysis and comparably good with previous works on IC system.

Chapter 5

The combination of 20 mmol/L acetic acid, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 3.5) was enabled a separation of 14 metals (K, Ba, Na, Ca, Mg, Mn, Co, Ni, Zn, Cr, Cd, Al, Cu and Pb) in less than 7 min. However, separation of Fe did not prove possible with this buffer. Comparison of the method or results obtained were subjected to statistical analysis of variance (ANOVA) to determine variability between batch samples. Reproducibility was demonstrated ($F_{obs} < F_{crit.}$) for each metal cations at 95% confidence level. Good reproducibility for migration times (SD 0.07-0.19, n = 10; F_{obs}, 0.01-1.41 < $F_{crit.}$, 3.93), absorbance (SD 0.03-0.18, n = 10; F_{obs}, 0.01-1.16 < $F_{crit.}$, 3.93) and peak area (SD 0.008-0.02, n = 10; F_{obs}, 0.01-1.08 < $F_{crit.}$, 3.93), limit of detection was at sub-ppm and less sensitive between 0.12 to 0.94 ppm, satisfactory working ranges (1-40 ppm, R^2 = 0.9859 to 0.9977 except for Mn), baseline separation, peak efficiencies (204,494-609,667 theoretical plates) were obtained for Na, Ca, Mn, Co, Ni, Zn, Cr, Al, Cu and Pb. It was demonstrated that Ni has the highest number of theoretical plates (N), about 609,667 followed by Na, Ca, Mn, Zn, Cr, Al, Co, and Pb because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as acceptable to Cu has the lowest N value, about 204,494. The efficiencies obtained were excellent as compared to N value between 12,000 to 300,00

for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the *N* value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of *N* between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. The result obtained shows that the peak efficiencies were excellent and may be suitable for quantitative analysis.

A complete separation of 8 metal cations (K, Na, Ca, Mg, Co, Ni, Cd and Pb) was achieved in less than 8.5 min using 10 mmol/L acac, 10 mmol/L 4-methylbenzylamine and 50% methanol (pH 4.0). Good reproducibility for migration times (SD 0.10-0.23, n = 10; F_{obs}, 0.93-1.89 < $F_{crit.}$, 3.93), absorbance (SD 0.01-0.25, n = 10; F_{obs}, 0.01-1.80 < $F_{crit.}$, 3.93) and peak area (SD 0.002-0.05, n = 10; F_{obs}, 0.01-2.28 < $F_{crit.}$, 3.93). Sensitive detection limits (0.17-0.76 ppm), not satisfactory working ranges (1-25 ppm, the correlation coefficients, $R^2 < 0.95$), baseline separation, peak efficiencies (178,163-223,749 theoretical plates) were obtained for K, Na, Ca, Mg, Co, Ni, Cd and Pb. K has the highest number of theoretical plates (*N*), about 223,749 followed by Cd, Na, Ca, Ni, Co, and Mg meanwhile, Pb has the lowest *N* value, about 178,163. This values obtained was good as compared to *N* value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the *N* value about between 46,400 to 325,

600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of *N* between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. The result obtained shows that the method used is may not possible for quantitative analysis.

Separation of metal cations (K, Na, Ca, Mg and Cd) by oxalic acid not as good as other complexing reagent. Good reproducibility for migration times (SD 0.03-0.29, n = 10; F_{obs}, 0.92-2.63 < $F_{crit.}$, 3.93), absorbance (SD 0.09-0.18, n = 10; F_{obs}, 1.02-2.37 < $F_{crit.}$, 3.93) and peak area (SD 0.01-0.10, n = 10; F_{obs}, 0.90-2.22 < $F_{crit.}$, 3.93), sensitive detection limits (0.1-0.5 ppm), not satisfactory working ranges (0-1 ppm, the correlation coefficients, $R^2 < 0.96$) were obtained. In the previous work by Shi and Fritz (29) reported a good linear calibration curves were obtained for the metal cations in the 0.4 to 10 ppm concentration range. They used the buffer solution of 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3 for the separation of eight metal cations include K, Na, Pb, Mn, Co, Ni, Zn and Cd. However they obtain a relatively poor correlation coefficient of linear regression for zinc. Meanwhile, Shi and Fritz (30) obtained a linear calibration curves for the metal cations from 0.1 to 10 ppm for the separation of Sr, Mg, Mn, Co and Zn. They used 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 0.6 mmol/L 18-crown-6, 10% methanol at pH 4.3. In another development, Yang et al. (63) reported the linear calibration curves up to 20 ppm was obtained for the separation of K, Na, Ca, Mg and Mn. They also found that at high concentrations peak distortion occurred owing to overloading, which caused

