Separation, analysis and validation of metal ions in aqueous samples using capillary electrophoresis

ABSTRACT

Capillary electrophoresis (CE) has recently attracted considerable attention as a promising analytical technique for the separation and analysis of ions (cations and anions) in complex matrices. Most inorganic ions have weak absorption profiles in the UV-Vis wavelength range. These mostly non-absorption 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. In the determination of metal ions, several electrolyte systems can be employed. Indirect detection at 214 nm was performed with α-hydroxyisobutyric acid (α-HIBA)-4-aminopyridine background electrolyte (BGE) that has a characteristic absorbance in at 214 nm. This BGE was applied to separation, analysis and validation of metal ions in water samples. Several metal ions (K^+, Na+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, $Ni^{2+}$and Zn^{2+}) were successfully separated. The effect of electrolyte pH, applied voltage and injection time for the separation of cations was investigated.

Keywords: Water analysis; Background electrolyte composition; Complexation; Cations

1. INTRODUCTION

Inorganic and organic ions released to the environment may cause risks to the balance of nature. The ions 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].

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]. In this paper we report on α-hydroxyisobutyric acid (α-HIBA)-4-aminopyridine background electrolyte (BGE) employed for the separation, analysis and validation of the cations K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+} and Zn^{2+}. In addition , we have also included the investigation pertaining the effect of electrolyte pH, applied voltage and injection time for the separation of cations.

2. MATERIAL AND METHODS

2.1. Instrumentation

The CE analysis was performed on a Prince Technologies (Emmen, Netherlands) capillary electrophoresis system equipped with a power supply (0 - ± 35 kV) 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 applied voltage was +25 kV. Samples were introduced into the capillary by the hydrodynamic mode (50 mbar) for 12-24 s. Data acquisition and analysis (DAx) soft ware from Prince Technologies was used for the control of instrument settings. All experiments were conducted at 25^{o}C. The current was monitored for all evaluated back ground electrolytes and was in the range of 8 to 11 µA.

2.2 Materials

Manganese, Cadimuim, Zinc and Lead (all Spectro sol grades) were obtained from Fluka (Buchs, Switzerland). $MgCl_2.6H_2O$, Copper (Spectro Sol), Nickel (Spectro Sol) were obtained from Merck (Darmstadt, Germany). α-HIBA (98%), 4-aminopyridine (98%) and H_3PO_4 (85%) were from Aldrich (Steinheim, Germany). NaOH (99%) and HCl (99%) were from Riedel-deHaen (France). $CaCl_2.2H_2O$ (97%) and $FeSO_4.7H_2O$ (97%) were from NT laboratory supplies (Johannesburg). NaCl (99%) and KCl (99%) were from PAL Chemicals.

2.3 Samples

The standard aqueous samples were prepared and stored in polyethylene containers and acidified and then kept in refrigerator at +4 ^{o}C. For CE measurements all samples were first screened with CE and their concentration were estimated. When the peak resolution was not satisfactory (high ionic strength) or the ion zones were distorted, the samples were diluted with ultra purified water (Milli-Q water, prepared in the purification unit) and filtered through 0.45µm membranes to achieve better separation for quantification. The samples were not manipulated by pH adjustment or complexation, because we wanted to analyse them by simulating the natural water conditions as closely as possible.

2.4 Conditioning of the capillary

New capillaries were conditioned by purging with 1M NaOH solution (30 min), Milli-Q water-ultra pure water (30 min) and running electrolyte (30 min) and the sample was injected with the appropriate method. Capillaries were washed after every change of electrolyte with a 5 min rinse of 1 M NaOH solution and then with water and the appropriate electrolyte.

2.5 Standard mixtures

Metal cation standard solutions were obtained by dilution in Milli-Q water from 1000 mg/L stock solutions. The mixtures for capillary electrophoretic studies were prepared from the stock solutions to the concentrations needed. All mixtures were ultrasonicated daily before use. The stock solutions were kept in a refrigerator, at 4 ^{o}C.

