Indirect Capillary Electrophoretic Detection Methods of Cations and Anions

by

Aron H.Goitom

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Supervisor: Prof. AM Crouch

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my original work and I have not previously in its entirely or in part submitted it at any university for a degree.

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Abstract

Capillary electrophoresis (CE) has recently attracted considerable attention as a promising analytical technique for the separation of cations and anions in complex matrices. Determination of ions in aqueous samples using capillary electrophoresis can be accomplished with indirect UV detection. Most inorganic ions have weak absorption profiles in the UV-Vis wavelength range. These mostly non-absorbing species are commonly detected by indirect UV absorbance through addition of an absorbing co-ion (chromophore) into the electrolyte. Inorganic cations most often require an additional complexing agent to selectively alter their similar mobilities and proper separation.

For optimal determination of alkali, alkaline, and transition metal ions, several electrolytes systems were studied. These include pyridine, imidazole and 4-aminopyridine as UV-absorbing species and glycolic acid, α-hydroxyisobutyric acid and their mixture were used as complexing reagents. A mixture of 10 metal ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, $Ni^{2+}$and Zn^{2+}) was successfully separated. Detection was performed at 210, 214 and 254 nm.

In the anion determination chromate and 2, 6 pyridine dicarboxylic acids (PDC) were used as back ground electrolytes for inorganic ions (F^-, Cl^- and SO_4^{2-}) and organic acids (tartaric acid, malic acid, succinic acid and citric acid) separations respectively. Electroosmotic flow (EOF) was reversed in the direction of the anode by adding Cetyltrimethylammonium bromide (CTAB) to the electrolyte. Highly alkaline conditions were used to confer a negative charge on inorganic and organic anions to

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promote their migration towards the anode. The detection wavelength was 200 nm. All peaks were completely resolved and well separated. The limit of detection (LOD) of cations and anions were in the range of 0.5 - 3 ppm and 2 - 3.5 ppm respectively. The described methods were used successfully in routine analysis of real samples. This includes the qualitative and quantitative analysis of an environmental water samples from the areas surrounding Stellenbosch, beverages and orange juice.

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Uittreksel

Kapillêre Elektroforese (CE) het in die onlangse verlede heelwat aandag getrek as 'n belowende analitiese tegniek vir die skeiding van katione en anione in komplekse monsters. Die bepaling van ione in waterige medium met kapillêre elektroforese word gedoen deur indirekte Ultraviolet (UV) deteksie aangesien meeste anorganiese ione swak absorbsie in die die sigbare UV (UV-Vis) golflengtegebied toon. Deteksie van hierdie meestal nie-absorberende spesies word algemeen gedoen deur indirekte UV absorbansie deur die byvoeging van 'n ko-ioon (chromofoor) tot die elektroliet. Anorganiese katione benodig dikwels 'n addisionele komplekserings reagens om selektief hulle eenderse mobiliteite te verander en sodoende goeie skeiding te bewerkstellig.

Vir die optimale bepaling van alkali-, alkali-aard- en oorgansmetaal ione is verskeie elektrolietsisteme bestudeer. Hierdie sluit in piridien, imidasool en 4-aminopiridien as UV absorberende spesies en glikoliensuur, α-hydroksie-isobottersuur asook 'n mengsel van die twee as komplekserings reagense. 'n Mengsel van 10 metaalione (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+} en Zn^{2+}) is sukselvol op hierdie wyse geskei. Deteksie is gedoen by golflengtes van 210, 214 en 254 nm.

Vir die anioon bepaling is chromaat en 2,6-piridiendikarboksielsuur gebruik as agtergrond elektroliete vir die skeiding van anorganiese anione (F^-, Cl^- en SO_4^{2-}) en organiese sure (tartaarsuur, malonsuur, suksiensuur en sitroensuur), onderskeidelik. Elektroosmotiese vloei (EOF) is omgekeer na die rigting van die anode deur byvoeging van setieltrimetielammoniumbromied (CTAB) by die elektroliet. Sterk

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alkaliese kondisies is gebruik om 'n negatiewe lading op die anorganiese en organiese anione te konsentreer en sodoende hul migrasie na die anode te bevorder. Die deteksiegolftengte hier gebruik was 200 nm.

Volkome resolusie en goeie skeiding is gerealiseer vir al die pieke. Die deteksielimiete (LOD) vir die katione en die anione was 0.5 – 3 ppm en 2 – 3.5 ppm, onderskeidelik. Die metodes wat beskryf word is suksesvol aangewend vir roetiene analise van werklike monsters. Dit sluit in kwalitatiewe sowel as kwantitatiewe analise van omgewingswater monsters uit die Stellenbosch area, koeldranke en lemoensap.

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Dedication

To my beloved Grand mother, Gabriel Habtikiel

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List of abbreviations

ACE	- Affinity capillary electrophoresis
AC	- Amperometric detection
APCAs	- Aminopolycarboxylic acids
BGE	- Back ground electrolyte
CCE	- Carbon composite working electrode
CD	- Conductivity detection
CE	- Capillary Electrophoresis
CEC	- Capillary electrochromatography
CEED	- Capillary Electrophoresis with Electrochemical detection
CGE	- Capillary Gel Electrophoresis
CIEF	- Capillary Isoelectric Focusing
CMC	- Critical micelle concentration
CME	- Chemically Modified Electrode
CZE	- Capillary Zone Electrophoresis
CITP	- Capillary Isotachophoresis
CTAB	- Cetyl trimethyl ammonium bromide
DTPA	- Diethylenetriaminepentacetic acid
DTPMP	- Diethylenetriaminepentamethylene phosphoric acid
E	- Potential
ED	- Electrochemical detection
EDTA	- Ethylene diamine acetic acid
EDTMP	- Ethylenediaminetetramethylene phosphoric acid
EOF	- Electroosmotic flow
ESI-MS	- Electron spray ionisation –Mass spectrometry

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HIBA	- Hydroxyisobutyric acid
HPCE	- High Performance Capillary Electrophoresis
i	- Current
i.d	- Inner diameter
IEEC	- Ion-exchange electrokinetic chromatography
MC	- Separation of microchip
MECC	- Micellar Electrokinetic Capillary Chromatography
MFE	- Mercury Film Electrode
MS	- Mass spectrometry
NACE	- Nonaqueous capillary electrophoresis
NMR	- Nuclear magnetic resonance
NTA	- Nitrillotriacetic acid
NTMP	- Nitrillotrimethylenephosphoric acid
o.d	- Outer diameter
PDC	- Pyridine dicarboxylic acid
SDS-PAGE	- Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TPP	- Tripolyphosphates
TTAB	- Tetra decyltrimetyl ammonium bromide

1. Introduction – Aims and objectives

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Inorganic and organic ions released to the environment may cause risks to the balance of nature. They are a significant hazard to human health when they are present in excess. Detection of these ions is imperative for monitoring environmental water samples and other sample matrices monitoring. The aim of this study was to determine cations and anions in aqueous samples. To achieve these new methods have to be developed for the analysis of cations and anions in environmental water samples, beverages and orange juice. Different methods would be developed for the study of cations and anions since they have different migration rate with respect to electroosmotic flow and require different pH values. Initially synthetic (made-up) samples would be analyzed to validate to the chosen methods. These methods would then be adapted for further investigation of cations and anions in environmental water samples, beverages and orange juice.

It is clear that a reliable and efficient analytical method for the determination of cations and anions is very important, not only in order to detect the ions present in various samples but also to determine the amount of those ions in those samples.

Currently, analysis of cations and anions could be also performed using high performance liquid chromatography (HPLC) and ion chromatography (IC). These separation techniques suffer from long analysis times, often require extensive sample preparation and high running cost since they consume high amount of chemicals. In recent years, some studies have investigated the use of capillary electrophoresis (CE) for the analysis of these compounds. However, the possibilities offered by this

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technique have not completely been investigated.

The analysis of cations and anions, in this study, would be determined by indirect capillary electrophoretic detection methods. Most inorganic ions have weak absorption profiles in the UV-Vis wavelength range. These mostly non-absorbing species are commonly detected by indirect UV absorbance through addition of an absorbing co-ion (chromophore) into the electrolyte. Inorganic cations most often require an additional complexing agent to selectively alter their similar mobilities in order to get good separation.

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2. Literature review

2.1 Introduction

2.1.1 Summary

This chapter provides a brief review of the role of detection in cation and anion analysis, the basic principles of metal-ligand complex, the separation mechanism in capillary electrophoresis (CE), a description of CE instrumentation, detection types, modes of CE operation, and some trouble-shooting guide. The basic CE principles discussed for cations are also applicable to anions.

2.1.2 The role of detection in cation and anion analysis

Inorganic and organic ions released to the environment may cause risks to the balance of nature. Cations and anions appear to contaminate the water supply systems (surface water and drinking water) and are found as concentrates in ecological systems [1].

Environmental pollutants are a significant hazard to human health, among them trace elements can be beneficial or harmful effects depending on their concentration and chemical form in the living organisms. In addition to the common elements (Sodium, Calcium, Magnesium etc.), a number of trace elements (Selenium, Zinc, Molybdenum, Manganese, etc.) are considered essential with specific biological functions at relatively low levels. However when present in excess, these elements can be harmful [2, 3].

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Detection of cations and anions can be performed using direct and indirect modes of detection. Direct refers to an analytical signal related directly to the chemical or physical properties of each cation or anion (for example, the energy of emitted x-rays). Indirect mode of detection refers to signals that are non-specific for the cation or anion and are obtained from a transformed species containing the cation or anion (for example, the UV-Vis spectrum of complexed metal ion) or from a species containing no cation or anion from the sample (for example, the signal from the background electrolyte in indirect UV-Vis or fluorescence detection).

The indirect mode of detection requires UV-absorbing species (chromophore) and complexing reagents. In cation analysis, a complexing ligand must be added to the background electrolyte to provide adequate separation by enhancing the difference in mobility among the cations. In addition, the separation buffer or background electrolyte contains a chromophore that provides a background level of absorption at the detection wavelength. The chromophore is displaced by the analyte ions and a decrease in the background absorption is measured when the analyte is in the detector window [4]. The commonly used complexing reagents and UV-absorbing species are given in tables 1 and 6 respectively. The main detection types are discussed in section 2.1.5.3.

2.1.3 Metal-ligand complex

A metal complex or coordination compound is defined as a central atom or ion attached to the sheath of ions or molecules. The molecule or ion bonded directly to the metal ion is called a ligand. A ligand is derived from a Latin word *ligare* meaning to *bind*. Ligands can be monodentate, bidentate, tridentate etc. Ligands containing

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more than one-donor atoms are known as chelating agent (from Greek word *chelos* for *crab's claw*) [5].

2.1.3.1 Classes of chelating agents

Chelating agents may be either organic or inorganic compounds (table 1), but the number of inorganic chelants is small.

Table 1. Commonly used chelating agents [6].

Chelating agent	Uses	Properties
Hydroxycarboxylic acids - Citric acid, glycolic acid, - α-hydroxyisobutyric acid (α-HIBA)	Removal of hardness salts, metal cleaning, water treatment, pharmaceutical, biological, food process.	Are expensive but weak chelating agents
Aminopolycarboxylic acids(APCAs) - Ethylene diamine acetic acid (EDTA) - Diethylenetriaminepentacetic acid (DTPA) -Nitrillotriacetic acid (NTA)	Removal of hardness salts, boiler cleaning for industrial chemical process, brewery, power plant and diary applications, metal cleaning, rust removal, petroleum drilling fluids, wood and pulp processing.	Are stable at high temperature and pH, show strong affinity for metals, are somewhat expensive
Phosphoric acids - Ethylenediaminetetramethylene phosphoric acid (EDTMP) - Diethylenetriaminepentamethylene phosphoric acid (DTPMP) - Nitrillotrimethylenephosphoric acid (NTMP)	Water treatments e.g., scale and corrosion inhibition in cooling towers, wood pulp processing, metal plating, polymer processing.	Are stable over a wide range of temperatures and pH levels, are expensive
Polycarboxylic acids Gluconates, citrates, polyacrylates, polyaspartates	Processing at high pH levels, hardness –ion sequestration with low stability constants	Are expensive but weak chelating agents
Polyphosphates (Inorganic chelating agent) - Tri polyphosphates (TPP) - Haxameta phosphates	Water treatment, consumer applications such as cleaning products and cosmetics.	Less expensive than organic chelants, are often hydrolytically unstable at high temperatures and pH levels

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2.1.3.2 Hydroxycarboxylic acids:

The hydroxycarboxylic acids and their derivatives are important chemicals for the pharmaceutical, biological, food, and other industries. Various hydroxycarboxylic acids occur naturally [7]. The technological, practical, and industrial application of these compounds has led to numerous studies of their properties and characteristics.

The hydroxycarboxylic acids contain two donor groups, the hydroxyl and the carboxlate groups, and therefore are all potentially bidentate ligands. The proton and metal ion complexation constants of these ligands depend strongly on the relative positions of the donor groups in the molecule. The α-hydroxycarboxylic acids form considerably stronger complexes with most metal ions, through bidentate chelation involving both functional groups, than do the corresponding simple carboxylic acids. Hydroxyl groups more distant from the carboxylic groups do not generally participate in the formation of chelate complexes, and the ligand coordination to metal ions occurs via the carboxyl group only. The hydroxyl carboxylic acids form stable complexes with most metal ions [7].

2.1.4 Principles of capillary electrophoresis

Capillary electrophoresis (CE) is a relatively new separation technique, which combines aspects of both electrophoresis and chromatographic principles. Like electrophoresis the separation depends upon differential migration in an electric field. As in chromatography detection is accomplished as the separation progresses with resolved zones producing an electronic signal as they migrate past the monitor point of a concentration-sensitive detector. CE has diverse and rapidly growing number of applications. CE has been applied to solve problems related to forensic chemistry,

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food chemistry, clinical chemistry, biochemistry, pharmaceutical science, neuroscience, molecular biology, and environmental science. It has been applied successfully to the separation and analysis of a variety of simple and complex molecules ranging from ions, vitamins, drugs, amino acids, and nucleotides, to peptides, aligonucleotides. More recently CE has been used in gene diagnosis and in the human genome project [8]. Scientists are devoting a major effort to build a high–through put DNA sequencing system based on capillary electrophoresis [9].

CE in conjunction with other instrument like, electrochemical detection (CE-ED) [10], Electron spray ionisation – Mass spectrometry (ESI-MS) [11] and Nuclear magnetic resonance (NMR) [12] has increased the range of its application.

2.1.4.1 Electrophoresis

Electrophoresis (from Greek words electron = electron and phoresis = carrying) is a well-established method in which ions are separated due to the difference in their migration rates under an external field [13]. Tiselius introduced electrophoresis as a separation technique in 1937. Placing protein mixtures between buffer solutions in a tube and applying an electric field, and he found that sample components migrated in a direction and at rate determined by their charge and mobility [15].

Separation efficiency in free solutions, as performed by Tiselius, was limited by thermal diffusion and convection. For this reason, electrophoresis traditionally has been performed in anti-convective media, such as polyacrylamide or agarose gels. Gels in the slab or tube format have been used primarily for the size- dependent separation of biological macromolecules, such as nucleic acids and proteins. A gel

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provides physical support and mechanical stability in the separation. Although it is one of the most widely used separation techniques, slab gel electrophoresis generally suffers from long analysis times, low efficiencies, and difficulties in detection and automation [14, 15].

2.1.4.2 Capillary Electrophoresis

CE is an instrumental approach to electrophoresis in which sample components placed between two buffers solutions are separated in a narrow bore-tube capillary (figure 1) with inside diameter ranging from 2 to 200 μm (usually from 25 to 75 μm employed) and a length usually between 10 to 100 cm.

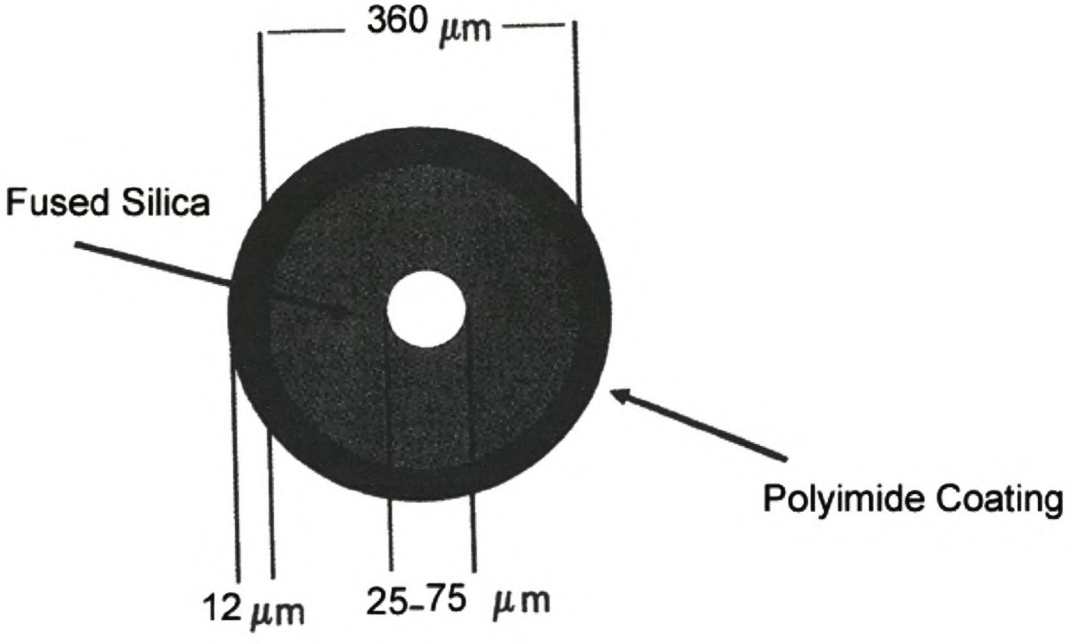

Fig. 1. Cross - sectional view of a fused silica capillary [16].

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The separation is based on the electrophoretic mobility of the analyte species induced by the large potential (10 - 30 kV) applied across the capillary. This migration takes place under the combined effects of electrophotertic and electro osmotic flows (EOF) generated by applying an electric field across the capillary [17]. A schematic diagram of capillary electrophoresis is shown in figure 3.

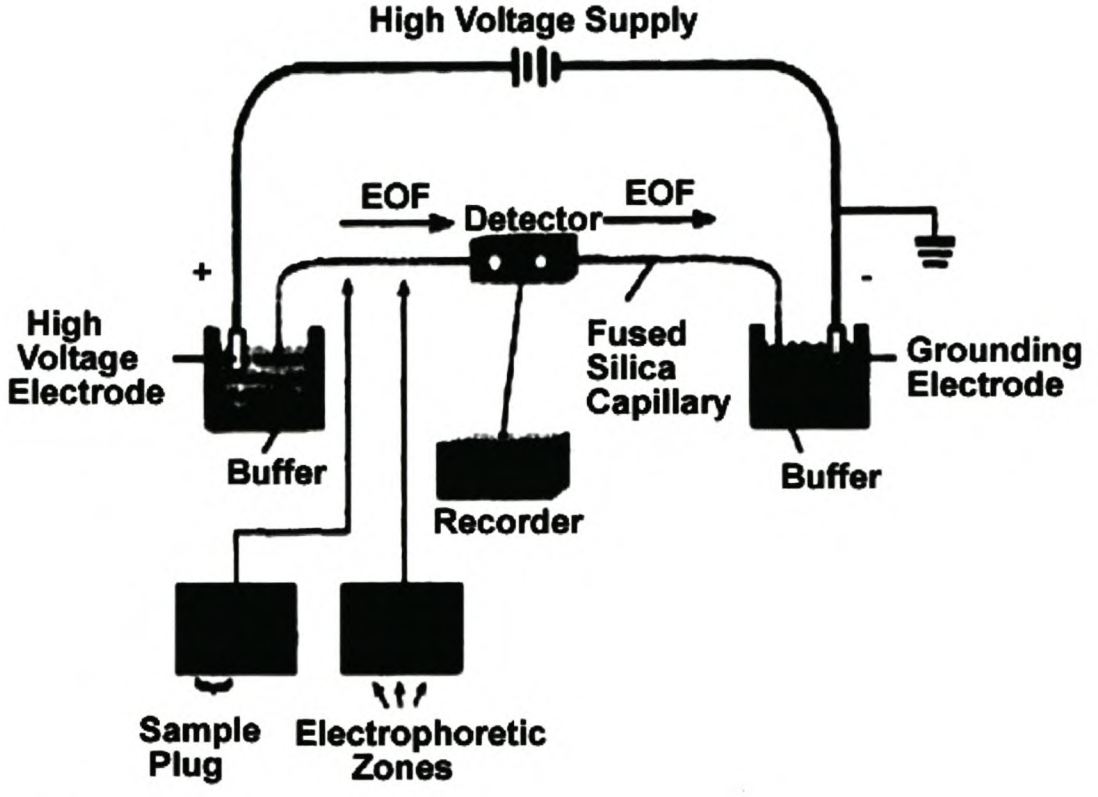

Fig. 2. Schematic diagram for capillary electrophoresis system [8].

The principle of separation in electrophoresis is that charged molecules migrate in the presence of an electric field. When an electric field is applied, a charged molecule experiences a force, F_e, equal to the product of its net charge, q, and the electric field strength E:

$$F_e = qE \qquad (1)$$

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The charged molecule also experiences a drag force in the opposite direction of its motion, F_d, which is proportional to its velocity, v, and its translation friction coefficient *f*:

$$F_d = fv \qquad (2)$$

When the molecule is electrophoresing, the drag force acting on the molecule will counter balance the electrostatic force. Thus, the velocity of the analyte at steady-state ($F_e = F_d$) can be expressed as:

$$v = \frac{qE}{f} \qquad (3)$$

The electrophoretic mobility μ is defined as the velocity of the analyte divided by the electric field:

$$\mu = \frac{v}{E} = \frac{q}{f} \qquad (4)$$

Electrophoretic mobility, μ, is also described by the following equation.

$$\mu = \frac{v}{E} = \frac{q}{6\pi r \eta} \qquad (5)$$

Where: η = solution viscosity

r = ion radius

Therefore, differences in the mobility of the molecules will arise from either differences in their frictional properties (dependent on their size and shapes) or their net charges. It is these variations between the molecules that make separation by electrophoresis possible [18-20].

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2.1.4.3 Characteristics and limitation of capillary electrophoresis

2.1.4.3.1 Characteristics

Capillary electrophoresis has the following characteristics

- High speed of analysis [21].
- High separation efficiency [22].
- Nano scale separation technique. The power of CE in micro-separation is linked to the very small injection volume [23].
- Low consumption of electrolytes and samples [24].
- Capillaries can be easily conditioned with buffer before the start of the analysis [25].
- It has very simple electropherograms [26].
- Method robustness and simplicity [27].
- Simpler chemistry [28].
- It has high resolving power and quantitative capability [29].
- It uses cheap columns [30].
- Good tolerance to sample matrix, e.g., to high pH values [31].
- The ability to resolve complex mixtures efficiently [32].
- Unambiguous identification of species oxidation state [33].
- The availability of sensitive detectors for determining analytes migrating from the column [34].
- It has uniform flow profile [35].
- Feasibility and automation [36].
- Greater flexibility [37].
- Proved to be a good choice for investigation of samples in aqueous media since usually no more than a simple dilution of samples is needed [38].

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- Poses versatility of application [39].

2.1.4.3.2 Limitation of CE

CE suffers from the following disadvantages.

- CE is the high detection limit for the usually involved on column UV detection (ppm range) which is a serious limitation for its application to environmental analytes, i.e, relatively poor concentration detectability of CE, particularly using UV detector [40].
- Provides insufficient information for structural elucidation of unknown compounds, especially in complex multicomponent mixtures [41].
- Instability and irreproducibility of migration times and peak areas, manipulation of separation selectivity is difficult and detection options are limited [31].