insufficient selectivity of separation, so that the calibrations were no longer useful. At pH 4.5 the calibration line for manganese was linear only 6 ppm owing to the insufficient separation between manganese and magnesium. Recently, N. Shakulashvili et al. (191) reported a good linear calibration curves from 0.75 to 80 ppm for the separation of sixteen metal cations using 10 mol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5. For this work, peak efficiencies between 52,341-265,047 theoretical plates were obtained for K, Na, Ca, Mg and Cd. As can be seen that Na has the highest number of theoretical plates (*N*), about 265,047 followed by K, Ca, Mg and Cd compared to Cd has the lowest *N* value, about 52,341. This values obtained was acceptable as compared to *N* value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the *N* value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of *N* between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. In general, the result obtained shows that the method used is may not possible for quantitative analysis as compared to the previous works.

Chapter 6

An excellent separation of 13 metals (K, Ba, Na, Ca, Mg, Mn, Cd, Cr, Fe, Co, Ni, Zn and Cu) was achieved in less than 8.5 min using the buffer conditions of 20 mol/L lactic acid, 15 mmol/L LiOH, 10% MeOH (pH 4.3) by conductivity detection.

Comparison of the method or results obtained were subjected to statistical analysis of variance (ANOVA) to determine variability between batch samples. Reproducibility was demonstrated ($F_{obs} < F_{crit.}$) for each metal cations at 95% confidence level. Good reproducibility for migration times (standard deviation, SD 0.01-0.05, n = 10, F_{obs}, 0.90-1.8 < $F_{crit.}$, 3.93), conductivity (SD 0.03-0.23, n = 10; F_{obs}, 0.90-1.26 < $F_{crit.}$, 3.93) and peak area (SD 0.06-0.47, n = 10; F_{obs}, 0.90-2.09 < $F_{crit.}$, 3.93), sensitive detection limits (0.4-2.9 ppm), satisfactory working ranges (1-100 ppm, R^2 = 0.9905 to 0.9960) were obtained for K, Na, Ca, Mg, Mn and Fe. The overall linearity of ten metals from group I, group II and transition metals that are found to be up to 100 ppm in this work are excellent compared to the previous work as discussed below. Shi and Fritz (29) reported a good linear calibration curves were obtained for the metal cation studies in the 0.4 ppm to 10 ppm concentration range. They used the buffer solution of 2 mmol/L phthalic acid, UV-Cat1, 20% methanol at pH 3.3 for the separation of eight metal cations include K, Na, Pb, Mn, Co, Ni, Zn and Cd. However they obtain a relatively poor correlation coefficient of linear regression for Zn. Meanwhile, Shi and Fritz (30) obtained a linear calibration curves for the metal cations from 0.1 to 10 ppm for the separation of Sr, Mg, Mn, Co and Zn. They used 15 mmol/L lactic acid, 10 mmol/L 4-methylbenzylamine, 0.6 mmol/L 18-crown-6, 10% methanol at pH 4.3. In another development, Yang et al. (63) reported the linear calibration curves up to 20 ppm was obtained for the separation of K, Na, Ca, Mg and Mn. They also found that at high concentrations peak distortion occurred owing to overloading, which caused insufficient selectivity of separation, so that the calibrations were no longer useful. At pH 4.5 the calibration line for manganese was linear only 6 ppm owing to the insufficient separation between manganese and magnesium. Recently, N. Shakulashvili et al. (191) reported a good linear calibration curves from 0.75 to 80 ppm for the

separation of sixteen metal cations using 10 mol/L 4-aminopyridine, 6.5 mmol/L HIBA at pH 4.5.

For this work, peak efficiencies were obtained between 311,782 to 770,735 theoretical plates for K, Na, Ca, Mg, Mn and Fe. Mg has the highest number of theoretical plates (*N*), about 770,735 followed by Ca, Na, Fe and K because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as acceptable to Mn has the lowest *N* value, about 311,782. This values obtained was excellent as compared to *N* value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the *N* value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of *N* between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr.