2.6 Calibration solutions

A calibration curve was prepared with 5 mg/L, 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L concentrations by measuring 25 µL, 125 µL, 250 µL, 500 µL and 1000 µL respectively of each cation in 5 mL volumetric flasks and diluting with Milli-Q water to the mark. The calibration curves were measured only at the ranges suitable for real samples. The linear ranges of each ion were measured at specified injection time.

3. RESULTS AND DISCUSSION

The separation of metal ions was accomplished using different complexing agents and UV-absorbing species. The most important optimization parameter for the separation of cationic compounds is the choice of a suitable background electrolyte. In this study, α-hydroxyisobutyric acid and 4-aminopyridine BGEs were used. The different concentrations of complexing agent can change the migration order of metal ions since metal complexes have different stability thus mobility at different concentration of complexing agent.

3.1 Separation of metal ions

In the separation method, α-hydroxyisobutyric acid and 4-aminopyridine were used as the complexing agent and background electrolyte respectively for the separation of a mixture of ions. A mixture of 10 metal ions could be separated efficiently using 4-aminopyridine (Figure 1a). All the peaks were completely resolved. Using this method it was possible to detect Cd^{2+} and Pb^{2+} ions. Table 1 shows the quantitative data for migration time, peak areas and corrected peak areas of the metal ions in the standard mixture.

Table 1. 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%)

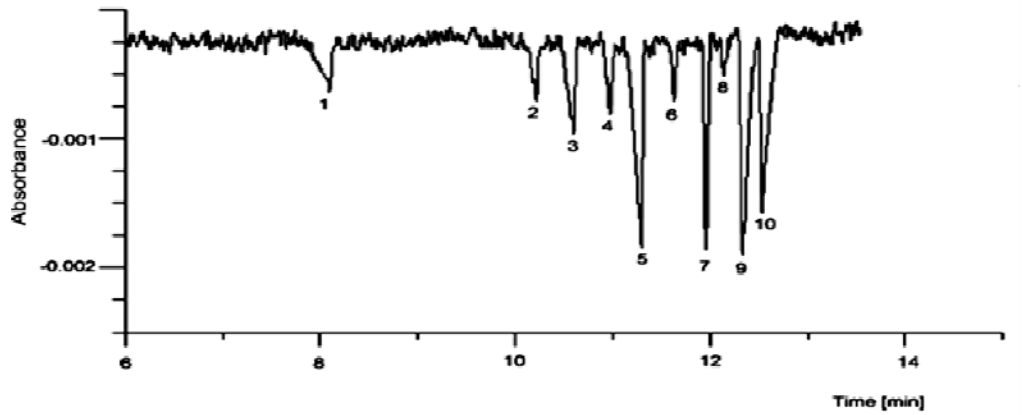


Fig.1a Electrophoretic separation of 10 metal ions. Carrier electrolyte, 10 mM 4-amino pyridine and 6.5 mM α-hydroxylisobutyric acid (pH 4.5); hydrodynamic injection 18 s; voltage, 25 kV; wavelength, 214 nm. Peaks according to sequence: 1= K^+, 2 = Ca^{2+}, 3 = Na^+, 4 = Mg^{2+}, 5 = Mn^{2+}, 6 = Fe^{2+},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