2.1.4.4 Electroosmotic flow (EOF)

One of the most distinguishing properties of capillary electrophoresis is electroosmotic flow (EOF), which is the bulk flow of a liquid in the capillary. Fused Silica capillary columns are used with ionizable silanol (SiOH) groups. Above about pH 2, the silanol groups ionize to produce a negative charge on the capillary surface, called the zeta potential. This surface charge creates an electrical double layer, from the accumulation of cations along the walls [42]. The EOF is directed towards the cathode as long as the capillary walls remain negatively charged. This means that cationic species move by electrophoresis in the same direction as the EOF. However, anionic electrophoretic mobilities and EOF have different directions [43]. The relationship of the EOF and zeta potential can be expressed as:

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$$v_{EOF} = (\varepsilon\zeta/\eta)\, E \qquad (6)$$

$$\mu_{EOF} = \varepsilon\zeta/\eta \qquad (7)$$

Where: v_{EOF} = EOF velocity

μ_{EOF} = EOF mobility

ζ = zeta potential

ε = dielectric constant

2.1.4.4.1 Alteration of Electroosmotic flow (EOF)

A great deal of work has been done to manipulate EOF. For example, the separation of proteins in free zone electrophoresis was accomplished by adjusting the pH of the buffer to 8 - 11, where the capillary wall and many proteins are electronegative and repel one another to minimize surface interaction. The addition of NaCl reduces EOF by decreasing double-layer thickness [44].

In addition to simple manipulation of the ionic composition of the buffer several chemicals have been added to the buffer to alter the zeta potential developed across the capillary/water interface. The direction of the EOF is reversed through the addition of cationic surfactant Cetyltrimethylammoniumbromide (CTAB) [38] or Tetra decyltrimetylammoniumbromide (TTAB) [45].

2.1.4.4.2 Control of Electroosmotic flow (EOF)

EOF can be suppressed using different ways. The main ones are described in table 2.

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Table 2. Methods to control electroosmotic flow [46].

Variable	Result	Comment
Electric field	Proportional change in EOF	• Efficiency and resolution may decrease when lowered • Joule heating may result when increased
Buffer pH	EOF decreased at low pH and increased at high pH	• Most convenient and useful method to change EOF • May change charge or structure of the solute
Ionic strength or buffer concentration	Decrease zeta potential and EOF when increased	• High ionic strength generates high current and possible Joule heating • Low ionic strength problematic for sample adsorption • May distort peaks shape if conductivity different from sample conductivity • Limits sample stacking if reduced
Temperature	Changes viscosity	• Often useful since temperature is controlled instrumentally
Organic modifier	Changes zeta potential and viscosity (usually decrease EOF)	• Complex changes, effect most easily determined experimentally • May alter selectivity
Surfactant	Adsorbs to capillary wall via hydrophobic and/or ionic interactions	• Anionic surfactants can increase EOF • Cationic surfactants can decrease or reverse EOF • Can significantly alter selectivity
Neutral hydrophilic polymer	Adsorb to capillary wall via hydrophobic interactions	• Decreases the EOF by shielding surface charge and increasing viscosity
Covalent coating	Chemical bonding to capillary	• Many modifications possible (hydrophilicity or charge • Stability often problematic

2.1.4.5 Migration time

In order to be separated analytes must have different electrophoretic velocities. The time required for a solute to migrate to the point of detection is called the migration time [47]. The migration time or residence time is given as:

$$t_m = \frac{l}{vE} = \frac{lL}{\mu V} \qquad (8)$$

Where: μ = electrophretic mobility

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V = applied voltage

l = effective capillary length (to the detector)

L = total capillary length

t = migration time

E = electric field

V = velocity

2.1.4.6 Dispersion

Separation in electrophoresis is based on differences in the electrophoretic mobility of analytes. During analysis, the analytes are focused into zones, and the difference in migration distance necessary to resolve two zones depends on the width of these zones. Dispersion, spreading of the analytes zone and increases zone length (band broadening) should be controlled [48, 49]. Dispersion results from differences in solute velocity within that zone, and can be defined as the baseline peak width, W_b, for a Gaussian peak,

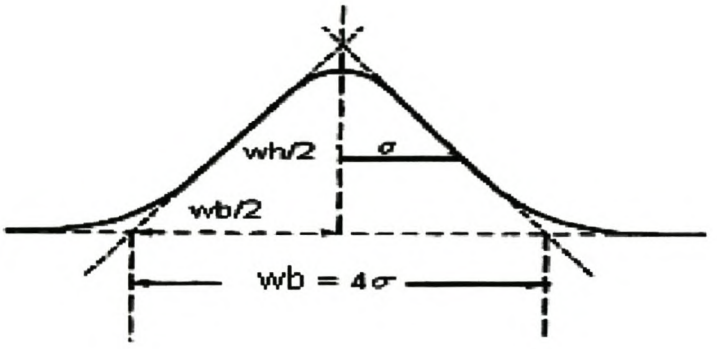

Fig.3. The typical Gaussian peak showing half peak height ($w_h/2$), peak width (w_b) and standard deviations of the peak (σ).

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$$W_b = 4\sigma \qquad (9)$$

Where: σ = standard deviation of the peak (in time, length, or volume).

The efficiency, expressed in number of theoretical plates, N, can be obtained by

$$N = \left(\frac{l}{\sigma}\right)^2 \qquad (10)$$

Where: l = capillary effective length

and can be related to the height equivalent to a theoretical plate (HEPT), H, by

$$H = \frac{l}{N} \qquad (11)$$

Under ideal conditions (that is, small injection plug length, no solute- wall interactions, and so on) the sole contribution to solute- zone broadening in HPCE can be considered to be longitudinal diffusion (along the capillary). Radial diffusion (across the capillary) is unimportant due to the plug flow profile. Similarly, convective broadening is unimportant due to the anti-convective properties of the capillary. Thus, the efficiency can be related to the molecular diffusion term in chromatography as:

$$\sigma^2 = 2Dt = \frac{2DlL}{\mu eV} \qquad (12)$$

Where: D = diffusion coefficient of the solute

Substituting equation (12) into equation (10) yields a fundamental electrophoretic expression for plate number

$$N = \frac{\mu Vl}{2DL} = \frac{\mu El}{2D} \qquad (13)$$

From equation (13), the reason for the application of high fields is evident. This

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follows simply because the solute spends less time in the capillary at high field and has less time to diffuse. In addition, this equation shows that large molecules such as proteins and DNA, which have low diffusion coefficients, will exhibit less dispersion than small molecules.

The theoretical plate number can be determined directly from an electropherogram, using, for example,

$$N = 5.54 \left[\frac{t}{w_{1/2}} \right]^2 \qquad (14)$$

Where: t = migration time

$w_{1/2}$ = temporal peak width at half height

Note that equation (14) should only be used for Gaussian peaks. Any asymmetry should be taken into account, for example, by use of central moments.

In practice, the measured efficiency, equation (14) is usually lower than the calculated efficiency, equation (13). This is because the theoretical calculation accounts only for zone broadening due to longitudinal diffusion.

2.1.4.6.1 Sources of band broadening

Diffusion is not the only cause for band broadening. Adsorption of solutes at the wall, temperature effects, detection and sample injection can also lead to dispersion according the following equation.

$$N = \left(\frac{t^2_{mig}}{\sigma^2_{Tot}} \right) \qquad (15)$$

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Where σ^2_{Tot} is the zone variance due to all sources of band broadening.

In CE, σ^2_{Tot} is given by:

$$\sigma^2_{Tot} = \sigma^2_{Diff} + \sigma^2_{Inj} + \sigma^2_{Det} + \sigma^2_{Heat} + \sigma^2_{Ads} + \ldots \quad (16)$$

Where σ^2_{Diff}, σ^2_{Inj}, σ^2_{Det}, σ^2_{Heat}, σ^2_{Ads} are the variances due to longitudinal diffusion, sample injection, detection, joule heating, and adsorption respectively [50]. Some of the key sources of dispersion along with their importance and remedy are given in table 3.

Table 3. Sources of band broadening.

Cause	*Solution*
Longitudinal diffusion	Minimized by increasing the voltage
Adsorption	Use coated capillary or buffer additive
Injection size	Reduce injection size
Joule heating	Reduce voltage
Detection window	Reduce slit width if possible

2.1.4.7 Resolution

The measure that describes the degree of separation of a pair of subsequently migrating analytes is the resolution. It is dimensionless and the definition used in most cases is:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2} = \frac{t_2 - t_1}{4\sigma} \quad (17)$$

Where: t = migration time

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w = base line peak width (in time)

σ = temporal standard deviation (subscripts 1 and 2 refer to the two solutes).

The numerator in the equation (17) describes the separation process in terms of differential and the denominator the dispersive processes acting against it.

Separation in HPCE is primarily driven by efficiency, not selectivity. This is in contrast to chromatography in which the opposite is usually true. Due to very sharp solute zones, small differences in solute mobility (<0.05% in some cases) are often sufficient for complete resolution. Of course, the extent of dispersion is immaterial if mobility differences are realized [51, 61].

The resolution of two components can be expressed with respect to efficiency

$$R = \left[\frac{1}{4N}\right]^{1/2}\left[\frac{\Delta\mu}{\bar{\mu}}\right] \qquad (18)$$

Where: $\Delta\mu = \mu_2 - \mu_1$

$$\bar{\mu} = \frac{\mu_2 + \mu_1}{2} \qquad (19)$$

Substituting equation (13) into equation (18) yields a commonly cited theoretical equation for resolution that does not require explicit calculation of efficiency. It also describes the effect of EOF on resolution.

$$R = \left[\frac{1}{4\sqrt{2}}\right]\Delta\mu\left[\frac{v}{D\left(\mu + \bar{\mu}_{eof}\right)}\right]^{1/2} \qquad (20)$$

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2.1.5 Instrumental and operational aspects of high performance capillary electrophoresis (HPCE)

The commercial HPCE systems available have no well-defined standard instrument specifications, and the apparatus from different companies may be quite different from each other with respect to technical data. A typical commercial HPCE system consists, however, basically of the high voltage power supply (max.30 kV), two buffer reservoirs, electrodes, capillary tube, capillary temperature controller, capillary filling system, detector, and data acquisition equipment. The following comprise a discussion of more general parameters such as capillary conditioning selected, detection types and injection modes in HPCE.

2.1.5.1 Capillary conditioning

One of the most important factors leading to good reproducibility is capillary conditioning. Proper conditioning of capillary surface ensures a consistent and repeatable EOF. The most reproducible conditions are encountered when no conditioning other than with buffer is employed. However, adsorption of sample to the surface and changes in EOF often do not allow this.

Base conditioning to remove adsorbates and refresh the surface by deprotonation of silanol groups is most commonly employed. A typical wash method includes flushing a new capillary with 1 M NaOH for 20 minutes. Before each injection, capillaries are conditioned by washing with 0.1 M NaOH for 5 - 10 minutes, with Milli- Q water (since many organic ions are poorly soluble in organic solvents, and precipitated buffer crystals can block the capillary) for 5 - 10 minutes and with background electrolyte solution for 5 - 10 minutes. Before each analysis only the last two steps are performed. In some cases, the capillary is flushed with pure organic solvents e.g.,

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methanol or DMSO, prior to the final conditioning step with electrolyte solution to eliminate water [52].

2.1.5.2 Sample injection

There are basically two different methods of sample introduction into the capillary– either hydrodynamic injection or electrokinetic injection.

2.1.5.2.1 Hydrodynamic injection

Hydrodynamic injection is the most widely used injection technique. It is simple to employ and usually guarantees that the proper amount of sample enters the capillary.

Hydrodynamic injection is accomplished in one of four ways:

- By elevating the capillary at the sample (inlet) end, permitting sample introduction by siphoning
- By applying pressure on the individual sample vial
- By applying vacuum on the detector-side buffer reservoir
- Injecting by syringe and employing a splitter to reduce the volume introduced into the capillary.

The volume of material injected per unit time (V_t, nL/s) is determined by the Poiseuile equation [53]

$$V_t = \frac{\Delta P D^4 \pi}{128 \eta L} \qquad (21)$$

Where: ΔP = the pressure drop

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D = the capillary internal diameter

η = the viscosity

L = the total length of the capillary

For gravity based- injections, ΔP is given by:

$$\Delta P = \rho g \Delta h \qquad (22)$$

Where: ρ = the density of the sample solution

g = the gravitational constant

Δh = the height difference between the liquid levels in the sample vial and in the detector-side buffer reservoir.

The consequences of an open-ended injection system and the Poiseuille equation mean that changes in the experimental conditions will result in variations of the amount of the material injected. If the temperature is increased, more material enters the capillary because of the decreased viscosity of the BGE. If the capillary is lengthened, the amount of material injected decreases due to the increase of backpressure.

Hydrodynamic injection is generally useful for capillaries with i.d.'s ranging from 25 to 100 μm. For smaller i.d. capillaries; high-pressure injection must be used to keep the injection time reasonably short. For large-diameter capillaries, the injection pressure must be reduced to maintain an injection time of over 1 s. Shorter injection times may adversely affect precision [54].

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2.1.5.2.2 Electrokinetic injection

Electrokinetic is the simplest injection method in CE; the capillary inlet is immersed in the sample solution and high voltage is applied for a brief period (typically a few seconds). If no EOF is present, sample ions enter the capillary by electrophoretic mobility alone. If EOF is present, sample ions will be introduced by a combination of electrophoretic mobility and EOF.

This mode is generally termed electrokinetic injection and the quantity (Q) of a solute injected is given as [53]:

$$Q = (\mu_{ep} + \mu_{eo})\, \pi r^2 ECT \qquad (23)$$

Where: μ_{ep} = the electrophoretic mobility

μ_{eo} = the electroosmotic mobility

r = the capillary radius

E = the field strength

t = the time of injection

C= the concentration of each solute

Electrokinetic's injection offers two advantages. First, only like charges will enter the capillary (in the absence of EOF). This enables discrimination against compounds of opposite charge, simplifying the separation problem. Second, zone sharpening can be achieved using the stacking principle, which is on capillary sample concentration

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based on the electric field strength differences between the sample zone and the running buffer [51]. Electrokinetic injection suffers from matrix effects, in that many sample matrices contain components such as proteins, can adsorb onto the capillary wall and change the magnitude of EOF [55].

2.1.5.2.3 No injection

The most frequent cause of no injection is a plugged capillary. This can result from evaporation of water at the capillary tip, which allows salt crystals to form. If the polyamide is not removed from the capillary tip, a shard of that material can enter and plug up the capillary. It is also possible that material from unfiltered sample or material that is insoluble in BGE can plug the capillary. If the capillary is plugged, the observed current is usually zero or thereabout. Cutting a few millimetres of capillary from the end or replacing the capillary should remedy the situation. No injection can also occur if an empty or incorrect sample vial is used, if an incorrect vial is called for in the method, if the vial cap is missing or badly leaking, or if the external pressure source is not activated. It is also possible that the capillary is broken. Breaks usually occur at the detection window. Check that the voltage polarity is correct set [56].

2.1.5.3 Detection

Perhaps the most rapidly developing aspect of capillary electrophoresis is detection. The ability to detect trace amount of a wide variety of solutes will dictate the future of capillary electrophoresis. Although on column UV absorbance and fluorescence have been the most commonly used detection modes, there has been a flurry of new detection and derivatization schemes developed. The main detectors are discussed below [57].

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2.1.5.3.1 UV absorbance

UV detection is most common in capillary electrophoresis, as it is simple and reliable. Many of the early reports of capillary electrophoresis with high field strength (150 V/cm) used UV detection with large diameter capillaries. Due to the need for small detection volumes, UV detection is accomplished on column, such that the path length is defined by the diameter of the capillary. This limits the sensitivity of absorbance methods, since sensitivity is proportional to path length. UV detection has remained the most popular method of detection, even with its limited sensitivity [58].

2.1.5.3.2 Fluorescence

Fluorescence detection is most easily adapted for use in CE, since its sensitivity is not path-length dependent. On column detection is accomplished simply by imaging the excitation source onto the column and collecting the emission at an angle perpendicular to the incident light. This allows the use of much narrower columns as low as 11 μm when lasers are employed as excitation source. The excitation source can be as simple as an arc lamp where the excitation wavelength is isolated with glass filters and then focused onto the capillary. The major pit fall with fluorescence detection is that it is only possible for use with compounds that have a chromophore or fluorophore [59-60].

2.1.5.3.3 Mass spectrometry (MS)

Mass spectrometry (MS) is another detection technique common in macro scale separation that has been applied to micro column separations. This technique is attractive because of its general applicability and its ability to yield structural

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information. Coupling micro column to MS is not as straightforward as coupling to optical detection methods because the on-column scheme cannot be used. Therefore the technology for routine use of MS in micro separation is still being developed [61].

2.1.5.3.4 Electrochemical detection (ED)

The growing popularity of ED has become evident within the last few years. Principally, there are three ED systems suitable for sensitive detection of inorganic ions (metal ions), i.e., amperometric, conductometric and potentiometeric detection. For all of these, investigations into simpler and more robust cell arrangements continued to be the main trend. Amperometric detection appears to be by far the most suited for CE of the metal analyte ions because a high- sensitivity response can be expected for a wider range of metal analytes. It can attain detection limits as low as 10^{-11} M for metal ions (table 4). Most recent advances in amperometric detection involved a simplified detector cell with a single (not electrophoretic) electrode, a field-portable CE instrument which incorporates, in a single unit, both amperometric and potetiometric detection, and a sensor for non-electro active cations employing a modified graphite electrode (with a mixed-valence ruthenium-iron cyanide) [62].

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Table 4. Detection types for capillary electrophoresis [63-64].

Method	*Advantage*	*Draw back*	*Detection limit (mol L^{-1})*
UV-Absorbance	Universal	Relatively low sensitivity	10^{-4}-10^{-6}
Fluorescence	Sensitive	Limited number of fluorophores	10^{-6}-10^{-9}
Mass spectrometry	Selective	Limited number of buffer	10^{-8}-10^{-9}
Amperometry	Sensitive, Selective	Stability, different electrodes are needed for different species	10^{-5}-10^{-11}
Condutometry	Universal	Maintenance of electrodes	10^{-4}-10^{-8}
Potentiometry	Universal	For lipophilic ions only	10^{-3}-10^{-11}

2.1.6 Modes of CE operation

Capillary electrophoresis (CE) can be accomplished with various modes, including the popularly employed Capillary zone electrophoresis (CZE), Micellar electrokinetic chromatography (MEKC), Capillary gel electrophoresis (CGE), Capillary isotachophoresis (CITP), Capillary isoelectric focusing (CIEF), Ion-exchange electrokinetic chromatography (IEEC) as well as some newly developed separation strategies that involve Affinity capillary electrophoresis (ACE), Capillary electrochromatography (CEC), Separation of microchip (MC) and using organic solvents as the separation medium-nonaqueous capillary electrophoresis(NACE).

This project focuses on Capillary zone electrophoresis mode of CE separation. The basic principle and application of Capillary zone electrophoresis is discussed in upcoming sections.

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2.1.6.1 Capillary zone electrophoresis (CZE)

CZE-based separation is the most widely used operational mode in CE. Its mechanism is based on the differences in the electrophoretic mobility of analytes. The analytes with different electrophoretic mobility will migrate in separated zones, and the analytes with the same electrophoretic mobility will co-migrate within the same zone in the capillary under the applied electric field. That is the origin for name *zone*. CZE provides high efficiency, resolution, speed and reduced consumption of reagents [65]. CZE is commonly used for the separation of inorganic ions (cations and anions). In the determination of metal ions, CZE is usually accomplished by the complexation method to enhance the difference in electrophoretic mobility of each metal ion. In the anion analysis a suitable BGE and surfactant for reversal of EOF should be used.

2.1.6.1.1 CZE for metal ion separation

CE has recently been developing very rapidly. It has become established as a powerful technique for metal ion separation [66].The first paper on the CE analysis of inorganic cations was published by Hjerten in 1967(separation of Bi^{3+} and Cu^{2+}) with lactic acid as the complexing agent [67], but the real boom in CE started after 1990 in close connection with rapid development of the instrumentation [68].

One of the problems in the analysis of metal ions by CE is that most of the transition metal cations have almost the same mobility due to their similar size and identical charge (see table 6). The electrophoretic mobility of cation, $\mu_{ep\ (ion)}$, can be related to the limiting ionic equivalent conductivity, λekv, by eq. (24),

$$\mu_{ep\ (ion)} = \lambda_{ekv}/F = q_i/6\pi\eta r_i \qquad (24)$$

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Where: F = the Faraday constant ($F=9.6487x10^4$as mol^{-1});

λ_{ekv} = the stokes law (cm^2 $mol^{-1}ohm^{-1}$)

q_i = the charge of the hydrated cation

η = the dynamic viscosity of the electrolyte (g cm^2s^{-1}),

r_i = the radius of the hydrated cation (cm).

The hydrated ionic radii of selected inorganic cations, calculated using eq. (24) are listed in table 5.

Table 5. Hydrated ionic radii r_i (in cm) of the selected inorganic cations [69].

Cation	10^8 r_i	Cation	10^8 r_i	Cation	10^8 r_i
Ba^{2+}	2.572	Gd^{3+}	3.651	Ni^{2+}	3.244
Ca^{2+}	2.750	Ho^{3+}	3.706	Pb^{2+}	2.297
Cd^{2+}	3.039	K^+	1.114	Pr^{3+}	3.520
Ce^{3+}	3.530	La^{3+}	3.530	Rb^+	1.058
Co^{2+}	3.150	Li^+	2.117	Sm^{3+}	3.587
Cs^+	1.044	Mg^{2+}	3.088	Sr^{2+}	2.742
Cu^{2+}	3.000	Mn^{2+}	3.168	Tu^{3+}	3.757
Dy^{3+}	3.745	Na^+	1.624	Yb^{3+}	3.745
Er^{3+}	3.728	Nd^{3+}	3.540	Zn^{2+}	3.046
Eu^{3+}	3.624	NH_4^+	1.104		

Obviously, the enhancement of separation selectively is the only alternative with which to achieve a satisfactory resolution. Generally, there are two main approaches in this direction that imply the addition of a complexing ligand to either the carrier electrolyte or a sample solution before introduction into the capillary.

Complexation of the cation to be separated with auxiliary ligands is used to change the selectivity of the separation and/or to facilitate the detection.

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2.1.6.1.2 Choice of derivatization ligands

In the application of CE to analysing metal complexes, the selection and utilization of the ligands are undoubtedly of key importance in the research and the core of the experimental design. A good derivitization reagent should fulfil the following requirements:

(i) It must be easily synthesized and purified.

(ii) It must form stable, sensitive and single-state complexes with multielements under controlled conditions.

(iii) The formed complex should be stable during electrophoresising in the capillary

(iv) The maximum absorptive wavelengths of the complexes investigated in an electrophoretic should be close to each other, far away from that of the ligand for the demand of detection [70].

2.1.6.1.3 Selection of the background electrolyte (BGE)

Choice of the BGE is most important in developing a method employing CZE with indirect UV detection since it must conform to the following requirements:

- The BGE must contain an absorbing co-ion of the analyte.
- Since sensitivity is directly related to the molar absorptivity of the BGE, this should be high.
- The peak shape of the analyte can be affected by differences between its mobility and the mobility of the BGE. Consequently, mismatching the ionic mobilities of the BGE and sample ions can produce peaks exhibiting fronting or tailing. Therefore the mobility of the BGE should be similar to that of the analyte of interest.

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- Migration time reproducibility is dependent upon reproducible mobilities, which in turn is pH dependent. Therefore, the BGE should have a good buffering capacity at the operating pH [71].

The commonly used UV-Vis absorbing electrolytes are described below.

Table 6. Frequently used UV-Vis absorbing electrolytes [72].