Chapter 7

Lactic acid was found as an excellent complexing reagent for the separation metal cations in acid mine discharges by CE-UV/Vis detection. Comparison of the method obtained in this work were subjected to statistical analysis of variance (ANOVA) to determine variability between batch samples. Reproducibility was demonstrated ($F_{obs} < F_{crit.}$) for each metal cations at 95% confidence level. The results obtained for metal cations were acceptable as compared to ICP-OES (SD 2.1-54.4; F_{obs} 0.21-2.24 < $F_{crit.}$,

3.93 but for Fe, F_{obs} 3.47 > F_{crit}. but K was not detected). The calibration curves plotted were acceptable (1-20 ppm, R^2 = 0.9907 to 0.9928) for determination of K, Na, Ca, Mg, Mn and Fe. It was demonstrated that K, Na, and Fe are very sensitive relatively as compared to Mg (0 – 25 ppm) and Mn but Ca is less sensitive at range between 0 to 20 ppm. Low concentration sensitivity in absorbance-based detectors in CE may be due to a number of factors. First, from Lambert-Beer law (see Equation 7.1) the absorbance is proportional to path length and the path length in CE for the detection window is very short. This path length relies on the internal diameter of the capillary column (usually between 50 - 100 μm). Moring et al. (110) employed a *z-cell* with a 3 mm capillary section of path length and obtained a tenfold increase in sensitivity. In another development Tsuda et al. (111) used *rectangular* capillaries of 1000 μm path length with an increase of about 20-fold in sensitivity. Later Wang et al. (112) used a *multireflection cell* to enable an increase of 40-fold in peak height. The path length also can be increased using a *bubble cell* as demonstrated by Heiger (202) when he obtained a 3-fold increase in peak height. For this work, peak efficiencies (114,719-774,365 theoretical plates) were obtained for K, Na, Ca, Mg, Mn and Fe. Mn has the highest number of theoretical plates (N), about 774,365 followed by Mg, Ca and Na because its electrophoretic mobility is close to that of ionic 4-methyilbenzylamaine as compared to Fe has the lowest N value, about 114,719. This values obtained was excellent as compared to N value between 12,000 to 300,00 for K, Na, Ca, Mg, K and Mn in the work established by Yang et al. (63). They employed the buffer of 5 mmol/L imidazole and sulphuric acid for the above separation. In another development, Simunicova et al. (68) obtained the N value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions. Meanwhile, Stathakis (79)

was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Shakulashvili (191) obtained the highest number of *N* between 65,000 to 651, 000 for K, Na, Ba, Sr, Ca, Mg, Mn, Li, Fe, Co, Cd, Ni, Zn, Pb and Cr. The result obtained shows that the peak efficiencies for each metal cations are suitable for qualitative analysis but further development required for quantitative analysis.

In CE-Conductivity detection, lactic acid in conjunction with LiOH was found to be a useful combination for the separation of six metal cations in acid mine discharges with CE-conductivity detection. Further development need to be carried out for improving the resolution and accuracy of result in quantitative analysis. The results obtained for metal cations were not as good as compared to ICP-OES (SD 2.1-66.8; F_{obs} 0.41-2.19 < $F_{crit.}$, 3.93 but Fe was not detected.). The calibration curves plotted were acceptable (1-20 ppm, R^2 = 0.9907 to 0.9928) for determination of K, Na, Ca, Mg, Mn and Fe. The calibration graph for all metals are less sensitive at range between 0 to 20 ppm. Further analysis may be employed to improve the sensitivity using different approaches. Another option to enhance sensitivity in sample concentration is by Isotachophoresis (ITP). It is performed by sandwiching a sample between a leading and terminating, or trailing, buffer and by applying an electric field in the constant current mode. This method is unable to obtain the separation of anions and cations in a single run. The method employed can enhance sensitivity by up to 3 orders of magnitude (usually between 100-1000 enhancement). This method was carried out by Stegehius et al. (123) to obtain a sensitivity enhancement of the order of 100 times in the separation of fluorescein isothiocyanate-amino acids by ITP-CE. Meanwhile, ITP conditions were employed for the investigation of anions by Haber et al. (67). They