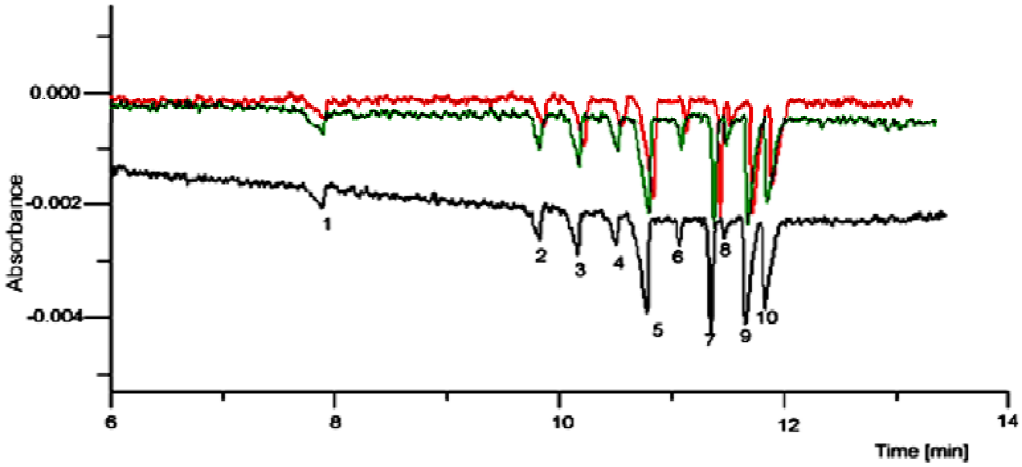


Fig.1b. The reproducibility (within runs) of metal ions over three runs using the same experimental conditions as in Fig. 1a. Peaks according to sequence: 1 = K^+, 2 = Ca^{2+}, 3 = Na^+, 4 = Mg^{2+}, 5 = Mn^{2+}, 6 = Fe^{2+}, 7 = Cd^{2+}, 8 = Pb^{2+}, 9 = Ni^{2+}, 10 = Zn^{2+}.

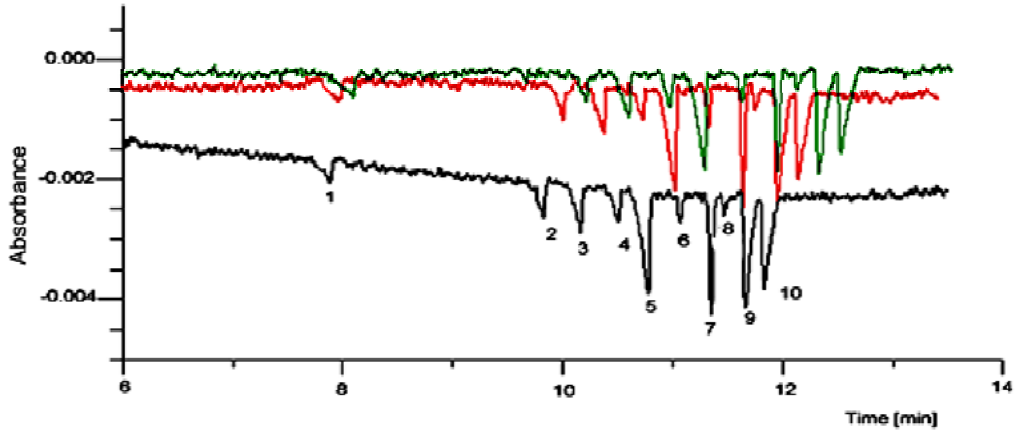


Fig.1c. The reproducibility of metal ions over three runs (between runs) using the same experimental conditions as in Fig. 1a. Peaks according to sequence: 1 = K^+, 2 = Ca^{2+}, 3 = Na^+, 4 = Mg^{2+}, 5 = Mn^{2+}, 6 = Fe^{2+}, 7 = Cd^{2+}, 8 = Pb^{2+}, 9= Ni^{2+}, 10 = Zn^{2+}.

Higher reproducibility was obtained over three runs (within runs) as shown in Figure 1b 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 in Figure 1c. 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 [5].

3.2. The analysis of synthetic and environmental samples

3.2.1 Analysis of synthetic samples

α-Hydroxyisobutyric acid-4-amino pyridine background electrolyte was chosen for the 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 [6] and contains suitable binding groups (carboxyl, hydroxyl) [7].

A high concentration of α-hydroxyisobutyric acid was used to decrease the electroosmosis flow (EOF) [8] 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 [9]. Optimum injection time was used to reduce overlap resulting from electro migration 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 [10]. The pH, the injection time and the separation voltage were the main factors affecting the separation of metal ions [11]. The effect of pH, applied voltage and injection on peak area and migration time has been studied.

3.2.2 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 [12, 13]. The electroosmosis flow (EOF) decreases with a decrease in pH [14, 15] 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 2 shows a plot of peak area as a function of pH.