S.No	UV-Vis absorbing electrolyte	S.No	UV-Vis absorbing electrolyte
1	Creatinine	6	4-aminopyridine
2	Imidazole	7	4-Methyl benzyl amine
3	Ephedrine	8	p-toluidine
4	Pyridine	9	Benzimidazole
5	Copper sulfate		

2.1.6.1.4 Methods of metal ion complexation

In principle, two experimental approaches are used in the CE analysis of cations, an off-line preparation of complexes prior to the CE analysis, and on line comlexation in the separation capillary [73].

2.1.6.1.4.1 On-line complexation

If complexes of low stability are rapidly formed, then on-capillary partial complexation can be used. A ligand is added to the running electrolyte and rapid equilibrium between the free metal ions and their complexes is established, with the most of the ions present in the free form. Owing to different complexation degrees with various charges on the complexes, the ions have different migration rates [74-78].

2.1.6.1.4.2 Off-line complexation

If the complexes of a metal with ligands are sufficiently stable under CE conditions, then off-capillary complexation is preferred. An excess of a strong complexing agent

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is added to the sample prior to the CE analysis. On–column UV-detection is possible as the fraction of the complexed ions is large. If there is a danger of the complex dissociation during the CE analysis, the complexation agent is added to the running buffer in a low concentration. The number of ligands do not affect the separation but the buffer components or competing complexing ligands do. Several conditions must be satisfied in off-line complexation. The ligand should react with a large number of metal cations and should suppress the original properties of the metals. The complexes should have a high UV absorbance, high solubility and a low electronegativity to prevent adsorption on the capillary wall.

Generally, partial complexation techniques gives significantly better separation selectivity, high efficiency and shorter analysis time [79-84].

2.1.7 Trouble shooting

Table 7 contains some troubleshooting guidelines. This table can be used as an aid in methods development as well as for troubleshooting of a previously established method. Most of these problems have been covered in the appropriate section of this chapter.

Table 7. Some troubleshooting guides [85].

Problem	Cause	Remedy
Base line too noisy	• Buffer solution polluted • Current too high • Detector lamp defect • pH too high • Broken capillary • Dirty detector window	• Replace buffer (perhaps degas or filtrate) • Cool capillary • Lower the voltage/current • Replace detector lamp • Lower pH • Change the capillary • Check the window
Base line shifts	• Different buffer solutions in capillary • pH too high • Too much strain ion window • Detector lamp defect	• Increase flush time for actual buffer solution • Lower pH • Bend capillary less • Replace detector lamp

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Continued

Small peak height	• Detector lamp off • Wrong wave length • Sample too old • Sample too much diluted • Detector is not sensitive • Plugged capillary • Air bubbles in capillary • Adsorption of substance on capillary wall • No voltage or voltage too low	• Switch lamp on • Check the wave length • Refresh the sample • Use more concentrated sample • Check the range of detector • Replace capillary or remove a few mm from the inlet side or retry • Flush with buffer at 2000 mbar, 1min • Increase the ionic strength of buffer • Turn voltage on or increase
Migration times drift	• Buffer evaporation • Buffer depletion Siphoning • Temperature of the capillary buffer has changed • Voltage is not stable • Inner wall of capillary change sample effect (bound disturb electrical field capillary)	• Replace buffer • Balance fluid levels between inlet and out let side • Reduce capillary diameter • Check the temperature of oven • Check voltage • Use the appropriate flushing procedures • Use wall coated capillary • Inject less sample • Increase buffer concentration • Change pH
Resolution gets worse	• Adsorption of substance on capillary wall	• Use the appropriate flushing procedures • Change to a wall coated capillary • Increase the ionic strength of the buffer
Broad peaks	• Large injection size • High ionic strength of sample solution • Siphoning • Solubility problem	• Reduce injection size • Dilute sample • Prepare sample in a low ionic strength buffer • Balance fluid levels of inlet and out let side • Add detergent and modifier to sample or buffer
Peak tailing	• Shard at capillary end	• Cut and clean capillary ends

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2.1.8 References

1. H.Siren, S.Vantsi, *J.Chromatogr.A*, 957 (2002), 17-26.
2. Song, L.G., *et al., J. Chromatogr., A,* 780 (1997), 297-328.
3. Y. –M.Liu, and J. -K.Cheng, *Electrophoresis*, 24 (2003), 1993-1994.
4. Carla Vogt, *et.al.*, *Fresenius J.Anal.Chem, 370* (2001), 316-318.
5. Douglas, B., *Concepts and models of Inorganic Chemistry*, 4 th edition, New York: John Wiley and sons, Inc.1994 pp 379-382.
6. Mark C., *et al.*, *Chemical engineering*, (1999), 86-90.
7. R.Portanova, *et al*, *Pure and Applied Chemistry*, **75**, No.4, 2003, pp 495-540
8. N.A.Guzman, *et al.*, *J. Chromatog.*, 697 (1997), 37-66.
9. J.Bashkin, *et al.*, *J. Capillary Electrophor.*, 3 (1996), 61-68.
10. Park, S.Lunte, C.E. *Anal. Chem.*, 67 (1995), 4366-4370.
11. Issaq, H., *Electrophoresis*, 18 (1997), 2438-2452.
12. Schewitz, J., *et al.*, *Analyst*, 123 (1998), 2835-2837.
13. F.Han, *et al.*, *J. Chromatogr., B,* 669 (1995), 103-112.
14. Weinberger Robert, *Practical Capillary Electrophoresis,* 2nd edition. Academic Press: New York (2000), 2.
15. N.Heiger David, *An Introduction to High Performance Capillary Electrophoresis*, Hewlett Packard: France (1992), 12.
16. Weinberger Robert, *Practical Capillary Electrophoresis,* 2nd edition. Academic Press: New York (2000), 62.

Stellenbosch University http://scholar.sun.ac.za

17. Y. -M.Liu, and J. -K.Cheng, *Electrophoresis*, 24 (2003), 1995.

18. W.M.Sunada, and H.W.Blanch, *Eletrophoresis*, 18 (1997), 2243-2254.

19. H.Kajiwara, *Journal of Chromatography*, 559 (1991), 345-356.

20. J W, Jorgenson, *Anal. Chem.*, 53 (1981), 1298-1302.

21. G.Bondonx, W.R.Jones, R.J.Jandik, *J. Chromatogr.*, 602 (1992), 76.

22. W.Liu, and H.K.Lee, *Electrophoresis*, 20 (1999), 2475-2483.

23. G.Dinelli, *et al*, *J. Chromatogr., A,* 773 (1996), 337-347.

24. P.K.Dasgupta, L.Bao, *Anal. Chem.*, 65 (1993), 1003.

25. A.Kumar Malik, W.Faubel, *Talanta*, 52 (2000), 341-346.

26. S.Mallet, *et al.*, *J. Chromatogr., A*, 853 (1999), 181-184.

27. K.D.Altria, *et al.*, *Journal of pharmaceutical and biomedical Analysis,* 13 (1995), 951-957.

28. Christa S.Burgisser, and Alant T.Stone, *Environmental Science Technology,* 31 (1997), 2656-2664.

29. M.Chiori, *et al.*, *Trends in analytical chemistry*, **17**, No.10 (1998), 623-631.

30. G.S.Wynia et al., *J. Chromatogr., A,* 773 (1997), 339-350.

31. P.R.Haddad, *J. Chromatogr., A*, 770 (1997), 289.

32. L.A.Dawson, *J. Chromatogr. B,* 697 (1997), 89-99.

33. K.R.Cooper, R.G.Kelly, *J. Chromatogr., A,* 850 (1999), 381-389.

34. Y. -S.Fung, and H. -S.Tung, *Electrophoresis*, 20 (1999), 1832-1841.

35. W.Lu, *et.al*, *J. Chromatogr., A,* 640 (1993), 433-440.

Stellenbosch University http://scholar.sun.ac.za

36. C.Desidero, S.Fanali, *J. Chromatogr., A,* 807(1998), 37-56.

37. W. -P.Yau, E.Chan, *Journal of biomed. Anal,* 28 (2002), 107-123.

38. V.Galli, *et al., J. Chromatogr., A,* 949 (2002), 367-372.

39. D.Scarcella, et al., *Forensic science International*, 89 (1997), 33-46.

40. S.Morales, R.Cela, *J. Chromatogr., A,* 846 (1999), 402.

41. Petra G., *et al, Anal. chem. news and Features*, May 1 (1999), 3315A.

42. Christain Gary, *Analytical chemistry*, 5th edition, John Wiley and Sons: New York (1994) 556.

43. E.Dabek –Zlotorzynska, *et al., Analytica Chimica Acta*, 359 (1998), 1-26.

44. Lauer, H.H., and Mc Manigill, D, *Anal. Chem.*, 587 (1986), 166-170.

45. Altria, K., and Simpson, C., *Anal.proc.*, 23 (1986), 453-454.

46. Huang, X, *et al., Anal.Chem.,* **61**. No.7 (1989), 766-770.

47. N.Heiger David, *An Introduction to High Performance Capillary Electrophoresis*, Hewlett Packard: France (1992), 21.

48. J.W.Jorgenson, and K.D.Lukacs, *High Resolution Chromatgr.,*.4 (1981), 202.

49. Bjergegaard, C., *et al., Chromatography and Capillary electrophoresis in food Analysis,* Royal society of chemistry: UK (1999), 211.

50. R.T.Kennedy, *Analytical Chimica Acta,* 400 (1999), 163-180.

51. N.Heiger David, *An Introduction to High Performance Capillary Electrophoresis*, Hewlett Packard: France (1992), 24-84.

52. S.Morales, R.Cela, *J.Chromatogr., A,* 846 (1999), 403.

Stellenbosch University http://scholar.sun.ac.za

53. Rose, D.J., *et al.*, *Anal. Chem.*, 60 (1988), 642.

54. Weinberger Robert, *Practical Capillary Electrophoresis,* 2nd edition. Academic Press: New York (2000), 325-327.

55. Wehr, T., *Capillary electrophoresis of proteins*, **80**, (1999). pp.14-15.Marcel Dekker: New York.

56. Weinberger Robert, *Practical Capillary Electrophoresis,* 2nd edition. Academic Press: New York (2000), 331.

57. Andrew G.Ewing, *et al.*, *Anal. Chem.,* **61**, No.4, (1989), 292A-301A.

58. Werner G.Kuhr, *Anal. Chem.*, **62**, No.12, (1990), 403R-413R.

59. Kuhr, W.G., *et al,. Anal. Chem.*, 60 (1988), 2642-2644.

60. Lukacs, K.D., *et al High Resolution.Chromatgr.Commun.*8 (1985), 407-411.

61. Andrew G.Ewing, *et al.*, *Anal. Chem.,* **66,** No.9, (1994), 529A.

62. A.R.Timerbaev, and O.A.Shpigun, *Electrophoresis*, 21 (2000), 4179-4191.

63. Carla Vogt. Gregory, L.Klunder, *Fresenius J. Anal.Chem,* 370 (2001), 328.

64. T.Kappes, P.C. Hauser, *Electroanalytsis,* **12**, No.3, (2000), 165-170.

65. B.Liu *et al., Journal of Chromatography A*, 834 (1999), 277-308.

66. C.Vogt, G.L.Klunder, *Fresenius Journal of Anal.Chem.*370 (2001), 316.

67. S.Hjerten, *Chromatogr.Rev.*, 9 (1967), 122,

68. V.Pacakova, *et al., J. Chromatogr., A*, 834 (1999), 257.

69. A.R.Timerbaev, *J. Capillary. Electrophor.*, 2 (1995), 165.

70. B.Liu *et al., J. Chromatogr., A,* 834 (1999), 287.

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71. T.Soga, G.A.Ross, *J. Chromatogr., A,* 767 (1997), 223-230.

72. Carla Vogt, *et.al.*, *Fresenius J.Anal.Chem, 370* (2001), 318.

73. A.Padarauskas, G.Schwedt, *J. Chromatogr., A*, 773 (1997), 351-360.

74. V.Pacakova, *et al.*, *J. Chromatogr., A,* 834 (1999), 259.

75. F.Foret, *et al*, *Electrophoresis*, 11 (1990) 780.

76. A.Weston, *et al.*, *J. Chromatogr,* 593 (1992), 289.

77. A. Weston *et al.*, *J. Chromatogr,*.602(1992), 249

78. Y.Shi, Js., *et al.*, *J. Chromatogr,*.640 (1993), 473.

79. V.Pacakova, *et al.,*. *J. Chromatogr, A,* 834 (1999), 261.

80. Motomizu, *et al., Anal. Sci.,* 7 (1991), 253.

81. A.R.Timerbaev, *et al, J. Chromatogr.*, 630 (1993), 379.

82. N.iki, *et al, Chem lett.*, 4 (1993), 701.

83. A.R Timerbaev, *et al*, *Analyst,* 119 (1994), 2795.

84. Conradi, *et al, J. Chromatogr, A,*.745 (1996), 103.

85. *Preliminary service manual*, Prince Technologies, (1996), pp. 58-61.

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3. Experimental procedures

3.1 Experimental conditions for metal ion detection

The CE analysis was performed on Prince Technologies (Emmen, Netherlands) capillary electrophoresis system equipped with power supply (0 - ±35kV) and UV detector (PU 4225 UV detector, Philips) with wavelength of 190 - 820 nm. Fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) of 75 µm I.D, (360 µm O.D) and 108.5 cm long (96.5 cm effective length) were used. The samples were injected using hydrodynamic mode (50 mbar). Data acquisition and analysis (DAx) soft ware from Prince Technologies was used for the control of instrument settings. All experiments were conducted at 25 ^{0}C. The current was monitored for all evaluated background electrolytes and was in the range of 7 to 11 µA.

A typical Capillary electrophoresis instrument used for the analysis of cations and anions is given in figure 4.

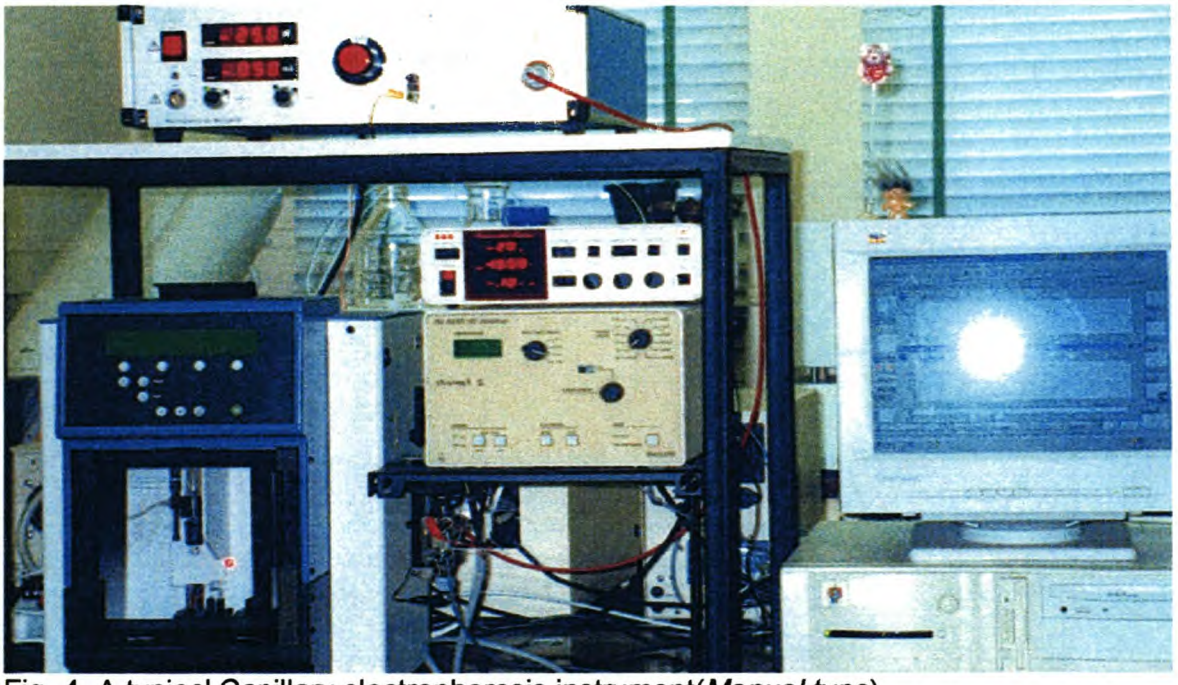

Fig. 4. A typical Capillary electrophoresis instrument(*Manual type*)

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All electrolytes were prepared daily using Milli-Q water through dilution of an appropriate amount of the UV-absorbing buffer compound and the complexing reagent.

Different methods have been investigated for the separation and detection of metal ions. The methods are listed below.

3.1.1 Glycolic acid-pyridine background electrolyte

In this method the following experimental conditions were used.

- Glycolic acid (13 mM), pyridine (10 mM)
- Analyte concentration : 10 ppm
- Applied voltage : +25 kV
- Wavelength : 254 nm
- Injection pressure : 50 mbar- hydrodynamic injection
- Injection time : 24 s
- pH : 4.0 adjusted with 0.1 M HCl
- Current : 7 µA

3.1.2 α-Hydroxyisobutyric acid-4-amino pyridine background electrolyte

Better base line noise, resolution and selectivity were obtained under the following experimental conditions.

- α-HIBA (6.5 mM) and 4-Amino pyridine (10 mM)
- Analyte concentration : 10 ppm

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- Applied voltage : +25 kV
- Wavelength : 214 nm
- Injection pressure : 50 mbar-hydrodynamic injection
- Injection time : 18 s
- pH : 4.5 adjusted with 0.1 M HCl
- Current : 9 µA

3.1.3 Glycolic acid-imidazole background electrolyte

This background electrolyte was used for the analysis of base metals and transition metals. In the analysis the following experimental conditions were used.

- Glycolic acid (15 mM), imidazole (12 mM)
- Analyte concentration : 10 ppm
- Applied voltage : +25 kV
- Wavelength : 214 nm
- Injection pressure : 50mbar- hydrodynamic injection
- Injection time : 24 s
- pH : 4.29 adjusted with 0.1 M HCl
- Current : 10 µA

3.1.4 α-HIBA-imidazole background electrolyte

This method was used for metal ions detection using the following experimental

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conditions.

- α-HIBA (7 mM) and imidazole (10 mM)
- Analyte concentration : 10 ppm
- Applied voltage : +25 kV)
- Wavelength : 210 nm
- Injection pressure : 50 mbar- hydrodynamic injection
- Injection time : 12 s
- pH : 4.5 adjusted with 0.1 M HCl
- Current : 9 µA

3.1.5 Mixtures of complexing agent and imidazole

- α-HIBA (7 mM), glycolic acid (13 mM)) and imidazole (10 mM)
- Analyte concentration : 10 ppm
- Applied voltage : +25 kV
- Wavelength : 214 nm
- Injection pressure : 50 mbar- hydrodynamic injection
- Injection time : 24 s
- pH : 4 adjusted with 0.1 M HCl
- Current : 8 µA

3.2 Experimental conditions for anion detection

The same equipment was also used for anion analysis. All experiments were

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conducted at 25 ^{0}C. The current was monitored for all evaluated back ground electrolytes and was in the range of 10 to 13 µA. All electrolytes were prepared daily using Milli-Q water through dilution of an appropriate amount of the UV-absorbing buffer compound and the complexing reagent

Different methods have been used for the separation and detection of inorganic and organic acids anions. The following parameters were used for inorganic and organic anions analysis.

3.2.1 The experimental condition for inorganic anions

In this method the following experimental condition was used.

- K_2CrO_4 (5 mM), Boric acid (3 mM), CTAB (35 µM) and EDTA(12 µm)
- Analyte concentration : 30 ppm
- Applied voltage : -20 kV
- Wavelength : 200 nm
- Injection pressure : 50mbar-hydrodynamic injection
- Injection time : 12 s
- pH : 8.2 adjusted with 1 M NaOH
- Current : 13 µA

3.2.2 The experimental conditions organic acid anions

In this method the following experimental condition was used.

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- PDC (5 mM), CTAB (0.5 mM)
- Analyte concentration : 30 ppm
- Applied voltage : -25 kV
- Wavelength : 200 nm
- Injection pressure : 50 mbar- hydrodynamic injection
- Injection time : 12 s
- pH : 5.1 adjusted with 1M NaOH
- Current : 10 µA

In both experimental conditions, a 75 µm capillary with total length of 96.5 cm (84.5 cm effective length) was used.

3.3 Reagents and chemicals

All chemicals and reagents were purchased commercially and used as received. The ultra pure water that was prepared in Milli-Q purification unit.

Table 8. List of reagents and chemicals used for the project.

Chemicals	**Manufacturer and Grade**
KCl	PAL(chemically pure reagent)
NaCl	PAL (analytical reagent -99%)
$MgCl_2.6H_2O$	Merck (99%)
$CaCl_2.2H_2O$	NT Laboratory Supplies(Chemically pure)
$FeSO_4.7H_2O$	NT Laboratory Supplies (Chemically pure -97%)
Manganese Solution (1000 ppm)	Fluka(spectro sol)
Cadmium Solution (1000 ppm)	Fluka(spectro sol)
2, 6 Pyridine dicarboxylic acid	ACROS ORGANICS (99%)

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Continued

Boric acid	Merck (99.8%)
Ethylene diamine acetic acid	NT (analytical reagent-99%)
NaF	ANALAR (analytical reagent-99%)
NH_4NO_3	NT (analytical reagent-99%)
Na_2SO_4	ANALAR (analytical reagent-99%)
Cetyl trimethyl ammonium bromide	Sigma (99%)
Zinc Solution (1000 ppm)	Fluka (spectro sol)
Lead Solution (1000 ppm	Fluka(spectro sol)
Nickel Solution (1000 ppm)	Merck (spectro sol)
Copper Solution (1000 ppm)	Merck (spectro sol)
2-Hydroxyisobutyric acid (α-HIBA)	Aldrich (98%)
4-Amino pyridine	Aldrich (98%)
Imidazole	Sigma (99%)
Glycolic acid	Fluka (99%)
Pyridine	Riedel-dehaen (99%)
HCl solution	Riedel-dehaen (37%)
NaOH	Riedel-dehaen (99%)
Ethanol absolute	Riedel-dehaen (99.8 %)
Phosphoric acid	Aldrich (85%)
K_2CrO_4	General-purpose reagent (99%)
Tartaric acid	Merck (99.5%)
Citric acid	Merck (99.5%)
Malonic	Sigma (95-100%)
Succinic acid	ACROS (99%)

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3.3.1 Preparation of Standard solutions

Preparation of Glycolic - pyridine buffer solution. 0.198 g of glycolic acid and 0.161 ml of pyridine were diluted with Milli-Q water to the mark in a 200 ml volumetric flask.

Preparation of Glycolic - imidazole buffer solution. 0.228 g of glycolic acid and 0.163 g of imidazole were diluted with Milli-Q water to the mark in a 200 ml volumetric flask.

Preparation of 2-Hydroxyisobutyric acid (α-HIBA) - imidazole buffer solution. 0.145 g of 2-Hydroxyisobutyric acid (α-HIBA) and 0.136 g of imidazole were diluted with Milli-Q water to the mark in a 200 ml volumetric flask.

Preparation of 2-Hydroxyisobutyric acid (α-HIBA) - 4-amino pyridine buffer solution. 0.135 g of 2-Hydroxyisobutyric acid (α-HIBA) and 0.188 g of amino pyridine were diluted with Milli-Q water to 200 ml in a volumetric flask.

Preparation of 2-Hydroxyisobutyric acid (α-HIBA), Glycolic acid and imidazole buffer solution. 0.145 g of 2-Hydroxyisobutyric acid (α-HIBA), 0.198 g of glycolic acid and 0.136 g of imidazole were dissolved in water and made up to 200 ml in a volumetric flask.