obtained the detection limits of 100 ppt using a conductivity detector. For this work, peak efficiencies between 83,163 to 101,909 theoretical plates were obtained for K, Na, Ca, Mg, Mn and Fe. Meanwhile, K has the highest number of theoretical plates (*N*), about 101,909 followed by Ca, Mg and Na Mn has the lowest *N* value, about 83,163. This values obtained was low compared to *N* value about 300,00 for Na in the previous work established by Yang et al. (63). In another development, Simunicova et al. (68) obtained the *N* value about between 46,400 to 325, 600 for Cs, NH_4^+, Rb, Na, Mg, Ca, Li, Sr, Ba and K using 5 mmol/L tartrate, benzimidazole and 18-crown-6 in their buffer conditions and K has the highest *N* value about 380,400. Meanwhile, Stathakis (79) was reported the efficiencies for Li, K, Mg, Ba, Zn, Pb, La, Sm, Eu, and Dy between 120 000 – 260 000 theoretical plate. Therefore, the method that have been developed for CE-Conductivity detection need a further development to improves the peak efficiency for quantitative analysis.

8.3. Suggestions for further work

This study clearly demonstrated that the reliable quantitative analysis of metal cations in group I, group II and transition metals is not possible via CE -UV/Vis and CE-conductivity detection. The method that have been developed using CE-UV/Vis and CE-conductivity detection should be re-evaluated for the possibility of quantitative analysis. The main weakness observed was the reproducibility of the metal cations, with poor limit and very high standard deviation. These were relatively poor when compared to other analytical techniques for quantitative analysis i.e ICP-OES. The methods used for CE separation are not suitable for quantitative analysis unless further work is carried out to obtain a resolution of metal cations at low concentrations (sub ppb level). The problems encountered in this studies may perhaps be overcome with a

different type of CE. Optimisation of pH, UV-absorber (4-methylbenzylamine), viscosity (e.g. methanol) and the concentration of a complexing reagent could result in better reproducibility. 4-methylbenzylamine was found very difficult to dissolve in water through out the investigations, which may affect the preparation of buffer composition for the determination of metal cations. Commercial UV-absorber may be purchased to overcome this problem. Over use of acetic acid for pH adjustment also may affect the reproducibility of metal cation determination.

The limit of detection observed in CE was primarily poor due to limited path length of capillary diameter and volume of a sample loading into the CE column (few nL) as compared to other analytical methods i.e. HPLC, AAS and ICP-OES. Stacking methods can be employed to increase the limit of detection of metal cations via UV/Vis detection, as discussed in Section 2.3.4.2. Isotachoporesis methods (ITP) also can be employed by sandwiching a sample between a leading and terminating, or trailing, and by applying and electric field in the constant current mode. This approach was successfully demonstrated using conductivity detection in the previous works (170) whereby the detection limit could be further lowered by a factor of 1000 or more (below 50 ppt).

A further investigation also may be carried using the separation of metal cations by CE with mass spectrometer (MS) detection. This techniques will improve the limit of detection and will increase the resolution of metal cations in acid mine discharges.

ANNEXES

Annex 1: References

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Annex 2: List of abbreviations and acronyms

AU	Absorbance unit
acac	acetylacetone
a	absorptivity
AAS	Atomic absorption spectroscopy
A^-	Sample ion
A	Independent of the flow velocity and characterises peak dispersion caused the Eddy diffusion
$[A^-]_{stat,.mob}$	The concentration of the sample ion in the mobile phase (mob.) and stationary phase (stat.)
ANOVA	Statistical analysis of variance
α	Selectivity
B	The longitudinal diffusion
b	The path length
C_i	The concentration of ions in equivalents/L
c	Sample concentration
CE	High performance capillary electrophoresis
CZE	Capillary zone electrophoresis
CITP	Capillary isotachophoresis
CGE	Capillary gel electrophoresis
CIEF	Capillary isoelectric focusing
CEC	Capillary electrochromatography
CIA	Capillary ion electrophoresis
CDTA	1,2-cyclohexanediaminetetracetic acid
CTAB	Cetyltrimethylammonium bromide
C	Comprises the resistance to mass-transfer between mobile and stationary phases
D_m	Diffusion coefficient of the solute
DAP	2,3-diaminopropionic acid
DAP.HCl	D,L-2,3-diaminopropionic acid monohydrochloride
Δv	The velocity different between two solutes
Δv_{AVE}	Average velocity
ΔH	The different in heights of the liquid in the sample and destination vial
ΔP	The pressure across the capillary
e	The charge per unit surface area
E	The applied electric field in unit $voltscm^{-1}$