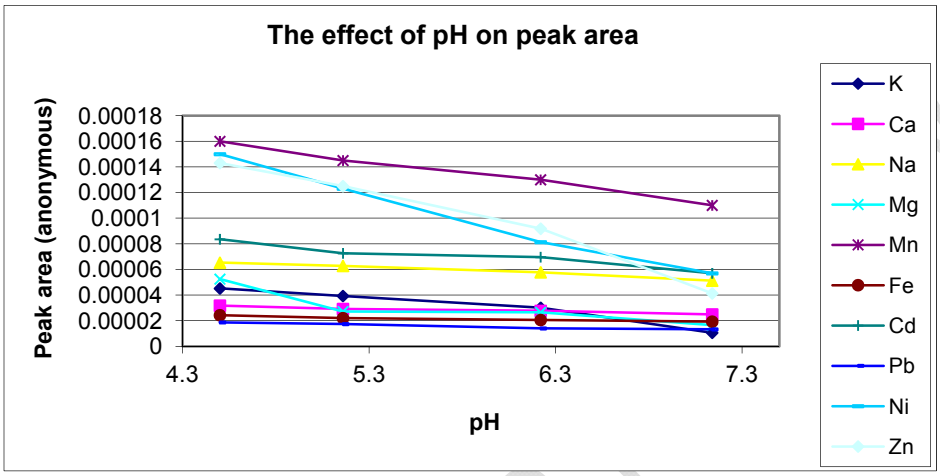


Fig. 2. The effect of pH of the background electrolyte on peak area. Experimental conditions are the same as in Fig.1a.

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 [16, 17, 18]. 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.

3.3.3 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) [19] is given by:

$$V = \mu E$$

The plot of the migration time as a function of applied voltage is given in Figure 3.

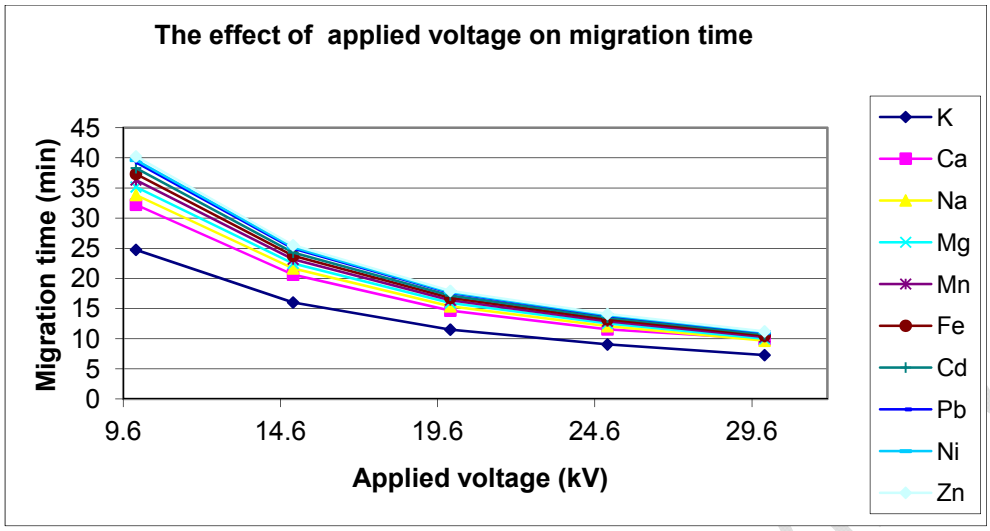


Fig. 3. The effect of applied voltage on migration time. Experimental conditions are the same as in Fig.1a.

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.

3.3.4 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 [20, 21]. 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 in Figure 4.