Preparation of 2, 6 Pyridine dicarboxylic acid - Cetyl trimethyl ammonium bromide buffer solution. 0.167 g of 2, 6 pyridine dicarboxylic acid and 0.03645 g

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Cetyl trimethyl ammonium bromide were dissolved in diluted water and made up to 200 ml in a volumetric flask.

Preparation of Potassium Chromate, Cetyl trimethyl ammonium bromide, Boric acid and Ethylene diamine acetic acid buffer solutions. 0.097 g of potassium Chromate, 0.0013 g of Cetyl trimethyl ammonium bromide, 0.0185 g of Boric acid and 0.00045 g of Ethylene diamine acetic acid were dissolved in water and diluted to 100 ml in a volumetric flask.

Preparation of metal standard solutions. Transition metals (Mn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}) were prepared by diluting 0.100 ml of the metal solution (each 1000 ppm) with Milli-Q water in a 10 ml volumetric flask. The common metals (K^{+}, Na^{+}, Mg^{2+}, Ca^{2+}) and Fe^{2+} standard solutions were prepared by weighing 0.1 g of the respective salt and diluting with Mill-Q water in a 100 ml volumetric flask. 10 ppm was prepared by diluting 0.100 ml of the metal solution with Milli-Q water in a 10 ml volumetric flask.

Preparation of calibration curve. A calibration curve was prepared with 5 ppm, 25 ppm, 50 ppm, 100 ppm and 200 ppm measuring 25 µl, 125 µl, 250 µl, 500 µl and 1000 µl respectively of each base metal ions in 5 ml volumetric flask. The same calibration procedure was used in the anion study.

Preparation of anion standard solutions. Anion standard solutions were prepared from sodium salts or free acids by weighing 0.01 g anion and diluting with Milli-Q

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water in a 10 ml volumetric flask.

Preparation of 1.0 M of NaOH Solution. 4 g of NaOH was dissolved in Milli –Q water and then diluted with to the mark in a 100 ml volumetric flask.

Preparation of 0.1 M of HCl Solution. 0.8 ml of Concentrated (37%) HCl was diluted with Milli-Q water to the mark in a 100 ml volumetric flask.

3.4 Capillary conditioning

The capillary was flushed before sample injection using a 1 M NaOH solution (10 min), Milli-Q water-ultra pure water (10 min) and running electrolyte (10 min) and the sample was injected with the appropriate method. Between each run the capillary was rinsed with running electrolyte for 4 min.

3.5 Sampling and sample handling

Environmental water samples were sampled from river, lake and reservoir areas surrounding the Stellenbosch. Tap water was used from a sink in the laboratory. The water samples were collected in polyethylene containers and acidified and then kept in refrigerator at 4 ^{0}c. Samples (Multimin and Mineral Max), which are used for animal protection, were also analyzed. The wine sample provided by the laboratory for separation sciences at Stellenbosch University. The orange juice and the beer were bought from the market.

The same environmental water sampling and sample handling was used from all water sites.

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Typical water sampling from University of Stellenbosch Gymnasium area (river) is shown in figure 5.

Fig. 5. Environmental water sampling near University of Stellenbosch Gymnasium area (river).

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4. Results and discussion for metal ions

The separation was performed using the Capillary zone electrophoresis (CZE) since it is a commonly used method for metal ion analysis [1]. The normal electro osmotic flow (EOF) phase was used [2]. The most important optimization parameter for the separation of cationic compounds is the choice of a suitable cationic background electrolyte (BGE) [3-5]. In these studies, three BGEs were used, that is, pyridine, imidazole and 4-aminopyridine. These background electrolytes all absorb strongly in the UV region and all have similar mobilities.

Determination of metal ions in aqueous samples using electrophoresis was accomplished with indirect UV-detection. For optimal determination of alkali, alkaline earth, and transition metal ions, several electrolyte systems were studied. Detection at different wavelength was performed with back ground electrolyte containing reagents with inherent absorbance in the UV range: pyridine, imidazole, and 4-aminopyridine. Glycolic acid and α-hydroisobutyric acid were also used as complexing reagents [6-8]. With these background electrolytes, a successful separation of mixture of cations (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, and Zn^{2+}) was obtained.

Inorganic cations are small and thus have higher charge densities (q/r ratios) than most organic ions. The problems connected with CE analysis of inorganic cations are due to small differences in their migration rates. Only the alkali metal ions exhibit large differences in their mobilities and can be easily separated. Bivalent and trivalent cations, such as those of the alkaline earth, rare earth and transition metals cannot

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be separated in a simple electrolyte as their mobilities are similar. An exception is the Pb^{2+} ion with higher mobility due to its smaller hydrated radius resulting from a lower degree of hydration. Hence complexation reaction of the cations is employed to enhance the differences in their mobilites [9, 10]. Different methods were developed for cations and are discussed below.

4. 1 Separation of metal ions using glycolic acid as the complexing agent and pyridine as background electrolyte:

Pyridine is a good UV-absorbing additive in CE cation separations. At λ = 254 nm the pyridinium cation has both good sensitivity and shows a stable background [11]. A mixture of 9 metals (as complexes) could be separated using pyridine as shown in (Fig. 6a). All peaks were completely resolved with a resolution R>1. Detection of Cd^{2+} in this system was problematic because of the very small peak height. Small peak distortion of Pb^{2+}, Ni^{2+} and Zn^{2+} peaks were caused by the formation of relatively stable complexes with glycolic acid with only small mobility [12].

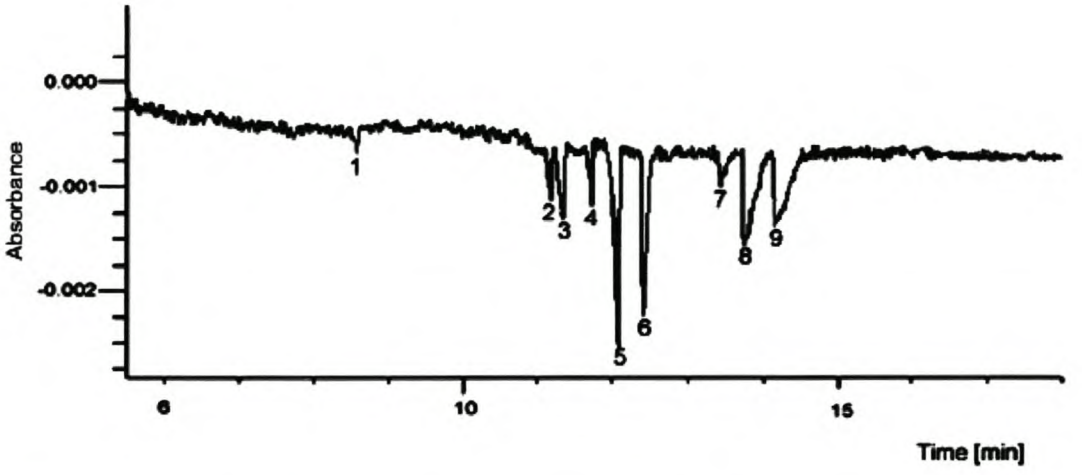

Fig. 6a. Electrophoretic separation of 9 metal ions. Carrier electrolyte, 10 mM pyridine – 13 mM glycolic acid (pH 4.0); hydrodynamic injection 24 s: voltage, 25 kV,wavelength, 254 nm. Peaks according to sequence: 1=K^{+}, 2=Na^{+}, 3=Ca^{2+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Pb^{2+}, 8=Ni^{2+}, 9=Zn^{2+}.

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The overlap of different electropherograms in figure 6b show the degree of reproducibility among the different runs for metal ions mentioned before.

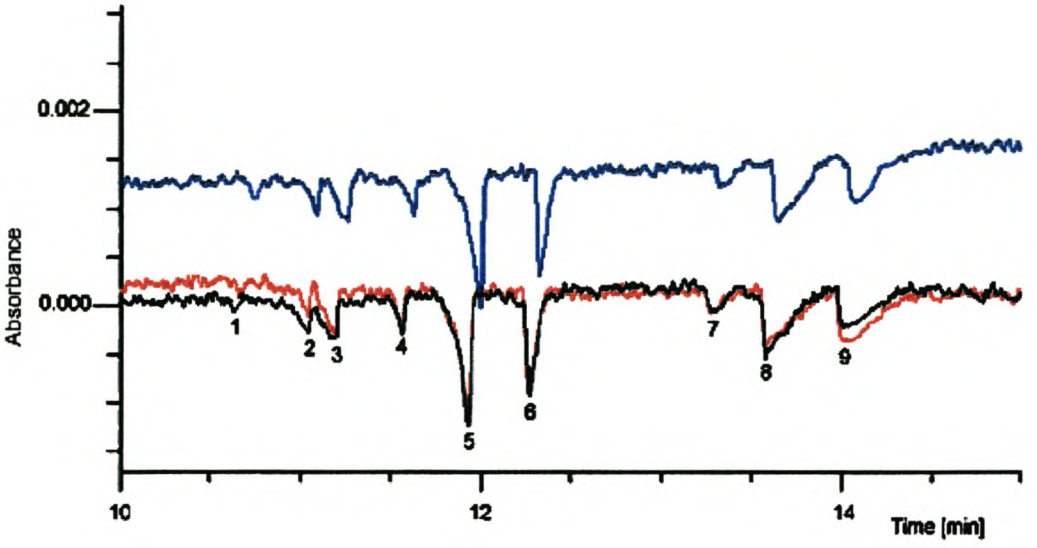

Fig. 6b. The reproducibility of metal ions over three runs (within runs). Experimental condition is the same as in fig. 1a. Peaks according to sequence: 1=K^{+}, 2=Na^{+}, 3=Ca^{2+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Pb^{2+}, 8=Ni^{2+}, 9=Zn^{2+}.

The migration time, peak area and corrected peak area is included in table 9. The corrected peak areas were obtained by dividing peak areas of each metal ion to its migration time. A lower relative standard deviation (RSD) was obtained for the corrected peak area (tables 9, 10, 11, 12 and 13).

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Table 9. The migration time, peak area and corrected peak area of metal ions at a concentration of 10 ppm (n = 6).

Metal ion	Migration time, min (RSD)	Peak area, (RSD)	Corrected peak area, (RSD)
K^+	8.61(±1.65%)	2.12E-05(±6.57%)	2.46E-06(±4.88%)
Na^+	11.21(1.89%)	2.47E-05(±9.70%)	2.20E-06(±8.09%)
Ca^{2+}	11.37(±1.90%)	5.02E-05(±4.06%)	4.41E-06(±3.92%)
Mg^{2+}	11.78(±1.77%)	3.36E-05(±9.86%)	2.85E-06(±9.45%)
Mn^{2+}	12.17(±1.75%)	1.27E-04(±12.89%)	1.04E-05(±11.51%)
Fe^{2+}	12.49(±1.77%)	9.69E-05(±12.11%)	7.74E-06(±10.21%)
Pb^{2+}	13.50(±1.86%)	2.76E-05(±11.46%)	2.04E-06(±10.44%)
Ni^{2+}	13.85(±1.89%)	1.17E-04(±13.99%)	8.42E-06(±12.56%)
Zn^{2+}	14.29(±1.94%)	1.23E-04(±12.21%)	8.62E-06(±10.61%)

As it shown in the above table, the relative standard deviations of the corrected peak areas were lower than the peak areas. Similar comparisons were obtained with other methods that are shown in the upcoming tables.

The main pitfalls of the method were that it was not sensitive enough for the detection of Cd^{2+}. The sensitivity of K^+ was also low. The reproducibility among the electropherograms of the metal ions was good as shown in figure 6b.

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4.2 Separation of metal ions using glycolic acid as the complexing agent and imidazole as background electrolyte

A mixture of 9 metal ions could be separated using imidazole as UV-absorbing species (fig 7a). All peaks were completely resolved. Detection of Pb^{2+} in this system was problematic because of its small peak height. Due to low absorbance of the Pb-glycolic acid complex. The detection sensitivity of Pb^{2+} can be improved using α-hydroxyisobutyric acid (α-HIBA) as complexing agent (fig 8a). The migration time, peak area and corrected peak area of the metal ions is shown in table 10.

Table 10. The migration time, peak area and corrected peak area of metal ions at a concentration of 10 ppm (n = 6).

Metal ion	Migration time, min (RSD)	Peak area, (RSD)	Corrected peak area, (RSD)
K^+	8.75(±1.39%)	3.85E-05(±10.17%)	4.39E-06(±9.36%)
Na^+	11.78(±1.63%)	9.30E-05(± 6.84%)	7.89E-06(±5.75%)
Ca^{2+}	11.94(±1.67%)	5.41E-05(4.13%)	4.53E-06(±5.19%)
Mg^{2+}	12.46(±1.71%)	5.30E-05(±6.27%)	4.25E-06(±5.83%)
Mn^{2+}	12.86 (±1.70%)	2.13E-04(±3.83%)	1.66E-05(±2.97%)
Fe^{2+}	13.63 (±1.72%)	4.08E-05(±13.16%)	2.99E-06(±12.13%)
Cd^{2+}	13.89 (± (1.72%)	1.11E-04(±14.05%)	7.99E-06(±13.44%)
Ni^{2+}	15.614(±1.83%)	2.25E-04(±6.74%)	1.44E-05(±6.39%)
Zn^{2+}	16.31(±1.83%)	2.20E-04(±4.98%)	1.35E-05(±5.12%)

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The electropherograms obtained using glycolic acid and imidazole as complexing agent and as UV-absorbing species respectively for mixtures of 9 metal ions are given below. The reproducibility of the method for three runs (red, black and blue) is shown in figure 7b. Peaks 8 and 9 are distorted due to the strong complexes formed between Ni^{2+} and glycolic acid and Zn^{2+} and glycolic acid.

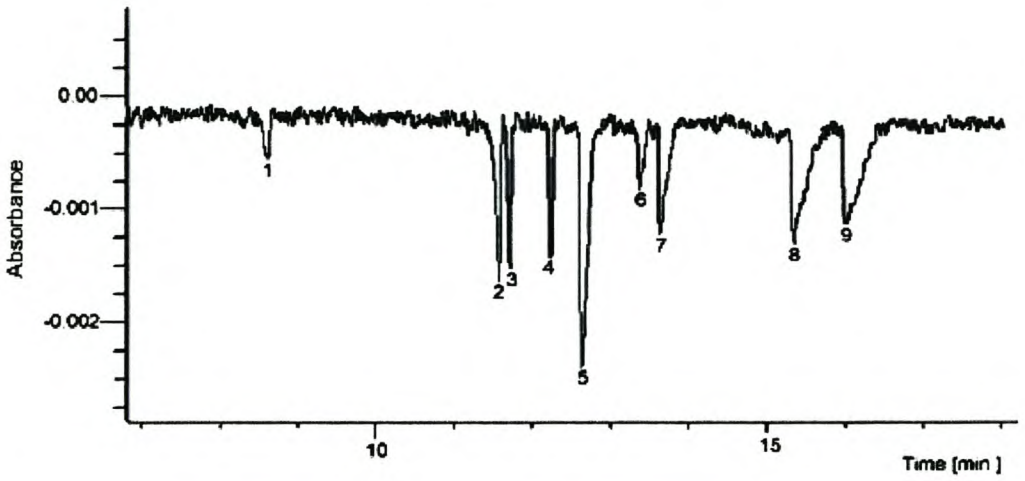

Fig. 7a. Electrophoretic separation of 9 metal ions. Carrier electrolyte, 12 mM imidazole – 15 mM glycolic acid (pH 4.29); hydrodynamic injection 24 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=K^+, 2=Na^+, 3=Ca^{2+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7= Cd^{2+}, 8=Ni^{2+}, 9=Zn^{2+}.

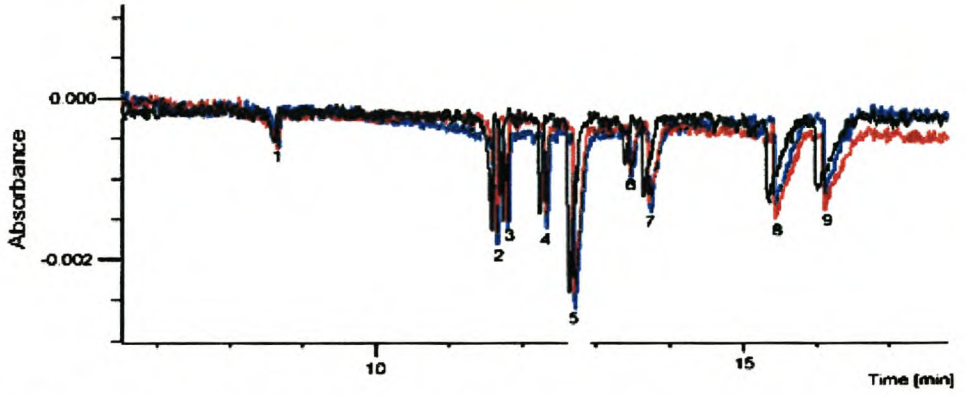

Fig.7b.The reproducibility of metal ions over three runs (within runs). Experimental condition is the same as in fig.6a. Peaks according to sequence: 1=K^+, 2=Na^+, 3=Ca^{2+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Cd^{2+}, 8=Ni^{2+}, 9=Zn^{2+}.

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4.3 Separation of metal ions using α-HIBA as the complexing agent and imidazole as background electrolyte

The use of a different complexing system influences the separation of the ions. With α-HIBA Cd^{2+} could be resolved. The K^{+} and Fe^{2+} peaks are small due to low sensitivity of the method towards those ions. The use of α-HIBA as a complexing reagent allows the detection of Pb^{2+} that could not be detected using glycolic acid as complexing reagent. The uses of different complexing agents affect the mobilities of the ions. The mobility of metal ions using α-HIBA as complexing agent is generally higher than when glycolic acid is used as complexing agent (tables 10 and 11). However, the order of migration time of the ions remains the same. The migration time, peak area and corrected peak area with α-HIBA - imidazole buffer are shown in table 11.

Table 11. The migration time, peak area and corrected area of the metal ions at a concentration of 10 ppm (n = 6).

Metal ion	Migration time, min (RSD)	Peak area, RSD	Corrected peak area, RSD
K^{+}	7.68(±0.46%)	2.66E-05(±9.21%)	3.46E-06(±9.24%)
Na^{+}	9.71(±0.45%)	2.33E-05(±11.01%)	2.4E-06(±10.56%)
Ca^{2+}	10.08(±0.43%)	3.67E-05(±2.33%)	3.64E-06(±2.28%)
Mg^{2+}	10.40(±0.42%)	1.96E-05(±10.64%)	1.89E-06(±10.57%)
Mn^{2+}	10.58(±0.40%)	8.39E-05(±2.24%)	7.93E-06(±1.88%)
Fe^{2+}	10.96(±0.40%)	1.56E-05(±10.73%)	1.42E-06(±10.61%)
Pb^{2+}	11.30(±0.38%)	4.53E-05(±5.41%)	4.01E-06(±5.43%)
Ni^{2+}	11.60(±0.56%)	5.67E-05(±10.57%)	4.88E-06(±10.26%)
Zn^{2+}	11.75(±0.41%)	3.60E-05(±4.88%)	3.06E-06(± 4.58%)

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The electropherograms showing a mixture of 9 metal ions and the overlapped for three runs are indicated below (fig 8a and 8b). In figure 8a, the peak shape for Fe^{2+}, Pb^{2+}, Ni^{2+} and Zn^{2+} is slightly distorted, showing the formation of strong complexes.

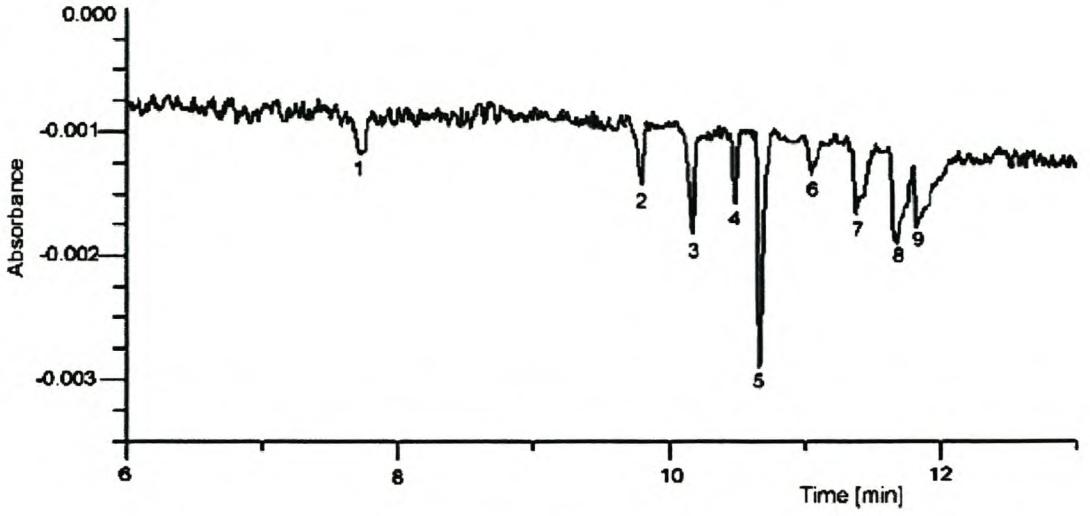

Fig 8a. Electrophoretic separation of 9 metal ions. Carrier electrolyte, 10 mM imidazole – 7 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 24 s: voltage, 25 kV, wavelength, 210 nm. Peaks according to sequence: 1=K^+, 2=Na^+, 3=Ca^{2+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Pb^{2+}, 8=Ni^{2+}, 9=Zn^{2+}.

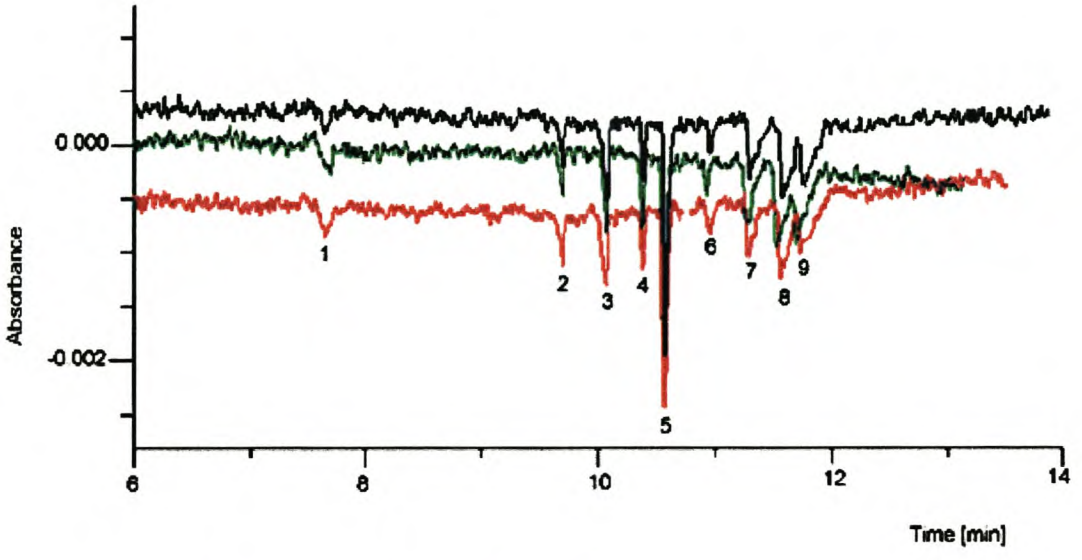

Fig. 8b. The reproducibility of metal ions over three runs using the same experimental conditions as in fig. 8a. Peaks according to sequence: 1=K^+, 2=Na^+, 3=Ca^{2+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Pb^{2+}, 8=Ni^{2+}, 9=Zn^{2+}.