E-	The eluent counter ion
$[E^-]_{stat,.mob}$	Eluent anion (counter ion) concentration in the mobile phase (mob.) and stationary phase (stat.)
EOF	Electroosmotic flow
EDTA	ethylenediamine tetraacetic acid
ε	Molar absorptivity in units of $L.mol^{-1}.cm^{-1}$
ε	The dielectric constant
UV	Ultraviolet
G	Gravitional constant
HETP	High equivalent to a theoretical plate
H	The plate height
HQS	8-hydroxyquinoline-5-sulfonic acid
HIBA	α-hydroxy isobutyric acid
HEC	hyrdroxyethylcellulose
η	The viscosity of the buffer
HIS	L-Histidine
HPLC	High performance liquid chromatography
HMB MES	2-[N-morpholino]-ethane sulfonic acid
HCl	hydrochloric acid
ICP	Inductively coupled plasma
IC	Ion chromatography
ID	Internal diameter
ICP-OES	Inductively coupled plasma optical emission spectroscopy
I_{in}	The intensity of light from the original light source
I_{out}	The intensity of light after passing through the capillary window
I	The current
κ	Conductivity of an ion in a dilute solution in S/cm
kV	kilovolt
K_A	Different equilibrium constant
λ_i^0	The ionic limiting equivalent conductivity
LOD	Limit of detection
l	The effective capillary length
L_p	The length of sample plug
L	The total capillary length
MT	Migration time
MEKC	Micellar electrokinetic chromatography
MS	Mass spectrometer
μ_{EOF}	The electroosmotic mobility

μ_{EP}	Electrophoretic mobility
μ_1 and μ_2	The electrophoretic mobilities of the solutes
μ_{AVE}	Average of the electrophoretic mobility
N	The number of theoretical plates
OD	Outside diameter
PEG	Polyethylene glycols
PAR	4-(2-pyridylszo)resorcinol
pK_a	pH at which concentrations of acid and base forms are equal
ppm	part per millions
ppb	Part per billions
ppt	Part per trillions
PDC	2,6-pyridine dicarboxylic acid
q	the analyte charge
Q_{inj}	Quantity injected
r	The analyte radius or the capillary inner diameter
R_s	Resolution RT Retention time
R^2	Correlation coefficients
R	The resistance
RSD	Relative standard deviation
ρ	The density of the buffer in the capillary
σ	The temporal the standard deviation subscript A and B refer to the two solutes
SD	Standard deviation
SDS	Sodium dodecylsulfate
S	Conductivity in unit of Siemens
t	The time duration
t_m	The migration time
TTAB	tetradecyltrimethyl ammonium bromide
TAPS	[N-tris(hydroxymethyl) methyl-3-aminopropane sulfonic acid]
TBAOH	tetrabutylammonium hydroxide
T	Transmittance
u	The overall migration rate of the mobile phase
V	Applied voltage
V_i	Volume of sample injected
ν_{OBS}	The observed electrophoretic velocity
ν_{eof}	The velocity of EOF
ν_{EP}	Electrophoretic velocity
w	The baseline peakwidth

$w_{1/2}$	Peak width at half-height
ζ	Zeta potential
δ	The thickness of the diffuse double layer

Annex 3: Example calculation for statistical analysis

A 3.1 Comparison of several means

This method is used in several applications in the thesis for the calculation of significant test because different batches of samples are measured. In all cases, 10 batch samples at different time duration are measured and given slight variations from one batch to another. Due to this reason analysis of variance (ANOVA) is used to separate and estimate the different causes of variation. For the particular examples shows in the thesis, it can be used to separate any variation which is caused by changing the controlled factor, from the variation due to random error. It can thus test whether altering the controlled factor leads to a significant difference between the mean values obtained. Reproducibility data (refer to Table 4.4, Chapter 4) on the migration time for metal cations i.e. potassium at different batches samples using 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0. This table shows the results obtained in an investigation into the stability of a mixtures standard solution stored under different sectional period and conditions. The values given are the reproducibility of migration time (in minute) from each sample at 10 ppm concentration. Three replicate measurements were made on each samples.