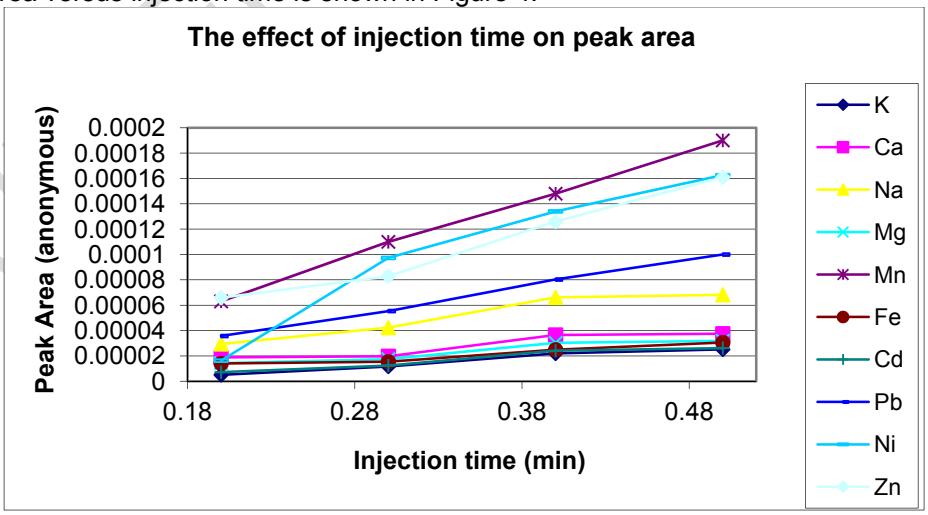


Fig.4. The effect of injection time on peak area. Experimental conditions are the same

as in Fig.1a.

3.3.5 Validation of the method

3.3.5.1 Limit of detection (LOD)

Table 2 shows with hydrodynamic injection for 24 s, at pH 4.5, with respect to each metal ion the following LOD was found. Lower limits of detection was obtained by injecting greater volumes, but at the expense of peak efficiency [22]. A higher limit of detection LOD was obtained for Ca^{2+}.

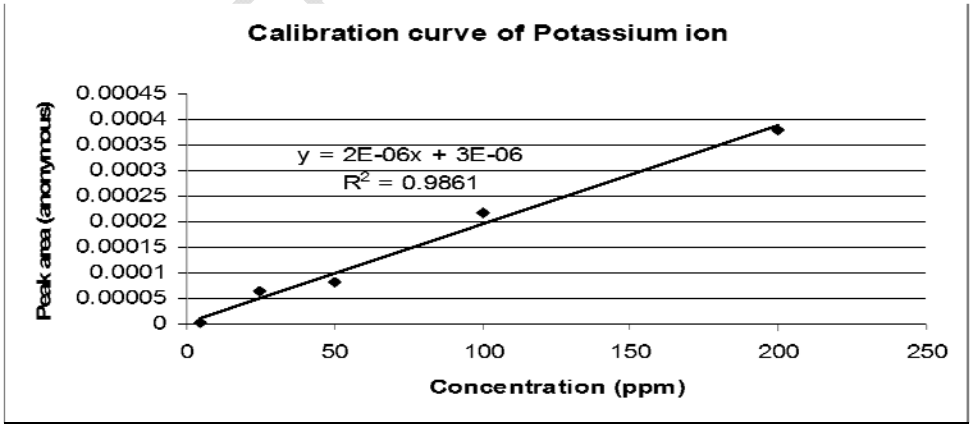
Table 2. The limit of detection of metal ions (ppm).

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

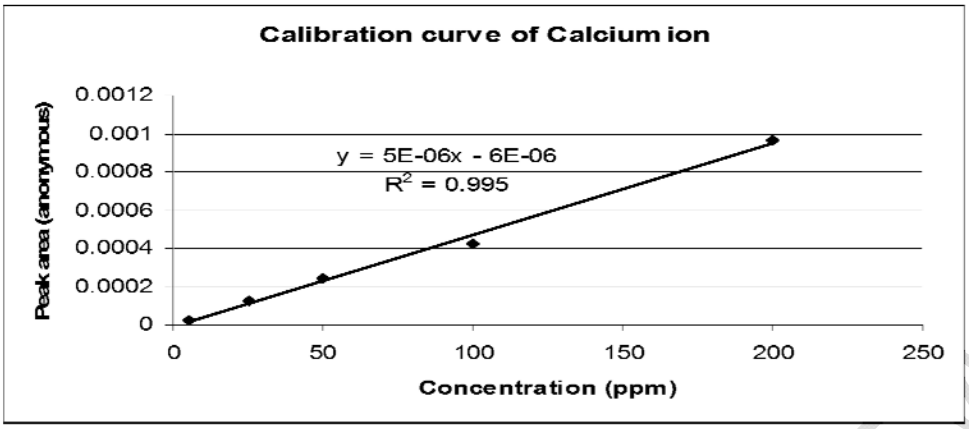
The limit of detection of each metal ion was determined by preparing standard solutions of each metal ion to the lowest concentration that can be obtained.