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4.4 Separation of metal ions using α-HIBA as the complexing agent and 4-amino pyridine as background electrolyte:

α-hydroxyisobutyric acid as complexing agent and 4-aminopyridine as background electrolyte is the most efficient method for separation of a mixture of ions. A mixture of 10 metals could be separated efficiently using 4-aminopyridine (Fig 9a). All peaks were completely resolved. With this method it was possible to detect Cd^{2+} and Pb^{2+}.

Table 12. The migration time, peak area and corrected peak area of metal ions at a concentration of 10 ppm (n = 6).

Metal ion	Migration time, min (RSD)	Peak area, (RSD)	Corrected peak area, (RSD)
K^+	7.99(±1.74%)	3.65E-05(±6.74%)	4.57E-06(±6.50%)
Ca^{2+}	10.04(±2.79%)	3.87E-05(±7.13 %)	3.86E-06(±5.26%)
Na^+	10.41(±2.83%)	6.47E-05(±4.85%)	6.22E-06(± 4.72%)
Mg^{2+}	10.77(±3.03%)	3.43E-05(±7.64%)	3.19E-06(±6.48%)
Mn^{2+}	11.07(±3.16%)	0.000138(±8.05%)	1.25E-05(±5.44%)
Fe^{2+}	11.38(±3.36%)	2.01E-05(±4.85%)	1.76E-06(±3.70%)
Cd^{2+}	11.69(±3.51%)	7.20E-05(±5.86%)	6.17E-06(±2.97%)
Pb^{2+}	11.83(±3.81%)	1.67E-05(±12.04%)	1.42E-06(±11.83%)
Ni^{2+}	12.023(±3.84%)	1.32E-04(±8.46%)	1.10E-05(±5.72%)
Zn^{2+}	12.21(±3.94%)	1.20E-04(±8.36%)	9.85E-06(±5.27%)

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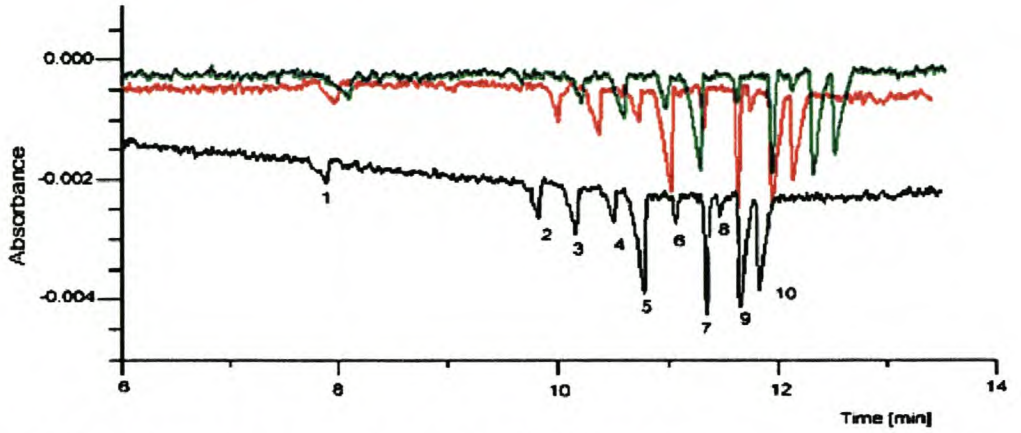

Fig. 9c. The reproducibility of metal ions over three runs (between runs) using the same experimental conditions as in fig. 9a. Peaks according to sequence: 1=K^{+}, 2=Ca^{2+}, 3=Na^{+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Cd^{2+}, 8=Pb^{2+}, 9=Ni^{2+}, 10=Zn^{2+}.

Higher reproducibility was obtained over three runs (within runs) as shown in figure 9b and the reproducibility decreases over three runs (between runs), in this plot a drift to some degree in migration time (especially with that of Ni^{2+} and Zn^{2+}) was observed (figure 9c). This may be due to capillary buffer temperature change, buffer evaporation and low stability of voltage over time that was also seen with other runs. Capillary electrophoresis suffers from instability and irreproducibility of migration times and peak areas with time [31].

4.5 Separation of metal ions using mixtures of the complexing agent and imidazole as background electrolyte

Mixture of complexing agent (α-HIBA and glycolic acid) with imidazole as UV-absorbing species have been investigated and 10 metal ions have been separated. A very small peak for Pb^{2+} was obtained but it was problematic to integrate.

The migration time, peak area and corrected peak area of the metal ions at a concentration of 10 ppm is shown in table below. Very good overlap among

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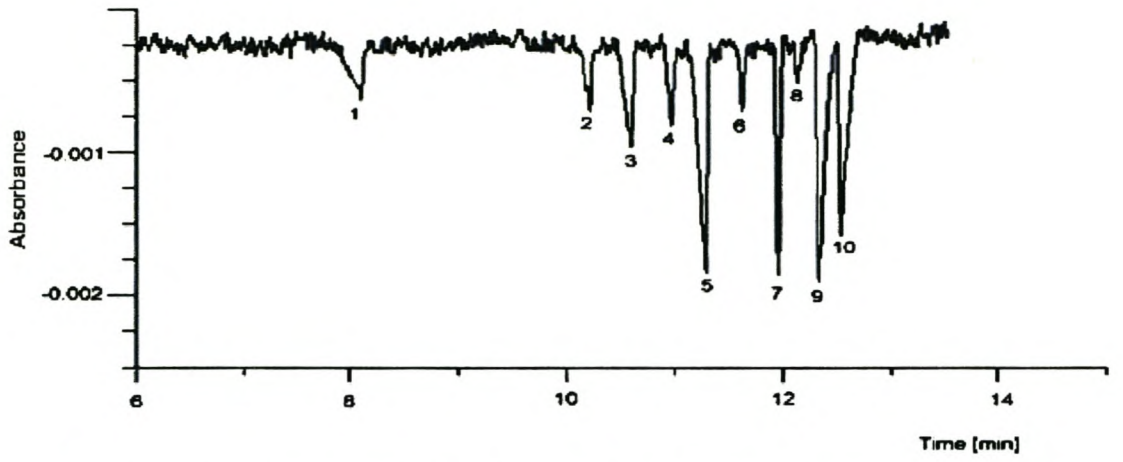

Fig 9a. Electrophoretic separation of 10 metal ions. Carrier electrolyte, 10 mM 4-amino pyridine - 6.5 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 18 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=K^{+}, 2=Ca^{2+}, 3=Na^{+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Cd^{2+}, 8=Pb^{2+}, 9=Ni^{2+}, 10=Zn^{2+}.

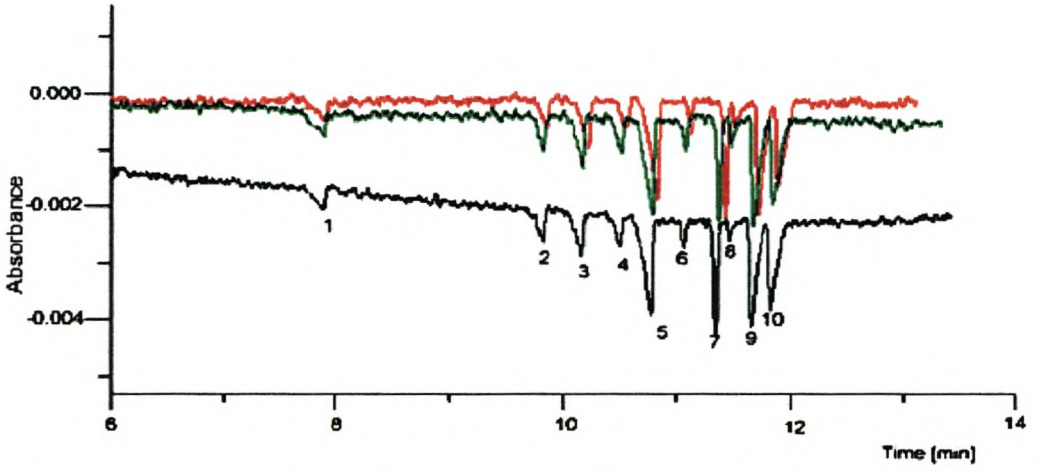

Fig. 9b. The reproducibility (within runs) of metal ions over three runs using the same experimental conditions as in fig. 9a. Peaks according to sequence: 1=K^{+}, 2=Ca^{2+}, 3=Na^{+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Cd^{2+}, 8=Pb^{2+}, 9=Ni^{2+}, 10=Zn^{2+}.

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electropherograms were obtained (fig. 10b)

Table 13. The migration time, peak area and corrected peak area of metal ions at a concentration of 10 ppm (n = 6).

Metal ion	Migration time, min (RSD)	Peak area, (RSD)	Corrected peak area, (RSD)
K^+	8.48(±0.81%)	3.40E-04(±3.28%)	4.01E-06(±3.17%)
Na^+	11.24(±1.02%)	5.33E-05(±6.49%)	4.74E-06(±6.94%)
Ca^{2+}	11.33(±1.05%)	3.76E-05(±7.62%)	3.31E-06(±6.99%)
Mg^{2+}	11.84(±1.07%)	5.26E-05(±3.07%)	4.45E-06(±3.51%)
Mn^{2+}	12.19(±1.08%)	2.13E-04(±6.40%)	1.75E-05(±6.59%)
Fe^{2+}	12.76(±1.10%)	1.07E-04(±4.84%)	8.36E-06(±4.60%)
Cd^{2+}	13.00(±1.06%)	2.59E-05(±2.37%)	1.99E-06(±2.06%)
Ni^{2+}	14.56(±1.16%)	2.10E-04(±3.01%)	1.44E-05(±3.20%)
Zn^{2+}	15.17(±1.08%)	2.10E-04(±3.01%)	1.38E-05(±2.06%)

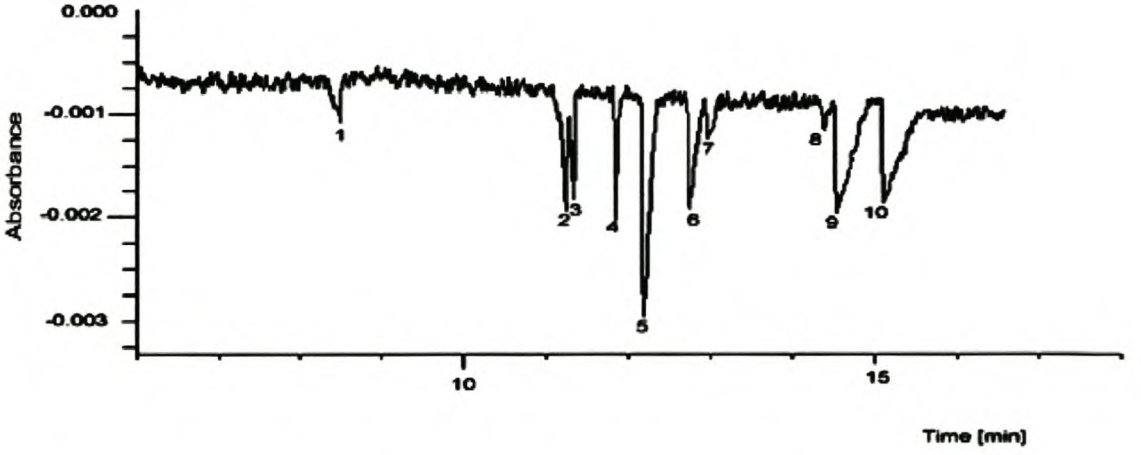

Fig. 10a. Electrophoretic separation of 9 metal ions. Carrier electrolyte, 10 mM imidazole – 7 mM α-hydroxylisobutyric acid and 13mMglycolic acid (pH 4.0); hydrodynamic injection 24 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=K^+, 2=Ca^{2+}, 3=Na^+, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Cd^{2+}, 8=Pb^{2+}, 9=Ni^{2+}, 10=Zn^{2+}.

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The peak for Pb^{2+}, Ni^{2+} and Zn^{2+} is distorted due to the formation of strong complexes. The relative standard deviations, RSD, for Ni^{2+} and Zn^{2} using a mixture of complexing agent are lower compared with the other complexing agents, showing lower peak distortion.

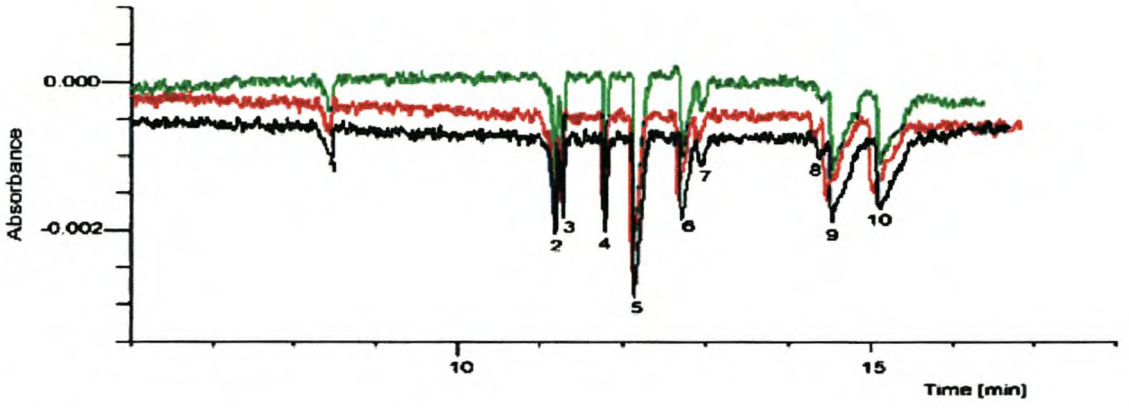

Fig. 10b. The reproducibility of metal ions over three runs (within runs). Experimental condition as in fig. 10a. Peaks according to sequence: 1=K^{+}, 2=Ca^{2+}, 3=Na^{+}, 4=Mg^{2+}, 5=Mn^{2+}, 6=Fe^{2+}, 7=Cd^{2+}, 8=Pb^{2+}, 9=Ni^{2+}, 10=Zn^{2+}.

Generally, good electropheragrams of a mixture of metal ions with different methods were obtained. The detection of Cd^{2+} and Pb^{2+} was problematic with glycolic acid complexing agent. The peak area for K^{+} was small. The mobility of metal ions was the highest for K^{+} and lowest for Zn^{2+} depending on the charge to mass ratio of each metal ion [9, 10]. The order of migration time for metal ions in the mixture was found to be similar to previous studies by other researchers [12].

4.6 The analysis of synthetic and environmental samples using α-Hydroxyisobutyric acid-4-amino pyridine background electrolyte system

4.6.1 Analysis of synthetic samples

α-Hydroxyisobutyric acid-4-amino pyridine background electrolyte was chosen for the

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analysis of real samples since all 10 metals could be analysed. α-Hydroxyisobutyric acid is a widely used complexing agent since it complexes with a large number of metal ions [13] and contains suitable binding groups (carboxyl, hydroxyl) [14].

A high concentration of α-hydroxyisobutyric acid was used to decrease the EOF [15] and enhance the resolution. An increase in buffer conductivity and running current resulted in a significant increase in the baseline noise and markedly decreased the analyte peak response[16]. Optimum injection time was used to reduce overlap resulting from electromigration dispersion that causes a broad peak. The more the concentration of the sample component, the more pronounced is this dispersion and therefore the broader the peak [17].

The pH, the injection time and the separation voltage were the main factors affecting the separation of metal ions [18]. The effect of pH, applied voltage and injection on peak area and migration time has been studied.

4.6.1.1 Effect of pH of the background electrolyte

Buffer pH has much influence on the separation selectivity of metal and controls the improvement of resolution of complexes [19, 20]. The electroosmosis flow (EOF) decreases with a decrease in pH [21-23] owing to a reduced dissociation of surface silanol groups. A decrease in the pH of BGE results in an increase in the difference of the migration times between two neighbouring cations. Changing the pH affects the selectivity and thus the peak area. A pH range between 3 - 6.5 has been studied. Figure 11 shows a plot of peak area as a function of pH.

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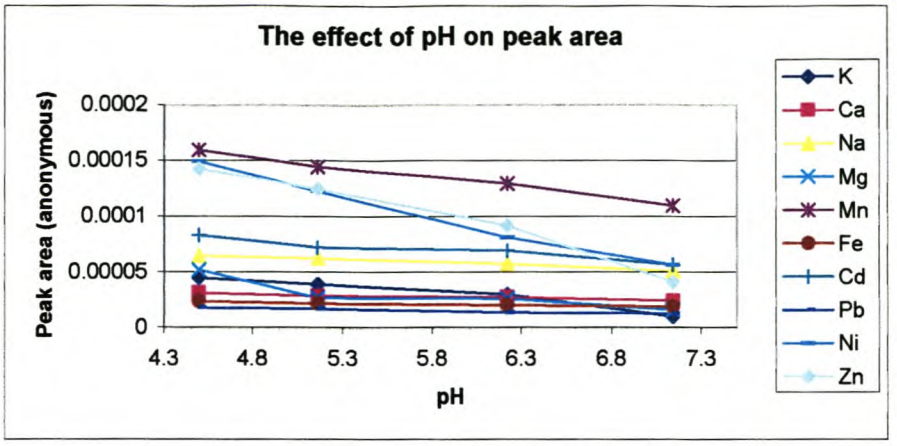

Fig. 11. The effect of pH of the background electrolyte on peak area. Experimental conditions are the same as in fig. 9a.

A decrease in pH results in an increase in peak area. The increased peak responses are caused by a decreased migration velocity of the sample zone through the detector because peak area is inversely related to the migration velocity [24, 25]. At pH 4.5 the largest peak area response, and consequently the highest sensitivity, were obtained for the cations. At pH below 4.5 a high noise and distortion in background was observed. Likewise, cations like, Mg^{2+}, Pb^{2+} and Zn^{2+}were not seen at pH lower than 4.5. This is because of the distortion of buffer solution and the complexes formed are not stable.

4.6.1.2 Effect of applied voltage

The increase in the applied voltage increases in the velocity of the ion and consequently its migration time. The relation between velocity of the ion (V), migration time (μ) and the applied voltage (E) [26] is given by:

$$V=\mu E$$

The plot of the migration time as a function of applied voltage is given in Figure 11.

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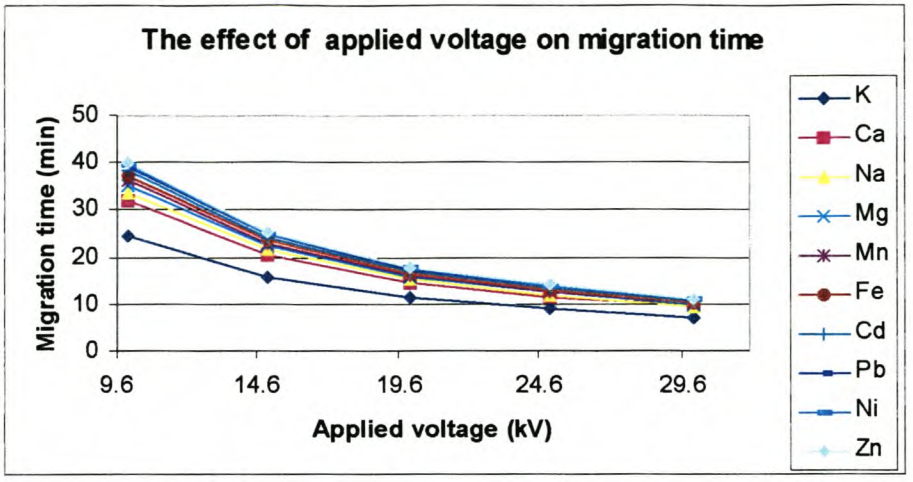

Fig.12. The effect of applied voltage on migration time. Experimental conditions are the same as in fig. 9a.

A change in the applied voltage has direct effect on the migration time. The migration time between the neighbouring cations changes when the applied voltage changes from 10 to 30 kV. From the above considerations, a positive voltage of 25 kV was selected for further experiments.

4.6.1.3 Injection time

The signals obtained were, in general, proportional to the injection time for metals investigated. However, peak broadening was found to occur at long injection time, leading to poor separation [27, 28]. The effects of varying injection time to 0.2, 0.3, 0.4 and 0.5 min was studied. High injection time was used since the sensitivity of the instrument is low. At higher injection time the peak area was increased. Therefore, in this study, 0.3 min for metal separation and 0.2 min for the calibration curve was used to reduce overlapping. The plot of peak area versus injection time is shown below.

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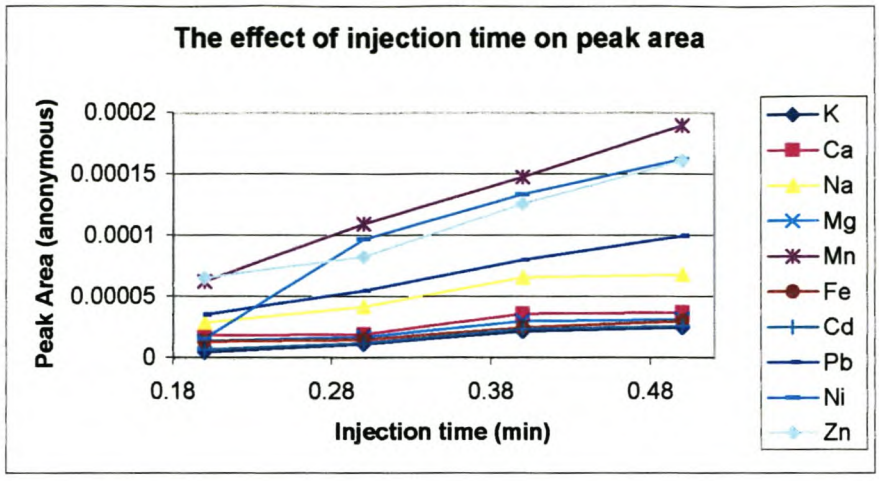

Fig.13. The effect of injection time on peak area. Experimental conditions are the same as in fig. 9a.

4.6.1.4 Validation of the method

4.6.1.4.1 Limit of detection (LOD)

With hydrodynamic injection for 24 s, at pH 4.5, with respect to each metal ion the following LOD was found (table 6). Lower limits of detection was obtained by injecting greater volumes, but at the expense of peak efficiency [29]. A higher LOD was obtained for Ca^{2+}.

Table 14. The limit of detection of metal ions (ppm).

Cation	LOD	Cation	LOD
K^+	3.0	Fe^{2+}	3.0
Na^+	2.5	Cd^{2+}	1.0
Ca^{2+}	0.5	Pb^{2+}	3.0
Mg^{2+}	2.5	Ni^{2+}	1.0
Mn^{2+}	1.0	Zn^{2+}	1.0

The limit of detection of each metal ion was determined by preparing standard

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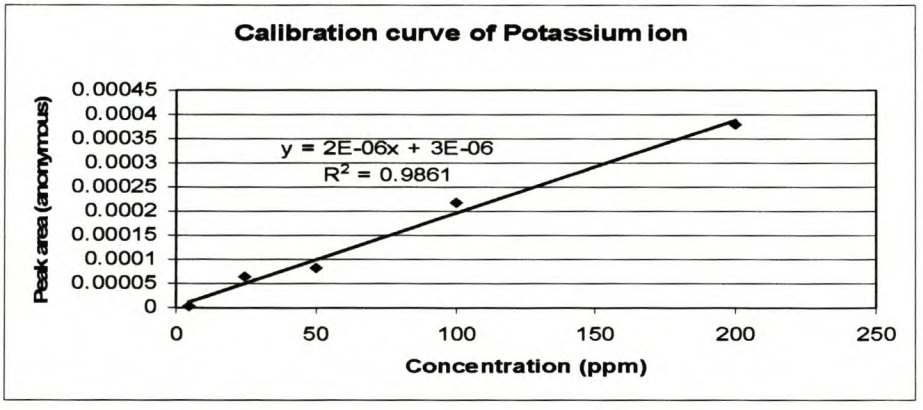
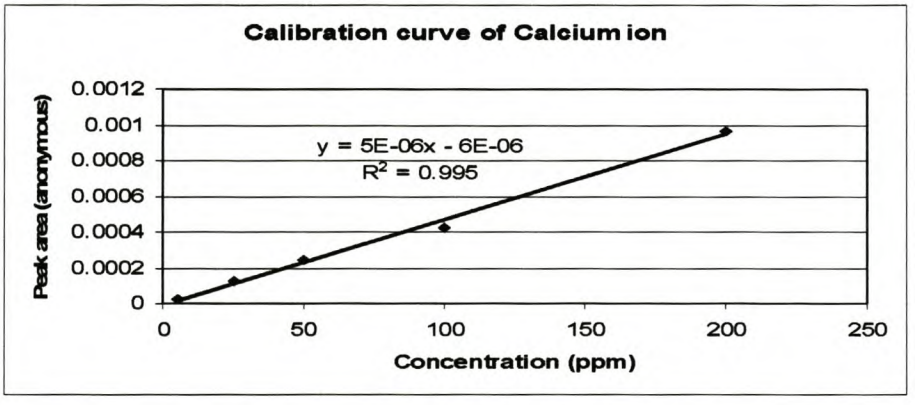
solutions of each metal ion to the lowest concentration that can be obtained.