Sample at different period (every 0.5 hrs.)	Replicate measurements			Mean
	Duplicate 1, MT (min.)	Duplicate 2, MT (min.)	Duplicate 3, MT (min.)	
SPL 1	3.62	3.61	3.72	3.650
SPL 2	3.61	3.66	3.70	3.66
SPL 3	3.59	3.62	3.69	3.63
SPL 4	3.62	3.65	3.70	3.66
SPL 5	3.61	3.65	3.72	3.66
SPL 6	3.63	3.67	3.70	3.67
SPL 7	3.59	3.62	3.70	3.64
SPL 8	3.61	3.65	3.69	3.65
SPL 9	3.64	3.68	3.70	3.67
SPL 10	3.58	3.69	3.72	3.66
Overall mean				3.65

Table A.1 Raw data for calcium on the migration time at different batch samples using 500 mmol/L acetic acid, 15 mmol/L 4-methylbenzylamine and 50% methanol at pH 4.0.

The null hypothesis adopted is that all the samples are drawn from a population with mean μ and variance σ^2_0. On the basis of this hypothesis σ^2_0 can be estimated in two ways, one involving the variation within the samples and the other the variation between samples.

(i) Within-sample variation

For each sample a variance can be calculated by using the formula

$$\sum \frac{(x_i - \bar{x})^2}{(n-1)} \qquad \text{(A.1)}$$

Using the values in the above Table A.1 we have:

Variance of sample 1 =

$$\frac{(3.62-3.650)^2+(3.61-3.650)^2+(3.72-3.650)^2}{3-1}=0.0037$$

Variance of sample 2 =

$$\frac{(3.61-3.657)^2+(3.66-3.657)^2+(3.70-3.657)^2}{3-1}=0.00203$$

Variance of sample 3 =

$$\frac{(3.59-3.657)^2+(3.62-3.657)^2+(3.69-3.657)^2}{3-1}=0.00263$$

Variance of sample 4 =

$$\frac{(3.62-3.657)^2+(3.65-3.657)^2+(3.70-3.657)^2}{3-1}=0.00163$$

Variance of sample 5 =

$$\frac{(3.61-3.657)^2+(3.65-3.657)^2+(3.72-3.657)^2}{3-1}=0.00310$$

Variance of sample 6 =

$$\frac{(3.63-3.657)^2+(3.67-3.657)^2+(3.70-3.657)^2}{3-1}=0.00123$$

Variance of sample 7 =

$$\frac{(3.59-3.657)^2+(3.62-3.657)^2+(3.70-3.657)^2}{3-1}=0.00323$$

Variance of sample 8 =

$$\frac{(3.61-3.657)^2+(3.65-3.657)^2+(3.69-3.657)^2}{3-1}=0.00160$$

Variance of sample 9 =

$$\frac{(3.64-3.657)^2+(3.68-3.657)^2+(3.70-3.657)^2}{3-1}=0.00093$$

Variance of sample 10 =

$$\frac{(3.58-3.657)^2+(3.69-3.657)^2+(3.72-3.657)^2}{3-1}=0.00543$$

Averaging these values gives:

Within-sample estimate of
$\sigma_0^2 = (0.00370+0.00203+0.00263+0.00163+0.00310+0.00231+$
$0.00123+0.00160+0.00093+0.00543)/10 = 0.00255$

This estimate has 20 degrees of freedom: each sample estimate has 2 degrees of freedom and there are 10 samples. Note that this estimate does not depend on the means of the samples; for example, if all the measurements for sample 1 were increased by say, 4, this estimate of would be unaltered. The general formula for the within-sample σ_0^2 is:

within-sample estimate o $\sigma_0^2 = \sum_i \sum_j \left(x_{ij} - \overline{x_i}\right)^2 / h(n-1)$ (A.2)

The summation over *j* and division by (n-1) gives the variance of each sample; the summation over *i* and division by *h* averages these sample variances. The expression in

Eq. (A.2) is known as a mean square since it involves a sum of squared terms divided by the number of degrees of freedom. Since in this case the number of degrees of freedom is 20 and the mean square 0.00255, the sum of the squared terms is 0.00255 x 20 = 0.05107.