3.3.5.2 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. 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 in Figure 5.



(a)



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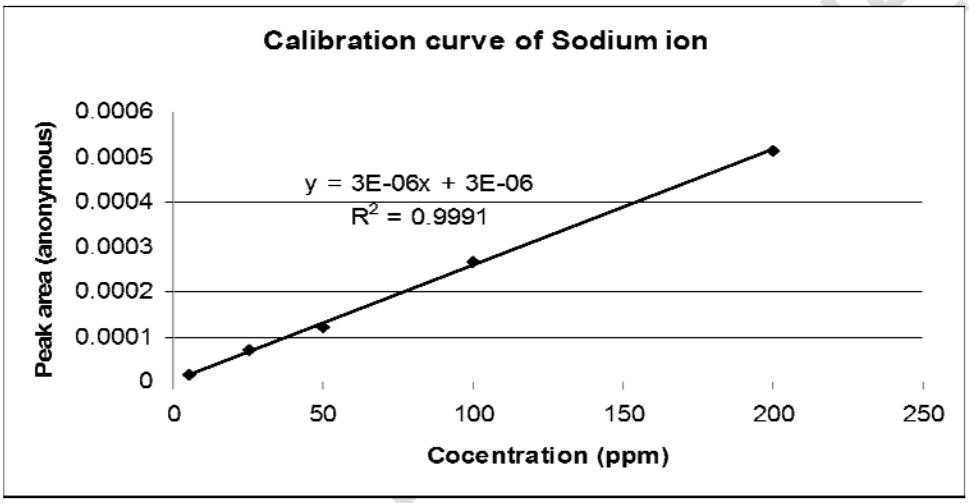

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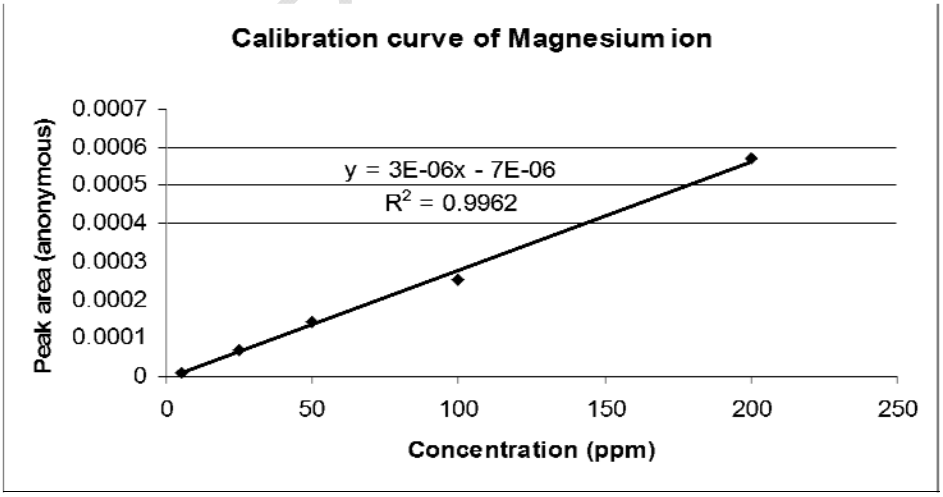

(d)

Fig. 5. Calibration curve of (a) K^+, (b) Ca^{2+}, (c) Na^+, (d) Mg^{2+}

4. CONCLUSION

Different methods have been developed for the separation, analysis and validation of metal ions in aqueous samples using capillary electrophoresis. The α-hydroxyisobutyric acid (α-HIBA)-4-aminopyridine background electrolyte (BGE) was effective since it results in for the detection of more number of metal ions. Using α-HIBA as complexing agent and 4-aminopyridine as UV-absorbing compound was the most efficient for the successful determination of all of metal ions. The potential of the method for quantitative and qualitative analysis of metal ions in complex matrix in environmental water sample is presented.

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