4.6.1.4.2 Linearity of the calibration line

Standard solutions containing 5, 25, 50,100, 200 ppm of each mixtures of metal was prepared under the optimised conditions to test the linearity of the response for the metals under the conditions of indirect detection [30]. Five injections were performed at each concentration level. Analytical calibration lines were calculated based on the measurement of the peak areas. Regression values greater than 0.9861 were obtained. The regression value for K^+ was low due to its small peak. The calibration curve for base metal ions is given below.



(a)



(b)

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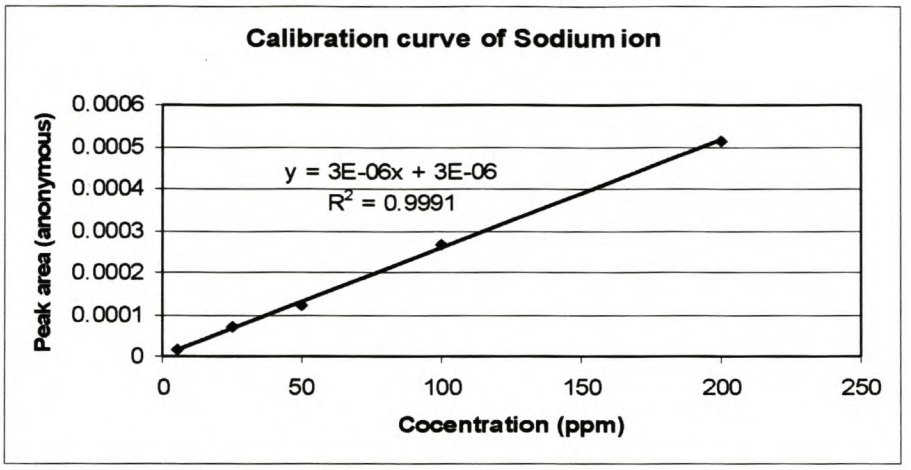
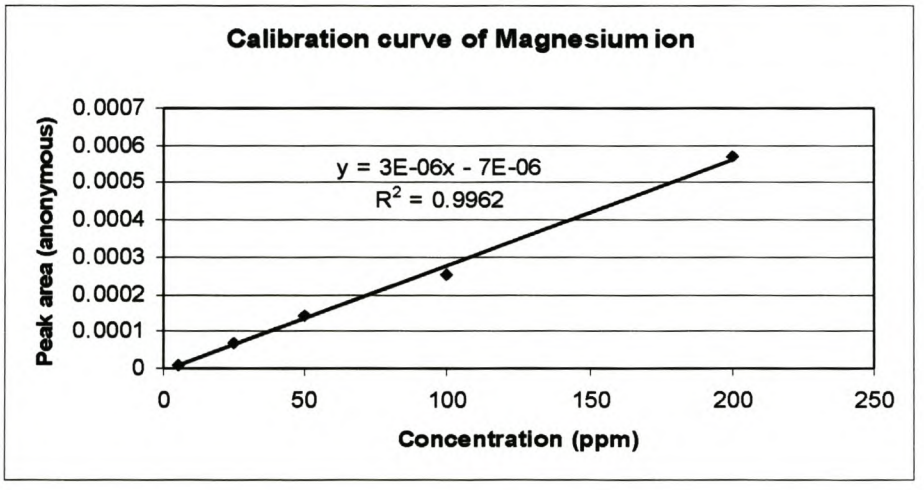


(c)



(d)

Fig. 14. Calibration curve of (a) K^+, (b) Ca^{2+}, (c) Na^+, (d) Mg^{2+}

4.6.1.4.3 Precision

The precision of the various methods was evaluated. Variations in migration time can be reflected as increased imprecision. Therefore, it is possible to calculate normalised areas by dividing the measured area of each peak by its corresponding migration time (corrected area) [31]. The relative standard deviations (RSD) for the

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migration time, peak areas and corrected areas are generally in the range of 0.38 - 3.94%, 2.37 - 14.05%, and 2.06 - 13.44% respectively.

4.6.2 Analysis of real samples

Samples from various locations were collected and identification of metal ions in those sample matrices has been performed using some of the developed methods. Identification of peaks was carried by the standard addition method, that is, the addition of single metal species to a metal mixture and identification of the peak with increased height [32, 33].

The two samples (Multimin and Mineral Max) that contain metals like sodium, Magnesium, copper and Zinc either in salt or chelated form and are useful for animal protection have been analysed and compared.

CE was run and a similar electropherograms (fig. 15 and 16) of the two samples have been obtained by decomplexing the chelated sample at lowest pH (≈1.0).

4.6.2.1 Multimin analysis

The sample was analyzed with glycolic acid-imidazole buffer solution and the electropherogram containing 4 metal ions was obtained.

The migration time, peak area, corrected peak area (table 15) and electropherogram (Fig. 15) of metal ions are given below.

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Table 15. The migration time, peak area, corrected peak area metal ions (n = 6).

Metal ion	Migration time, min (RSD)	Peak area, RSD	Corrected area, RSD
Na^+	12.32(±0.48%)	3.40E-03(±1.56%)	2.76E-04(±1.55%)
Mn^{2+}, Zn^{2+}	12.60(±0.56%)	1.15E-03(±4.68%)	9.00E-06(±4.21%)
Cu^{2+}	15.53(±0.71%)	8.95E-04(±8.75%)	5.00E-06(±9.06%)

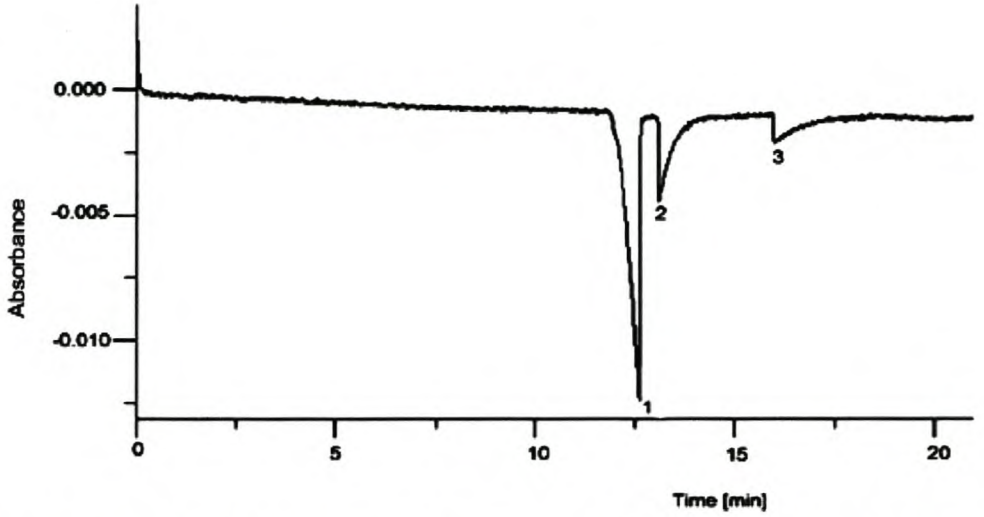

Fig. 15. Electrophoretic separation of metal ions. Carrier electrolyte, 12 mM imidazole – 15 mM glycolic acid (pH 4.29): hydrodynamic injection, 0.6 s: voltage 25 kV. Peaks according to sequence: 1=Na^+, 2=Mn^{2+} (Zn^{2+}), 3=Cu^{2+}.

Synthetic (standard) samples of respective metals have been injected to identify the peaks in the sample. The highest peak for Na^+ was obtained. The tail in the second peak is due to the overlap of Mn^{2+} and Zn^{2+}. Different attempts have been made to resolve the two peaks, that is, by varying the pH of the buffer and concentration of the sample. However, these attempts proved to be inconclusive. Strong peak distortion of Cu^{2+} peaks is caused by the formation of very stable complex with glycolic acid with only small mobility [11].

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4.6.2.2 Mineral Max analysis

A similar electropherogram of Mineral Max was obtained as it shown in figure 16.

Table 16. The migration time, peak area, corrected peak area of metal ions (n = 6).

Metal ion	Migration time, min (RSD)	Peak area, RSD	Corrected area, RSD
Na^+	12.33(±0.48%)	3.40E-03(±1.56%)	2.76E-04(±1.55%)
Mn^{2+}, Zn^{2+}	12.79(±0.57%)	1.08E-03(±5.16%)	8.40E-05(±5.47%)
Cu^{2+}	15.53(±0.71%)	8.94E-04(±8.72%)	5.00E-06(±9.10%)

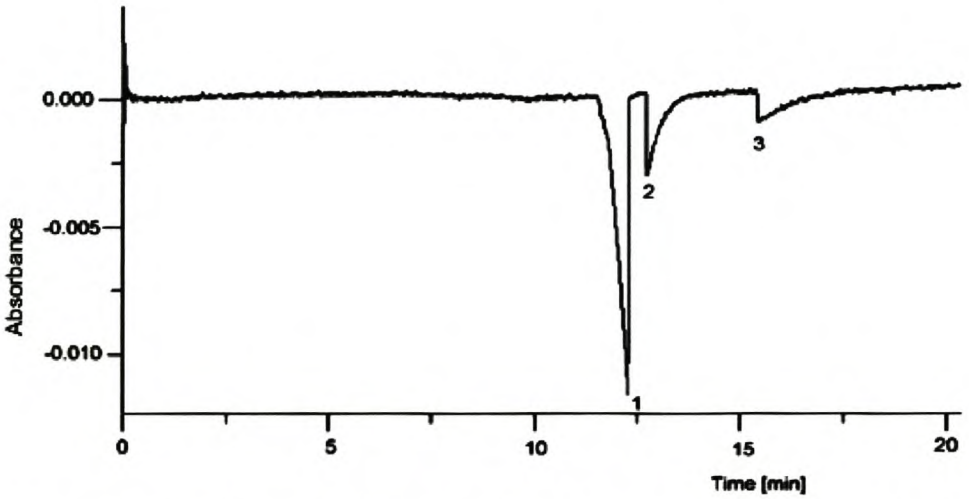

Fig. 16. Electrophoretic separations of metal ions. Carrier electrolyte, 12 mM imidazole – 15 mM glycolic acid (pH 4.29): hydrodynamic injection, 0.6 s: voltage 25 kV. Peaks according to sequence: 1=Na^+, 2=Mn^{2+} (Zn^{2+}), 3=Cu^{2+}.

The electrophergrams obtained from Multimin and Mineral max were overlapped to compare the two samples. The degree of overlap was very high as it shown in figure 17. The migration time and peak area were similar for the two samples (tables 15 and 16).

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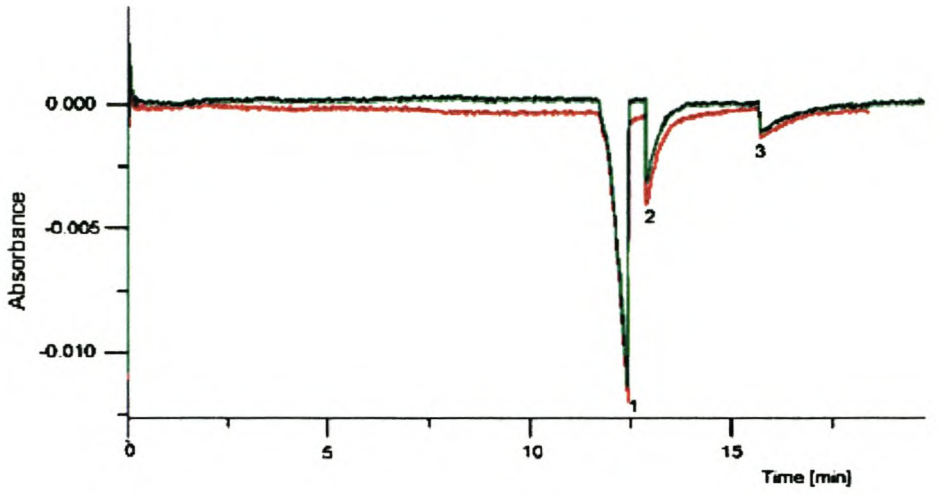

Fig. 17. The reproducibility between the two samples: Multimin (red electropherogram) and Mineralmax (green electropherogram). Carrier electrolyte, 12 mM imidazole – 15 mM glycolic acid (pH 4.29): hydrodynamic injection, 0.6 s: voltage 25 kV. Peaks according to sequence: 1=Na^+, 2=Mn^{2+} (Zn^{2+}), 3=Cu^{2+}.

It can be said that the two samples are identical with regard to Na^+ and Cu^{2+}. But it is difficult at this stage to conclude with respect to Mn^{2+} and Zn^{2+} since they show different degree of overlapping. It can be concluded the electropherogram of the samples are similar and very good reproducibility between the two samples has been obtained.

4.6.2.3 Environmental water samples

Environmental water samples were collected from different sites (table 17) and analyzed for the presence of the metal ions using α-HIBA - 4-aminopyridine buffer solution. The map (fig. 18) is included to show where environmental samples were taken.

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Table 17. List of environmental water samples.

Sample number	Type of water	Area taken
1	Reservoir water	Tokera
2	Small River	Village near Tokera
3	River	Boschendal
4	Main river	Village near Tokera
5	River	Franshock
6	River	Kastaing
7	Lake	Bridge house (water fall)
8	Lake	Kastaing
9	Lake	Berg
10	Lake	FBA
11	River	near university of Stellenbosch Gymnasium area
12	Tap water	Laboratory

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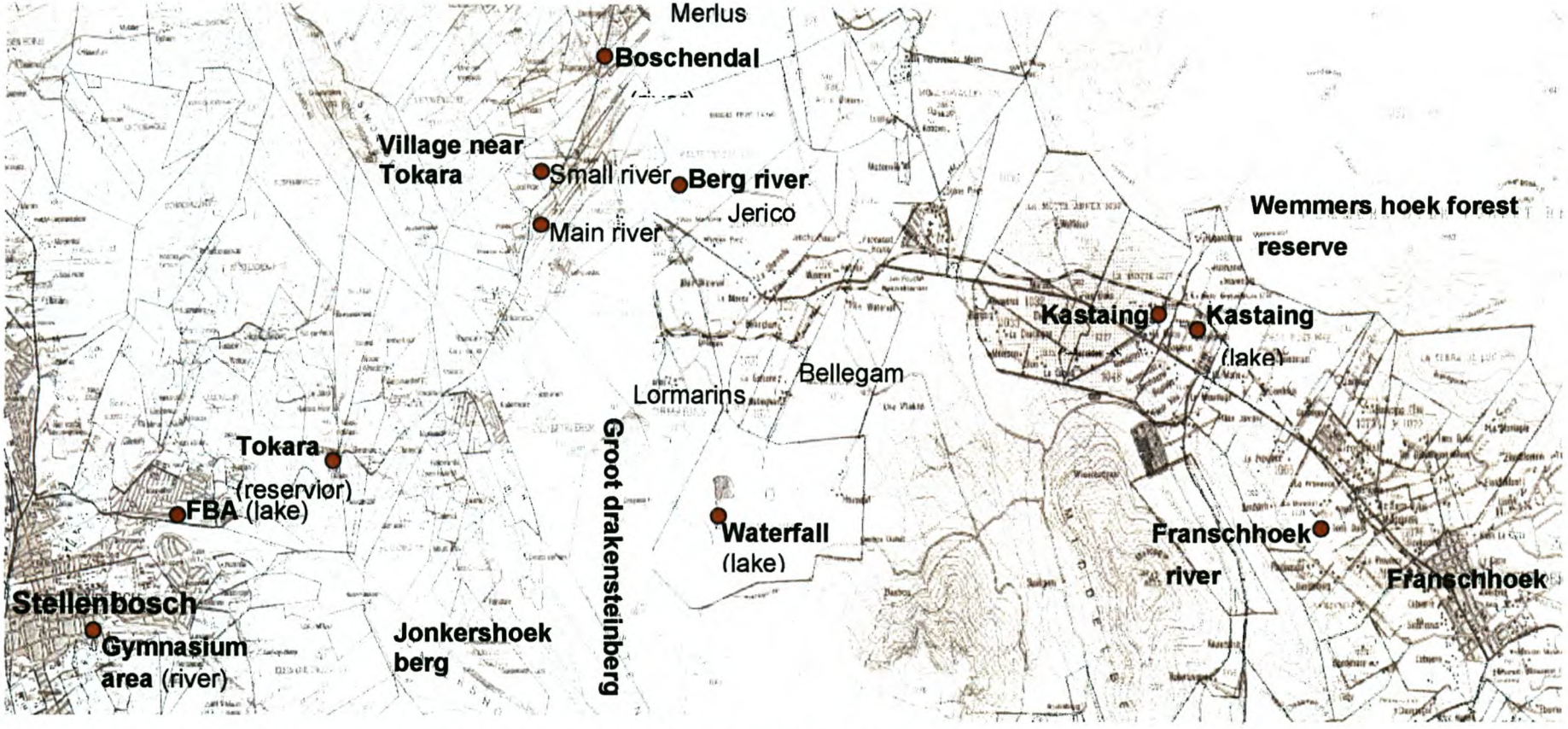

Fig. 18. Map (Topographical) showing different environmental water sites (Stellenbosch area).

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The electropherograms for metal ions in environmental water samples from different areas were obtained as follows. The electropherograms were enlarged to different scale to see the peaks that caused the peak distortion.

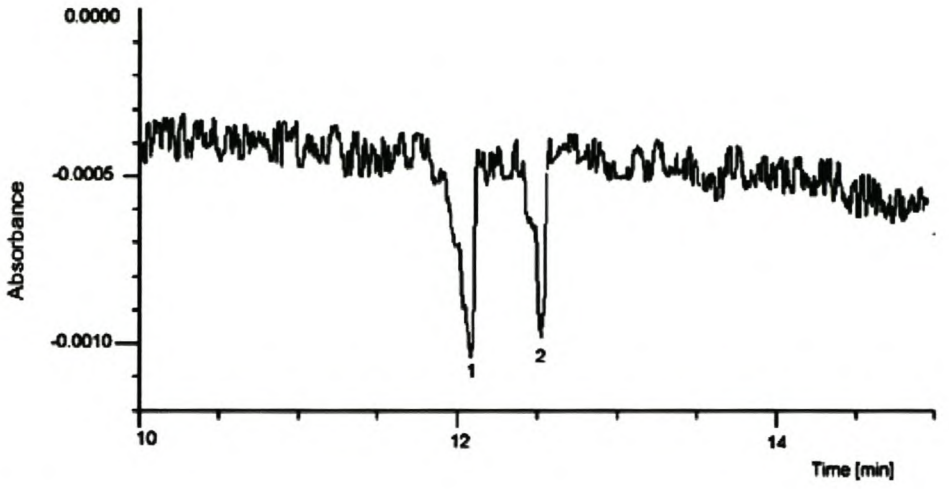

Fig. 19. Electrophoretic separation of metal ions in tap water from the laboratory. Carrier electrolyte, 10 mM 4-amino pyridine - 6.5 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 12 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=Ca^{2+}, 2=Na^{+}.

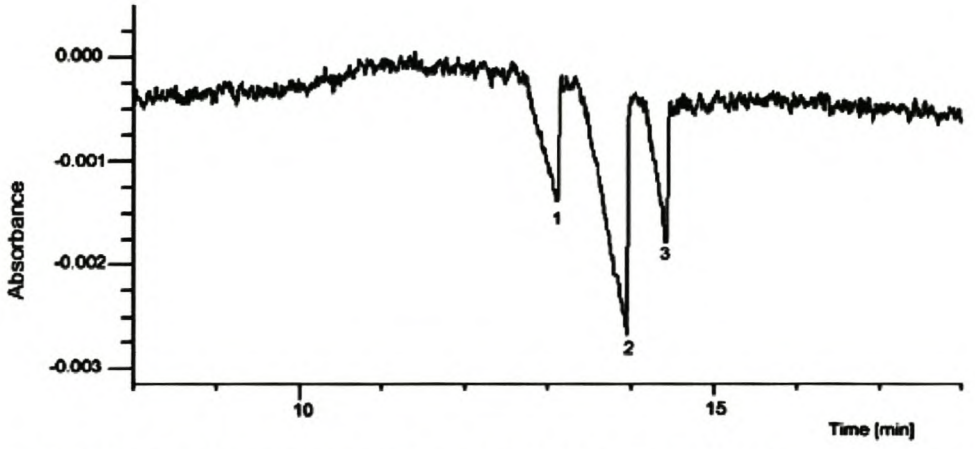

Fig. 20. Electrophoretic separation of metal ions in FBA-lake. Carrier electrolyte, 10 mM 4-amino pyridine - 6.5 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 12 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=Ca^{2+}, 2=Na^{+}, 3=Mg^{2+}.

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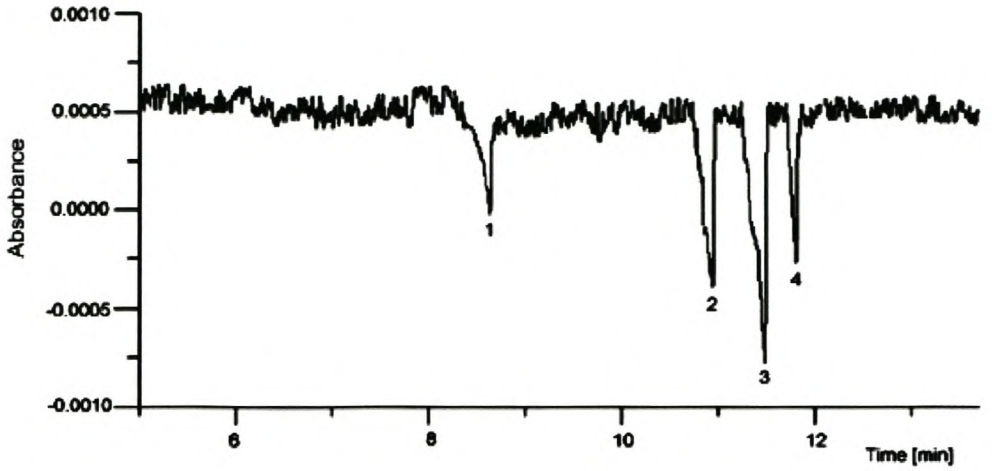

Fig. 21. Electrophoretic separation of metal ions in Kostaing - lake. Diluted 1:2 Carrier electrolyte, 10 mM 4-amino pyridine - 6.5 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 12 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=K^+, 2=Ca^{2+}, 3=Na^+, 4=Mg^{2+}.