(ii) Between-sample variation

If the samples are al drawn from a population which has variance σ_0^2, then their means come from a population with variance σ_0^2 / n. Thus, if the null hypothesis is true, the variance of the means of the samples gives an estimate of σ_0^2 / n. From Table A.1;

Sample mean variance =

$$\frac{(3.650-3.655)^2+(3.657-3.655)^2+(3.633-3.655)^2+(3.657-3.655)^2+(3.660-3.655)^2}{+(3.667-3.655)^2+(3.637-3.655)^2+(3.650-3.655)^2+(3.673-3.655)^2+(3.663-3.655)^2}{10-1}$$

$= 0.00016$

so

between sample estimate of $\sigma_0^2 = 0.00143$

The estimate has 9 degrees of freedom since it is calculated from 10 sample means. Note that this estimate of σ_0^2 does not depend on the variability within each sample, since it is calculated from the sample means. However, if, for example, the mean of

sample D were changed, then this estimate of σ_0^2 would also be changed. In general we have:

$$\text{between-sample estimate of } \sigma_0^2 = n\Sigma_i(x_i - \bar{x})^2 / (h-1) \qquad \text{(A.3)}$$

which is again a 'mean square' involving a sum of squared terms divided by the number of degrees of freedom. In this case the number of degrees of freedom is 9 and the mean square is 0.00016 so the sum of squared terms is 9 x 0.00016 = 0.00143.

Summarizing our calculations so far:

With-sample mean square = 0.00255 with 20 d.f.

Between-sample mean square = 0.000143 with 9 d.f.

If the null hypothesis is correct, these two estimates of σ_0^2 should not differ significantly. If it is incorrect, the between-sample estimate of σ_0^2 will be greater than the within-sample because of between-sample variation. To test whether it is significantly greater, a one-tailed *F*-test is used:

$F_{9,20} = 0.00016 / 0.00255 = \mathbf{0.06}$

From Table A.3 the critical value of *F* is 2.393 ($P = 0.05$). Since the calculated value of *F* is smaller than critical value this the null hypothesis is retained: the sample means do not differ significantly.

A3.2 Comparison of the means of two samples or new analytical methods

Another way in which the results of a new analytical method may be tested is by comparing them with those obtained by using a second (perhaps a reference) method. In this case we have two sample mean $\bar{x}_1$ and $\bar{x}_2$. Taking the null hypothesis that the two methods give the same results, we need to test whether $(\bar{x}_1 - \bar{x}_2)$ differs significantly from zero. If the two samples have standard deviations which are not significantly different, a pooled estimate of standard deviation can be calculated from the two individual standard deviations s_1 and s_2 by using the equation:

$$s^2 = \{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2\}/(n_1 + n_2 - 2) \qquad \text{(A.4)}$$

It can be shown that t is then given by:

$$t = (\bar{x}_1 - \bar{x}_2)/s\sqrt{(1/n_1 + \frac{1}{n_2})} \qquad \text{(A.5)}$$

Where t has $n_1 + n_2 - 2$ degrees of freedom.

However the technique that have been developed in this work e.g. CE-conductivity (Table 7.2) need to compare with the established method such as ICP-OES.

Example: Table 7.2 (Chapter 7) - Results for the analysis of acid mine discharges by CE-Conductivity and ICP-OES detection for calcium.

ICP-OES method; mean = 363.4; standard deviation 14.0

CE-conductivity method mean = 300.8; standard deviation 62.1

For each method 5 determinations were made. The null hypothesis adopted is that the means of the results given by the two methods are equal. From the equation Eq. (A.4) , the pooled value of the standard deviation is given by:

$$s^2 = (4 \text{ x } 62.1^2 + 4 \text{ x } 14^2)/8$$

$$= 2028.25$$

$$s^2 = 45.04$$

From Eq. (5):

$$t = (363.4 - 300.8)/ 45.04\sqrt{1/5 + 1/5}$$

$$= - 2.2$$

There are 8 degrees of freedom, so (Table A.2) the critical value of $|t|$ is 2.31 (P= 0.05). The observed value of $|t|$ is less than the critical value so the null hypothesis is retained; there is no evidence that the different between two methods is accepted.