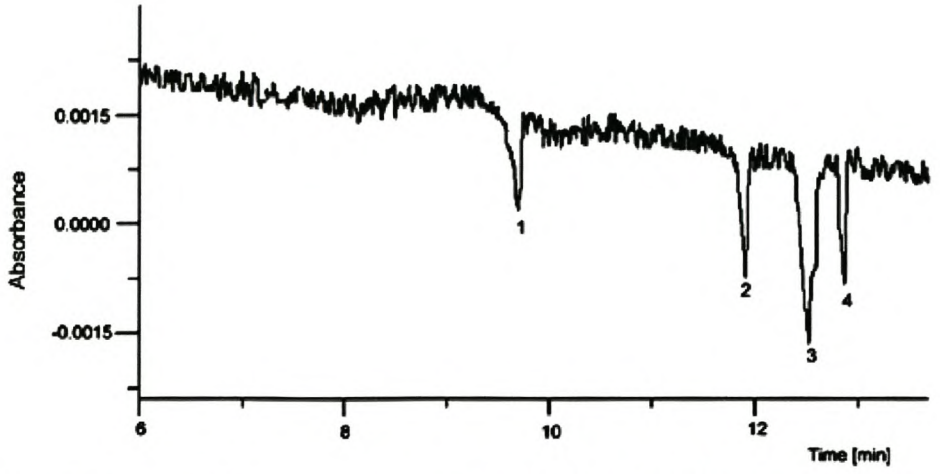

Fig. 22. Electrophoretic separation of metal ions in Village near Tokera - river. Carrier electrolyte, 10 mM 4-amino pyridine - 6.5 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 12 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=K^+, 2=Ca^{2+}, 3=Na^+, 4=Mg^{2+}.

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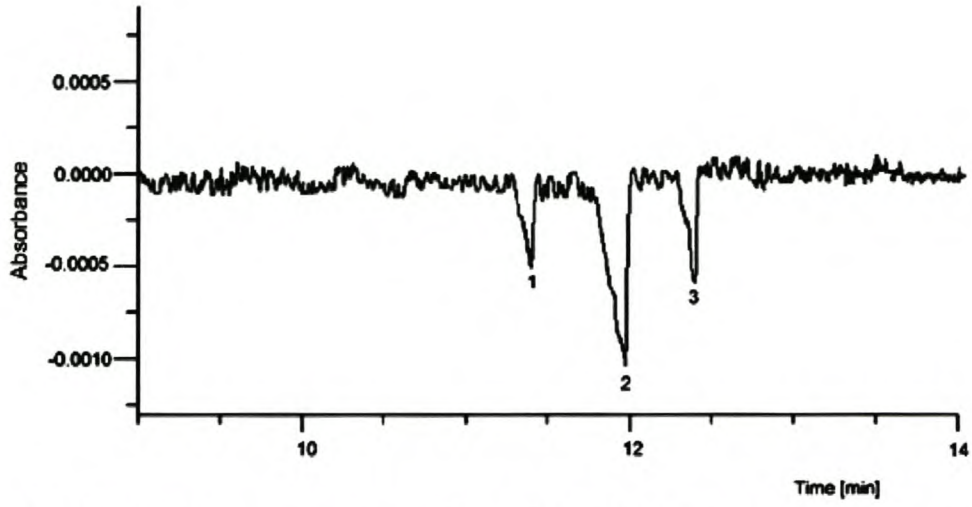

Fig. 23. Electrophoretic separation of metal ions in University of Stellenbosch gymnasium area-river. Carrier electrolyte, 10 mM 4-amino pyridine - 6.5 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 12 s: voltage, 25 kV, wavelength, 214 nm. Peaks according to sequence: 1=Ca^{2+}, 2=Na^{+}, 3=Mg^{2+}.

The peak shape for base metals in the environmental water samples is also affected by the concentration of each metal ion in the sample. The quantitative data of metal ions in environmental water samples have been presented as table 18.

Table18. Capillary electrophoresis results of metal ions from environmental samples (n=6).

Cation	Tab water	Village near Tokera -river	Kostaing -lake	FBA-lake	Gymnasium area-river
K^{+}	< LOD	1.42E+01(±3.88%)	3.60E+01(±6.09%)	< LOD	< LOD
Ca^{2+}	1.26E+01(±3.01%)	8.26(±4.72%)	4.32E+01(±9.31%)	5.27E+01(±7.56%)	8.26(±0.48%)
Na^{+}	8.27(±3.72%)	3.37E+01(±5.05%)	1.19E+02(±3.18%)	1.89E+02(±2.46%)	4.18E+01(±5.14%)
Mg^{2+}	< LOD	1.22E+01(±1.93%)	3.43E+01(±6.08%)	6.48E+01(±5.14%)	1.45E+01(±4.50%)

From the above table (table 18), the presence of base metal ions in tap water was low. A higher concentration of Ca^{2+}, Na^{+} and Mg^{2+} was found in water samples from

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Kostaing and FBA especially with Na^+. The source of the high levels of Na^+ in the lake water could not be determined.

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4.7 References:

1. C.Vogt, G.L.Klunder, *Fresenius J.Anal.Chem.*, 370 (2001), 316.
2. Y.-M.Liu, and J.-K.Cheng, *Electrophoresis*, 24 (2003), 1994.
3. T.Soga, G.A.Ross, *J.Chromatogr.*, A, 767 (1997), 223-230.
4. L.Geiser, *et al.*, *J.Pharm.Biomed.Anal.*, 00 (2003), 1-6.
5. Carla Vogt, and Gregory L.Klunder, *Fresenius J.Anal Chem.*, 370 (2001), 316-331.
6. A.Weston, P.R.Brown, P.Jandik, W.R.Jones, A.L.Hackenberg, *J.Chromatogr.*, 593 (1992), 289.
7. A.Weston, P.R.Brown, A.L.Hackenberg, P.Jandik, W.R.Jones, *J.Chromatogr.*, 602 (1992), 249.
8. M.Koberda, M.Konkowski, P.Youngberg,W.R.Jones, A.Weston, *J.Chromatogr.*602 (1992), 235.
9. V.Pacakova, *et al.*, *J. Chromatogr.*, A, 834 (1999), 257-275.
10. S.Motellier *et al*, *Analytica Chimica Acta,* 410 (2000), 12.
11. F.Han *et al., J.Chromatogr.* B, 669 (1995), 103-112.
12. N.Shakulashvili *et al., J.Chromatogr.*, A, 895 (2000), 210.
13. Y.-S, Fung, and K.M.Lau, *Electrophoresis*, 24 (2001), 2192-2193.
14. V.Pacakova, *et al.*, *J. Chromatogr., A,* 834 (1999), 260.
15. D.Scarcella, *et.al., Forensic Science International,* 89 (1997), 33-46.
16. Q.Yang *et al., J.Chromatogr.*, A, 673 (1994), 275-285.
17. F.Foret, S.Fanali,L.Ossicini and P.Bocek, *J.Chromatogr.*, A, 470 (1989), 299.

Stellenbosch University http://scholar.sun.ac.za

18. X.Xiong. S.F.Y.Li., *J.Chromatogr.*, A, 822 (1998), 130.

19. B.Liu *et al*, *J.*Chromatogr.A, 834 (1999), 301.

20. A.R.Timerbaev, O.p Semenova, *J.Chromatogr.,* A, 690 (1995), 141.

21. A.Weston, P.R.Brown, A.L.Hackenberg, P.Jandik, W.R.Jones,

J.Chromatogr., 602 (1992), 252.

22. H.-W.Chen, Z,-L. *Fang.Analytica Chimica Acta*, 394 (1999), 13-22.

23. Y.-S, Fung and K.M.Lau, *Talanta*, 45 (1998), 641-646.

24. D.K.Altria, *LC.GC*, 6 (1993), 164.

25. W.-P.yau, E.Chan., *J.Pharm.Biomed.Anal*, 28 (2002), 107-123.

26. W.M.Sunada, and H.W.Blanch, *Eletrophoresis*, 18 (1997), 2243-2254.

27. R.T.Kennedy, *Analytical Chimica Acta,* 400 (1999), 163-180.

28. Y.-S.fung, and H.-S.Tung, *Electrophoresis*, 20 (1999), 1832-1841.

29. A.Padarauskas, G.Schwedt, *J.Chromatogr.*, A 773 (1997), 359.

30. A.Kumar Malik,W.Faubel, *Talanta*, 52 (2000), 341-346.

31. K.D.Altria, *et al., J.Pahrm.Biomed.Anal.*, 13 (1995), 951-957.

32. N.Shakulashvili *et al., J.Chromatogr.*, A 895 (2000), 210.

33. K.D.Altria, *Trends in Analytical chemistry*, **17**, no.4, 1998.

34. H.Siren, S.Vantsi, *J.Chromatogr.A*, 957 (2002), 17-26.

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5. Analysis of anions

5.1 Introduction

The simultaneous separation of anions and cations in a single run is somewhat problematic due to the difference in the migration of ions to that of the electro osmotic flow (EOF). That is, where one class of analytes (cations) migrates in the same direction of EOF, the other class of analytes (anions) migrates opposite to the direction of the EOF. The use of indirect UV detection for simultaneous anion and cation determination demands the presence of anionic and cationic UV-absorbents in the background electrolyte (BGE) [2-4]. Likewise, most of these cationic UV chromophores can be used only in acidic medium (pH<6) due to their deprotonation or low solubility at higher pH-values [5]. At low pH cations are normally not or only weakly complexed. Hence different experimental conditions are required for the analysis of cations and anions.

The choice of electrolyte is extremely important to the success of any CE analysis. The mobility of the BGE should match as nearly as possible the mobility of the analytes of interest. Chromate or pyromellitate is commonly used as BGE for the analysis of inorganic anions since they have a similar high mobility [6-10]. How ever, for the analysis of lower mobility compounds such as short chain carboxylic acids (C1-C8), benzoate is more suitable [6]. Phthalate is the most popular BGE for the analysis of medium mobility of organic acids [6, 10, 11]. The mobility of 2,6 pyridine dicarboxylic acid (PDC) is similar to that of phthalate which indicated that it is a suitable BGE for the analysis of medium mobility anions [12].

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In this study of anion separation, the EOF was reversed in the direction of the anode by adding Cetyltrimethylammonium bromide (CTAB) to the electrolyte and highly alkaline conditions (greater than pH 8) were used to confer a negative charge on inorganic and organic anions so that these conditions promote their migration towards the anode[13]. The separation of inorganic and organic acids was achieved using chromate and PDC respectively as background UV-absorbing electrolytes. Thus different experimental conditions have been used for inorganic and organic anions.

5.2 Results and discussion

5.2 1 Analysis of the standard solutions

Synthetic solutions were analysed prior to the determination of anions in various sample matrices. The electropherogram of the synthetic inorganic and organic acid based on the suggested experimental conditions as outlined in section 3.2.1 is given below. A positive peak for nitrate ion was obtained.

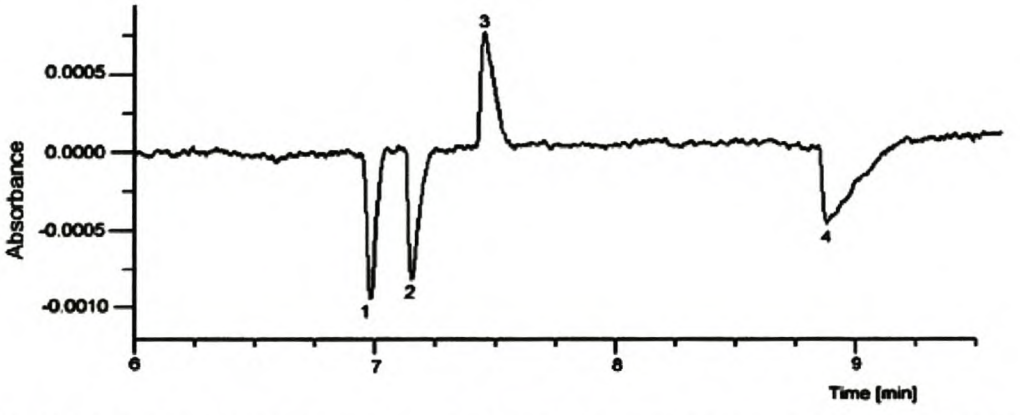

Fig. 24. Electropherograms of synthetic solutions of inorganic anions (25 mg/l). Ions in the electropherograms: (1) Cl^-, (2) SO_4^{2-}, (3) NO_3^- and (4) F^-. Experimental conditions as in section 3.2.1.

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In the above electropherogram a broad peak for F^- was observed. This is because of the mismatch of the F^- electrophoretic mobility with that of the background electrolyte [14]. The same effect was shown for succinic acid in the figure 25.

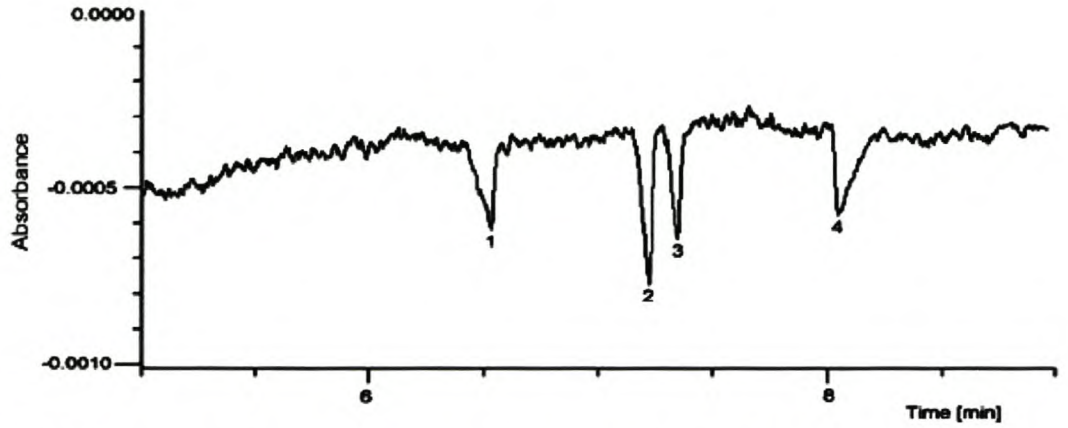

Fig. 25. Electropherograms of synthetic solutions of organic anions (25 mg/l). Ions in the electropherograms: (1) Tartaric acid$^-$, (2) Malonic acid$^-$, (3) Citric acid$^-$ and (4) Succinic acid. Experimental conditions as in section 3.2.2.

The order of migration of inorganic and organic anions agrees well with data previously determined by other researchers [10, 12]. For the anions the limit of detection was in the range of 2 - 3.5 ppm.

5.2.2 Analysis of real samples

The developed methods for anions were applied to the determination of inorganic and organic anions in water samples, orange juice, beer and wine. The samples were kept at low temperature (4 ^{0}c), degassed and filtered prior to injection. The determination of anions using other methods for example, ion chromatography, is possible. However, this demands sample preparations and long analysis times. CE is a good choice since it involves very simple sample preparation that consisted only of degassing the sample by sonication, and diluting it with Milli Q water prior to injection.

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For CE measurements all the samples were first screened with CE and their concentrations were estimated (with the standard solutions). When the peak resolution was not satisfactory (high ionic strength) or the ion zones were distorted, the samples were diluted 1:4, 1:10, 1:20 or 1:25 (v/v) (table 1) with purified water. The samples were not manipulated by pH adjustment or complexation to analyse them by simulating the real samples conditions as closely as possible. The ions in each electropherograms were identified by the standard addition, that is, by adding standard solution to the sample and observing peak's height increases.

Table 19. Samples, dilutions needed in the study.

Sample type	Dilution factor
River (Village near Tokera)	Undiluted
Lake (Kastaing)	Undiluted
Lake (FBA)	1:6
Orange juice	1:10 or 1:20
Beer	1:10
Wine (white wine)	1:10
Wine (red wine)	1:25

The analysis of inorganic anions and organic were performed using the methods given in sections 5.1 and 5.2 respectively. Calibration curves of anions were prepared using the standard solutions (5, 25, 50 100 and 200 ppm) and the regression value was >0.9816 (fig. 26).

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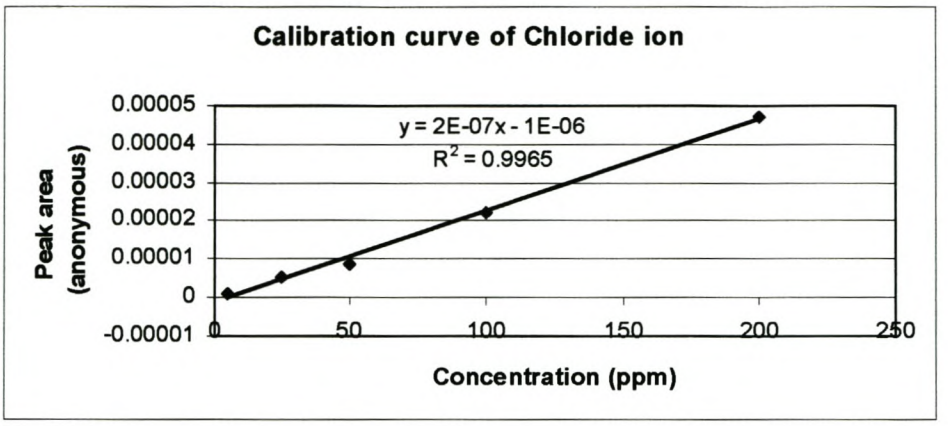
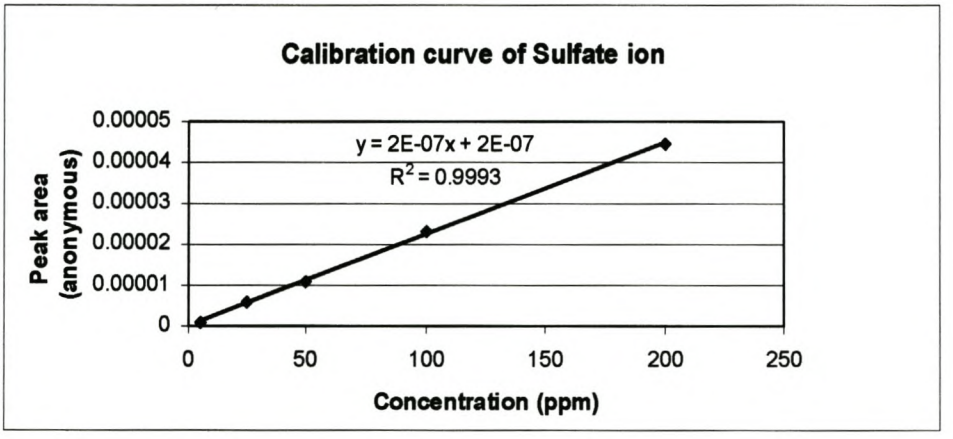
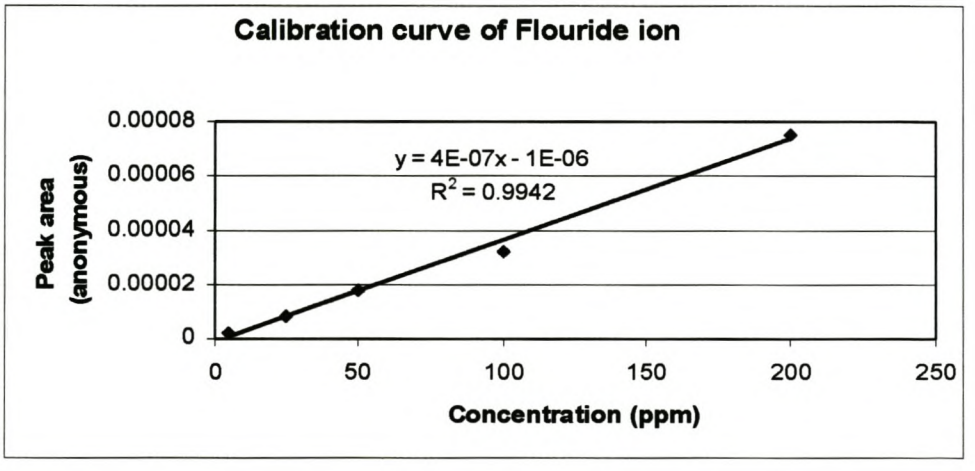


(a)



(b)



(c)

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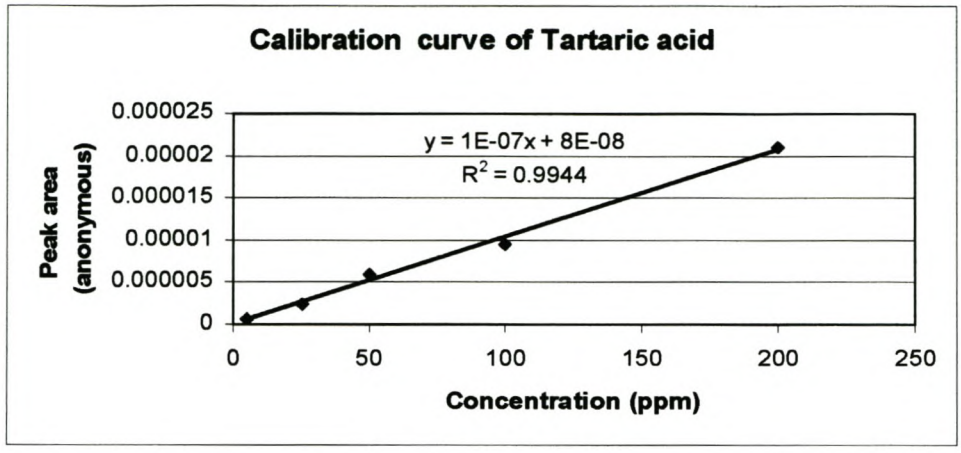
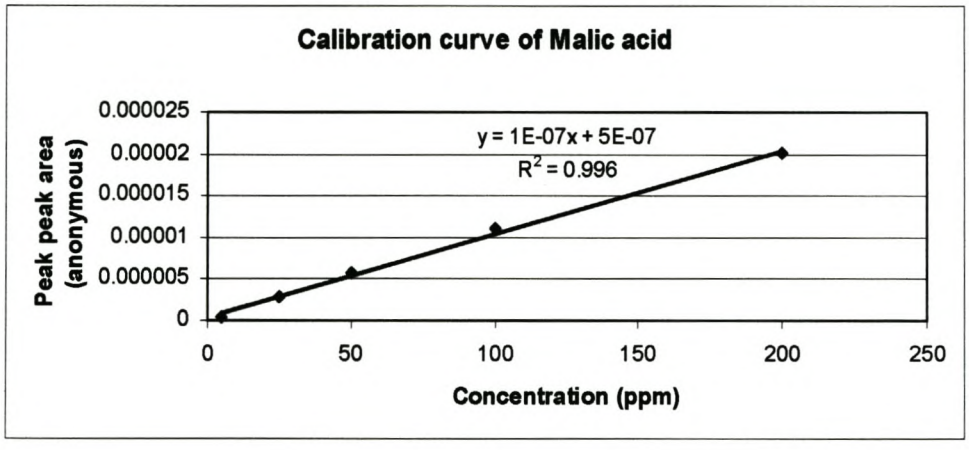
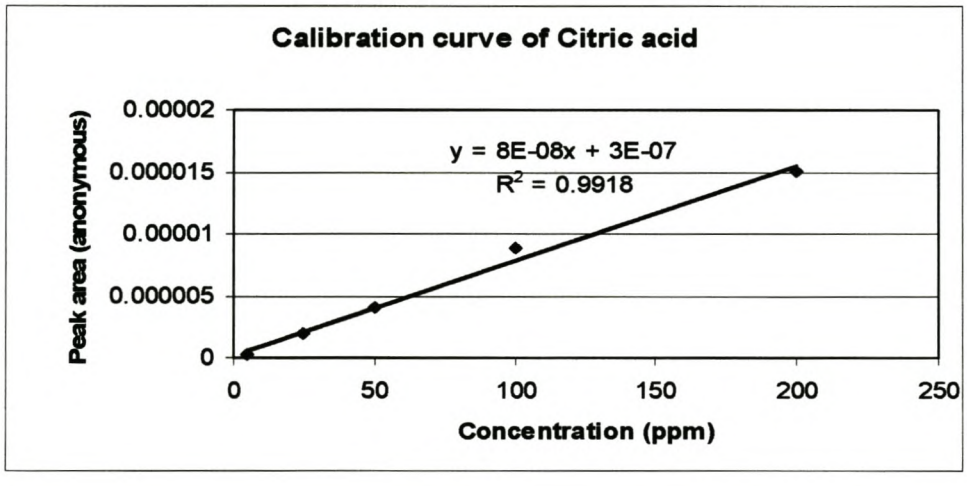


(d)



(e)



(f)

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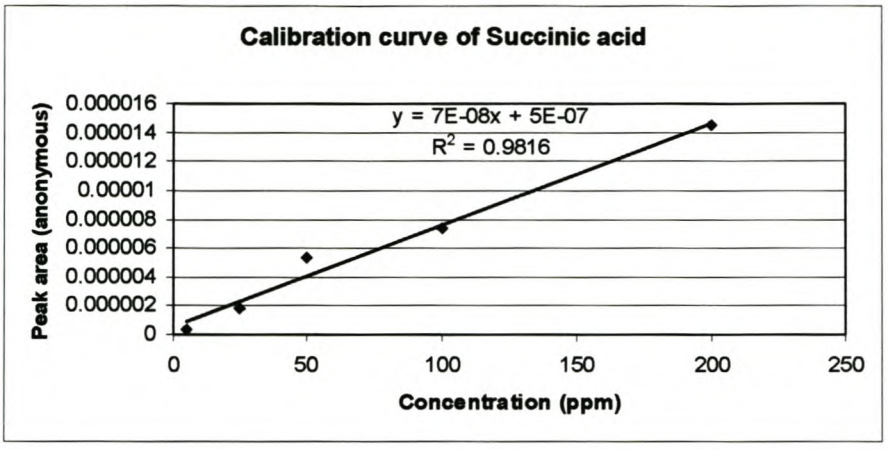

(g)

Fig. 26. Calibration curve of (a) Cl^-, (b) SO_4^{2-}, (c) F^-, (d) tartaric acid, (e) malic acid (f) citric acid, (g) succcinic acid

CE results of anions in the samples listed in table 19 were obtained as follows.

Table 20. Capillary electrophoresis results of anions in the samples listed in table 19.

Sample	Chloride, mg/l (RSD)	Sulfate, mg/l (RSD)	Tartaric acid, mg/l (RSD)	Malic acid, mg/l (RSD)	Citric acid, mg/l (RSD)	Succinic acid, mg/l (RSD)
Tokera	2.26E+01 (±2.81%)	5.44 (±6.28%)	-	-	-	-
Kastaing	7.16E+01 (±4.29%)	3.06E+01 (±2.03%)	-	-	-	-
FBA	6.08E+02 (±2.35%)	3.27E+01 (±6.86%)		-	-	-
Orange juice	1.37E+02 (±1.51%)	7.01E+01 (±6.49%)	-	2.27E+01 (±7.28%)	1.31E+03 (± 4.36)	-
Beer	2.06E+02 (±0.94%)	1.23E+02 (±3.99%)	-	-	-	-
Wine (white)	9.88E+01 (±2.95%)	4.57E+02 (±2.84%)	-	-	-	-
Wine (red)	-	-	1.43E+03 (±5.30%)	-	-	6.88E+02 (±7.99%)

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One of the wine types was used for organic acid analysis to check the potential applications of the method for the determinations of organic acids.

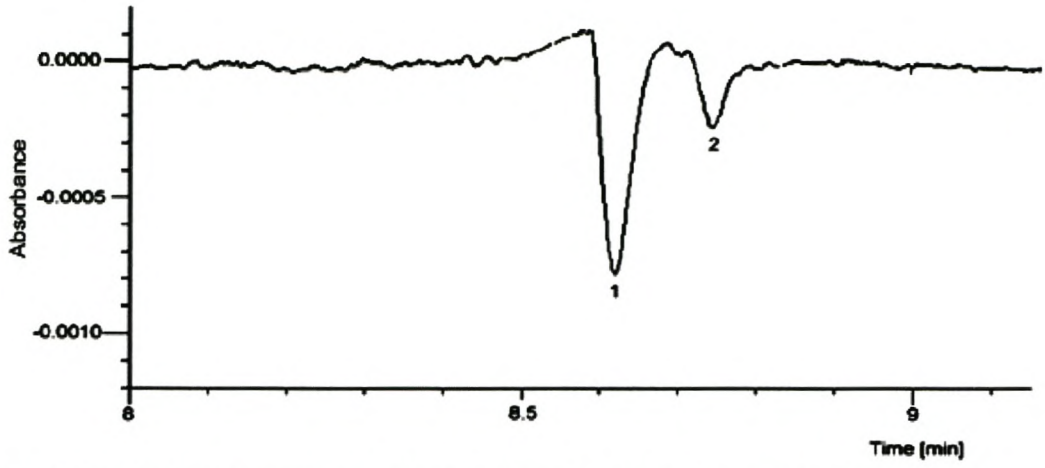

Fig. 27. Electropherograms of inorganic anions in Tokera area .Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in section 3.2.1.

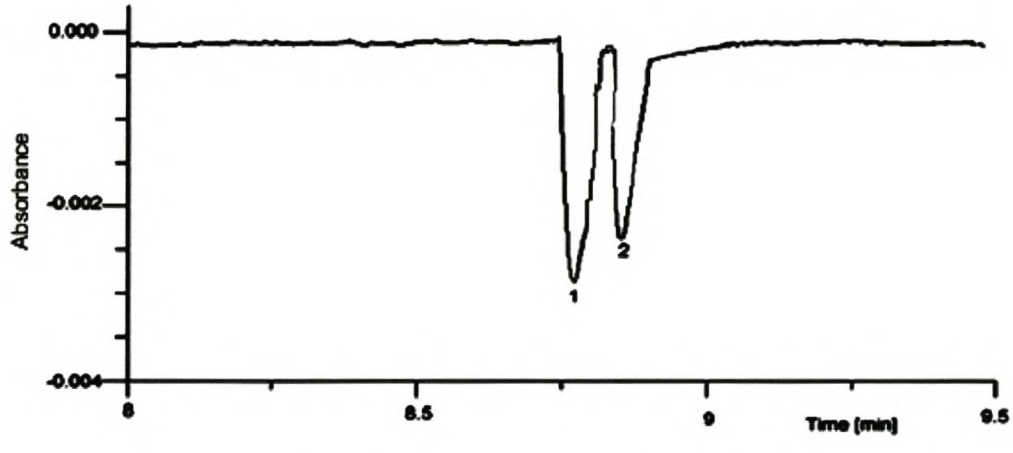

Fig. 28. Electropherograms of inorganic anions in Kastaing area .Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in section 3.2.1.

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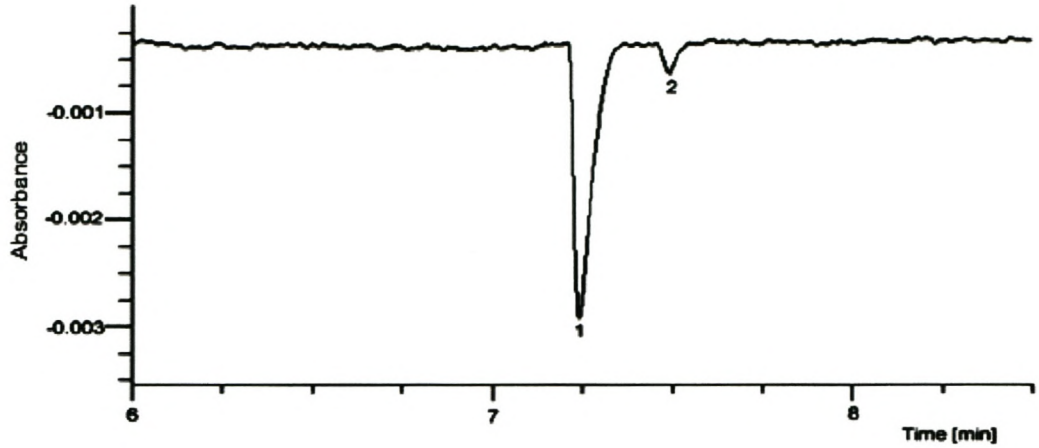

Fig. 29. Electropherograms of inorganic anions in FBA area (diluted 1:6). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in section 3.2.1.

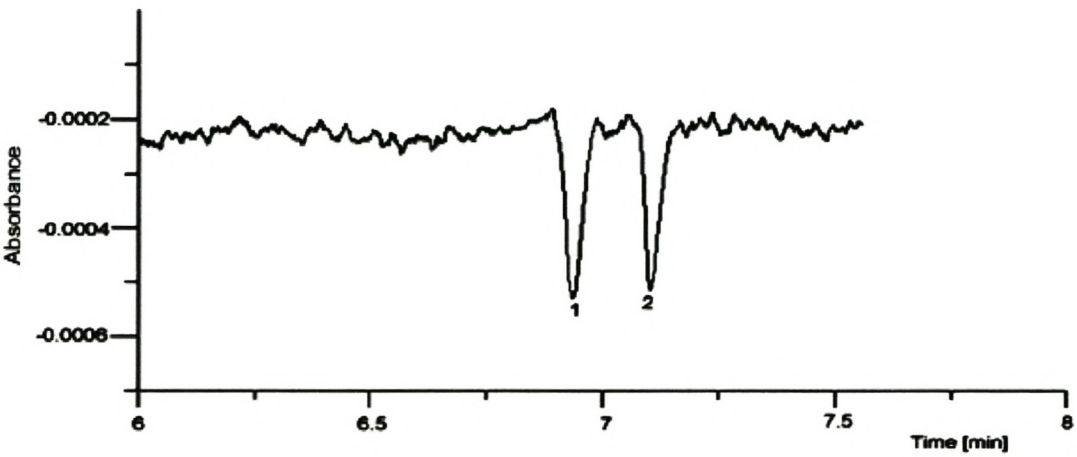

Fig. 30. Electropherograms of inorganic anions in orange juice (diluted 1:10). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in section 3.2.1.

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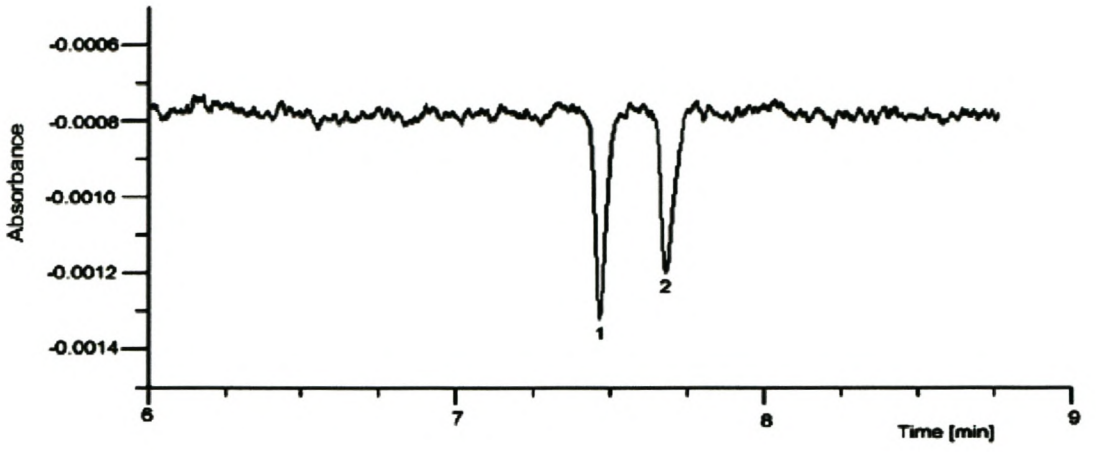

Fig. 31. Electropherograms of inorganic anions in beer (diluted 1:10). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in section 3.2.1.

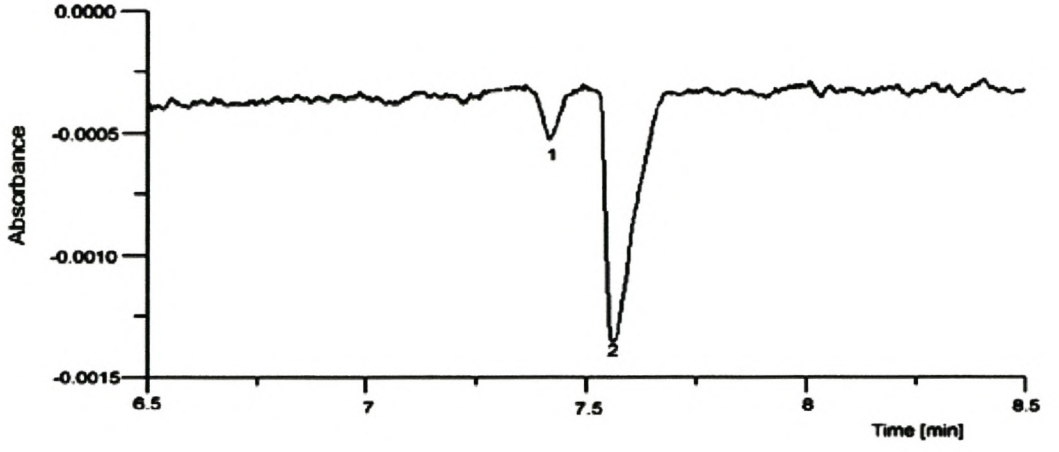

Fig. 32. Electropherograms of inorganic anions in white wine (diluted 1:10). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in section 3.2.1.

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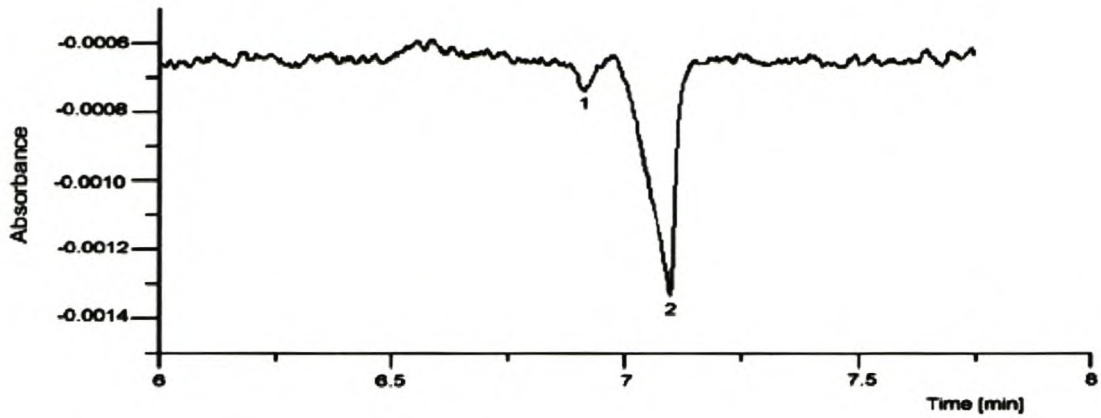

Fig. 33. Electropherograms of organic anions in orange juice (diluted 1:20). Ions in the electropherograms: (1) Malic acid and (2) citric acid^{-}. Experimental conditions as in section 3.2.2.

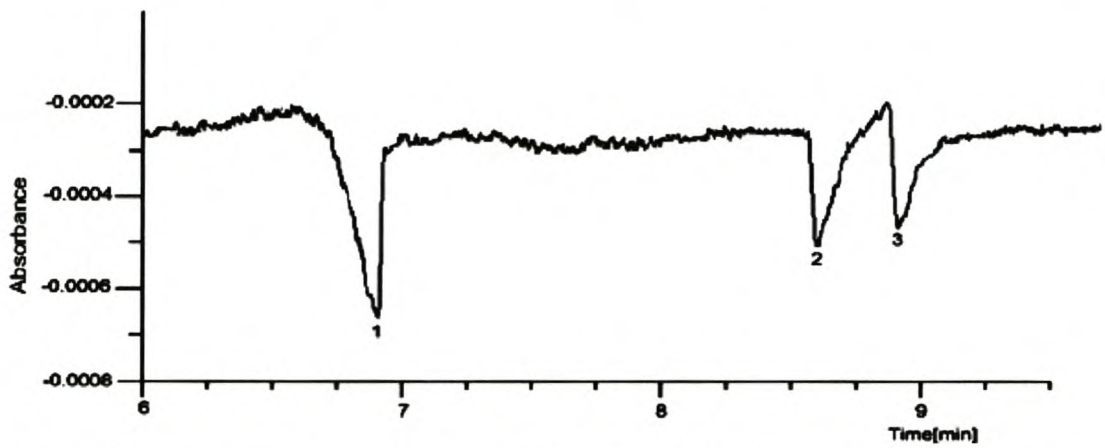

Fig. 34. Electropherograms of organic anions in redwine (diluted 1:25). Ions in the electropherograms: (1) tartaric acid (2) succinic acid and (3) unidentified peak. Experimental conditions as in section 3.2.2.

In the analysis of red wine, a sharp peak for tartaric acid was observed. A slight tailing of succinic peak was shown due to the mismatch of electrophoretic mobility with that of BGE. The peak labelled, as '3' could not be identified. It was not one of the injected synthetic solutions.

The methods developed for the determination of anions were efficient and useful for both qualitative and quantitative analysis in various samples. These include the analysis of inorganic anions and organic acids in environmental water, beverages

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and orange juice. Based on the results obtained, it could be applicable for the determination of anions in similar samples from other areas

Anions could be also determined using the high performance liquid chromatography (HPLC) and ion chromatography (IC). However, those techniques demand longer analysis time, high chemical consumptions and complex sample preparation. CE has comparative advantages over those techniques since it posse's short analysis times, simple sample preparations and low consumption of chemicals.

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5.3 References

1. Yiwei Deng, *Pergamon,* **32**, No.8 (1998), 2249.
2. P.Kuban *et al.*, *J.Chromatogr. A*, 836 (1999), 75.
3. I.Haumann *et al., J.Chromatogr.A*, 895 (2000), 269.
4. X.Xiong, and S.F.Y.Li, *J.Chromatogr.A,* 822 (1998), 125-136.
5. A.Padarauska et al., *Analytica Chimica Acta*, 374, No.23 (1998), 161.
6. J. Romano, E Jandik, W.R. Jones and EE. Jackson, *J.Chromatogr.*, 546 (1991), 411.
7. W.R. Jones, and P. Jandik, *J. Chromatogr.*, 546 (1991), 445.
8. W.R. Jones, *J. Chromatogr.*, 640 (1993), 387.
9. S.M. Cousins, ER. Haddad and W. Buchberger, *J. Chromatogr. A*, 671 (1994), 397.
10. E.Dabek-Zlotorzynska, and J.F. Dlouhy, *J. Chromatogr. A*, 671 (1994), 389.
11. E.Dabek-Zlotorzynska and J.F. Dlouhy, *J.Chromatogr. A*, 685 (1994), 145.
12. T.Soga, G.A. Ross, *J.Chromatogr. A,* 767(1997), 225.
13. T.Soga, G.A. Ross, *J.Chromatogr. A,* 837 (1999), 231.
14. T.Hiissa *et al., J.Chromatogr.* A, 853 (1999), 403-411.

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6. Conclusion

New capillary electrophoresis (CE) methods were developed for determination of both cations and anions in various samples using BGEs containing 10 mM pyridine, 12 mM imidazole and 10 mM 4-Amino pyridine 0.5 mM Chromate and 7 mM PDC as UV-absorbing cations, inorganic anions and organic acids.

Glycolic acid and α-hydroxyisobutyric acid were used as complexing reagents for cation separation to selectively alter their similar mobilities and proper separation. Different methods were developed for cation analysis. Using a glycolic acid - pyridine, glycolic acid - imidazole and α-hydroxyisobutyric - imidazole back ground electrolytes, we were able to separate only a mixture of 9 metal ions. A mixture of complexing reagents (glycolic and α-hydroxyisobutyric acids) with imidazole as UV-absorbing species was investigated and was found useful for the detection of a mixture of 10 metal ions though the peak for Pb^{2+} was small and difficult to integrate.

α-hydroxyisobutyric acid and 4-aminopyridine back ground electrolyte was used for further investigation as it was shown to be the combination where ± 10 metal ions could be seprated efficiently. Hence it was applied for qualitative and quantitative analysis of metal ions in tab water from laboratory and environmental water samples from the areas surrounding Stellenbosch. Successful detection of metal ions was obtained for tap water and environmental waters from FBA (lake), near the University gymnasium (river), a village near Tokera (river) and Kostaing (lake). A higher concentration of metal ions (Ca^{2+}, Na^{+} and Mg^{2+}) was obtained in the FBA area.

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Glycolic acid – imidazole back ground electrolyte was applied for detection of metal ions in Multimin and Mineral Max samples which are useful for animal protection. The metal ions were analysed and compared in the two samples. A similar electropherograms (as shown in fig 15) were obtained showing that the similarity of the two samples.

Chromate and PDC back ground electrolytes were developed for the analysis of inorganic anions and organic acids respectively. 0.5 mM or 35 μM CTAB was used as an EOF modifier. The developed methods were applied to analysis of real sample. The presence of anions in environmental water samples, beverages and orange juice were analysed. Inorganic anions like chloride and Sulfate ions were obtained in environmental water samples from FBA, Kastaing and Tokera areas. Higher concentration of Cl^- and SO_4^{2-} ions were found in the FBA area. This is in agreement with the cation analysis. Cl^- and SO_4^{2-} ions in Orange juice, beer and white wine were determined. High concentration of Cl^- and SO_4^{2-} ions in beer and white wine was obtained. The analysis of organic acids in orange juice and red wine was performed and high concentration of citric and tartaric acids in orange juice and red wine respectively was found.

The results obtained in the present study demonstrate the feasibility and advantages of using CE for the analysis of both cations and anions in environmental water samples from the areas surrounding Stellenbosch. In this work, data have been included on the qualitative and quantitative analysis of beverages and orange juice, showing the possible application of those methods for the determination of cations and anions on other sample matrices. The corrected peak area was shown to be a better quantitative parameter than the use of the peak height. The limit of detection

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(LOD) of cations and anions was in the range of 0.5 - 3 ppm and 2 - 3.5 ppm respectively. The use of longer injection time in hydrodynamic injection was shown to increase the sensitivity of the methods but suffered the loss of efficiency and resolution of the separation. Thus, 12 s was adopted for hydrodynamic injection.

The broad peak for ions Ni^{2+} and Zn^{2+} in the case of cations and F^- and succinic acid for anions were due to the mismatch of electrophoretic mobility of those ions and the background electrolyte. The higher stability of Ni^{2+} and Zn^{2+} with complexing reagent was also the cause for peak broadening.

In the environmental water samples only the base metal ions like K^+, Na^+, Ca^{2+} and Mg^{2+} could be determined. None of the transition metals was detected. This could be either due to low sensitivity of the UV-detector or the water samples contained none of the transition metals.

In summary, CE was shown to provide a quick, relatively sensitive, economic and reliable method for qualitative and quantitative determination of both cations and anions in various samples.

7. Future work

Capillary electrophoresis (CE) has high detection limit (ppm range), that is, it has low sensitivity since it involves UV detection for the analysis of ion in various sample matrices. This causes a serious limitation for its application to environmental analytes which are found in a very low concentration range. This can be overcome by coupling CE with other types of detectors like, electrochemical detection (CE-ED) and Electron

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spray ionisation–Mass spectrometry (ESI-MS) Thus one of the future works is to couple CE with electrochemical detection since it poses low running cost, easy to apply and high sensitivity.