Table A.2 The *t*-distribution

Value of t for a confidence interval of	90%	95%	98%	99%
Critical value of $\lvert t \rvert$ for P values of	0.10	0.05	0.02	0.01
Number of degrees of freedom				
1	6.31	12.71	31.82	63.66
2	2.92	4.30	6.96	9.92
3	2.35	3.18	4.54	5.84
4	2.13	2.78	3.75	4.60
5	2.02	2.57	3.36	4.03
6	1.94	2.45	3.14	3.71
7	1.89	2.36	3.00	3.50
8	1.86	2.31	2.90	3.36
9	1.83	2.26	2.82	3.25
10	1.81	2.23	2.76	3.17
12	1.78	2.18	2.68	3.05
14	1.76	2.14	2.62	2.98
16	1.75	2.12	2.58	2.92
18	1.73	2.10	2.55	2.88
20	1.72	2.09	2.53	2.85
30	1.70	2.04	2.46	2.75
50	1.68	2.01	2.40	2.68
∞	1.64	1.96	2.33	2.58

The critical values of $\lvert t \rvert$ are appropriate for a two-tailed test. For a one-tailed test the value is taken from the column for twice the desired P-value, e.g. for a one-tailed test, $P = 0.05$, 5 degrees of freedom, the critical value is read from the $P = 0.10$ column and is equal to 2.02.

Table A.3 Critical Values of the *F* for one-tailed test (P = 0.005)

V1 / 2	1	2	3	4	5	6	7	8	9	10	12	15	20
1	161.4	199.5	224.6	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0
2	18.51	19.00	19.25	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45
3	10.13	9.552	9.117	9.117	9.013	8.941	8.887	8.845	8.812	8.786	8.745	8.703	8.660
4	7.709	6.944	6.338	6.388	6.256	6.163	6.094	6.041	5.999	5.964	5.912	5.858	5.803
5	6.608	5.786	5.192	5.192	5.050	4.950	4.876	4.818	4.772	4.735	4.678	4.619	4.558
6	5.987	5.143	4.534	4.534	4.387	4.284	4.207	4.147	4.099	4.060	4.000	3.938	3.874
7	5.591	4.737	4.120	4.120	3.972	3.866	3.787	3.726	3.677	3.637	3.575	3.511	3.445
8	5.318	4.459	3.838	3.838	3.687	3.581	3.500	4.438	3.388	3.347	3.284	3.218	3.150
9	5.117	4.256	3.633	3.633	3.482	3.374	3.293	3.230	3.179	3.137	3.073	3.006	2.936
10	4.965	4.103	3.478	3.478	3.326	3.217	3.135	3.072	3.020	2.978	2.913	2.845	2.774
11	4.844	3.982	3.357	3.357	3.204	3.095	3.012	2.948	2.896	2.854	2.788	2.719	2.646
12	4.747	3.885	3.259	3.259	3.106	2.996	2.913	2.849	2.796	2.753	2.687	2.617	2.544
13	4.667	3.806	3.179	3.179	3.025	2.915	2.832	2.767	2.714	2.671	2.604	2.533	2.459
14	4.600	3.739	3.112	3.112	2.958	2.848	2.764	2.699	2.646	2.602	2.534	2.463	2.388
15	4.543	3.682	3.056	3.056	2.901	2.790	2.707	2.641	2.588	2.544	2.475	2.403	2.328
16	4.494	3.634	3.007	3.007	2.852	2.741	2.657	2.591	2.538	2.494	2.425	2.352	2.276
17	4.451	3.592	2.965	2.965	2.810	2.699	3.614	2.548	2.494	2.456	2.381	2.308	2.230
18	4.414	3.555	2.928	2.928	2.773	2.661	2.577	2.510	2.456	2.412	2.342	2.269	2.191
19	4.381	3.522	2.895	2.895	2.740	2.628	2.544	2.477	2.423	2.378	2.308	2.234	2.155
20	4.351	3.493	2.866	2.866	2.711	2.599	2.514	2.447	2.393	2.348	2.278	2.203	2.124

ν_1 = number of degrees of freedom of numerator and ν_2 = number of degrees of freedom of the denominator