CHAPTER 7

RESULTS AND DISCUSSION

7.1 Absorption spectra of background electrolyte

Imidazole (Figure 7.1) is chosen to use as a probe because it provides high molar absorptivity.

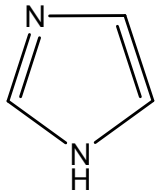

Figure 7.1 Structure of imidazole

The absorption spectrum of electrolyte containing 12 mM imidazole, 3 mM 18-crown-6 ether and 15 mM alanine at pH 6 was shown in Figure 7.2. Thus, the maximum absorbance at 206 nm was selected as detection wavelength.

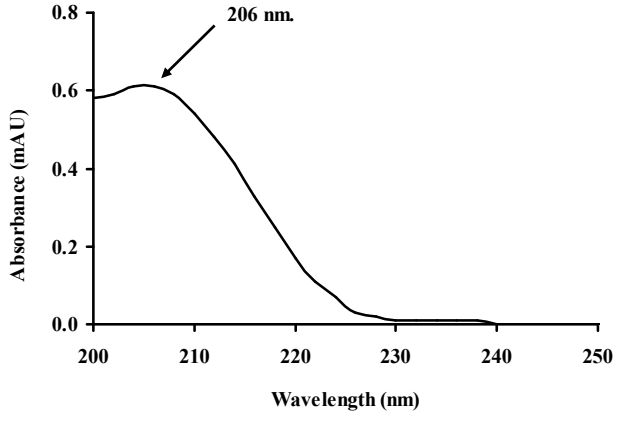

Figure 7.2 Spectrum of background electrolyte containing 12 mM imidazole, 3 mM 18-crown-6 ether and 15 mM alanine at pH 6.

7.2 Separation conditions

The advantages of imidazole were high molar absorptivity and its similar electrophoretic mobility to alkali and alkaline earth metal ions. Therefore, imidazole was suitable as an absorption probe in an electrolyte for the simultaneous determination of cations (Abe, et al., 2006; Carducci, et al., 2000; Gao, et al., 2008; Hopper, et al., 2005b; Johns, et al., 2004; Piovezan, et al., 2010; Sekar & Azhaguvel, 2007; Suarez-Luque, et al., 2006; Sze, et al., 2007; Warren & Adams, 2004). To find an alternative CZE method suitable for the determination of cationic NH_4^+, K^+, Ca^{2+} and Mg^{2+} in fertilizers, the preliminary experiment started with background electrolyte composed of imidazole and 18-crown-6 ether in acetic acid at pH 4.3. The separations were conducted with detection at the cathodic end of the capillary. The electropherogram of standard NH_4^+ and K^+ showed an interference peak at 1.42 min. (Figuer 7.3a and 7.3b), whilst there was no interference peak for Ca^{2+} and Mg^{2+}. The further experiment was done in electrolyte consisted of imidazole and 18-crown-6 ether in acetic acid at pH 6. The same interference peak still appeared for NH_4^+ and K^+, whilst migration of cations was faster because proton produced from dissociation of acetic acid increased EOF of electrolyte (Figure 7.4).

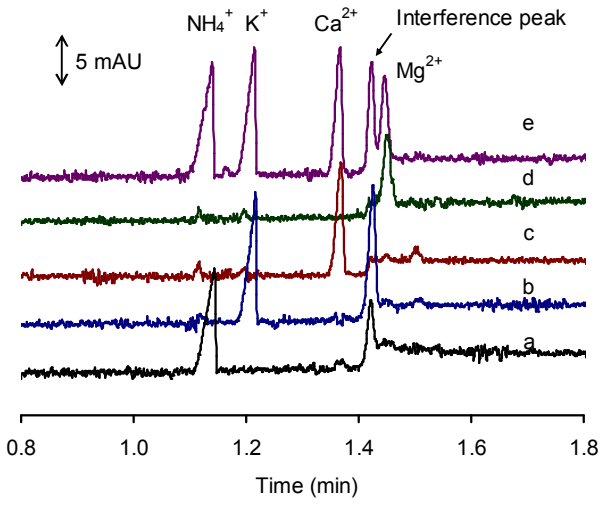

Figure 7.3 Electropherogram of standard (a) NH_4^+ (2.5 mg L^{-1}), (b) K^+ (5 mg L^{-1}), (c) Ca^{2+} (1.25 mg L^{-1}), (d) Mg^{2+} (0.5 mg L^{-1}) and (e) mixture of NH_4^+ (2.5 mg L^{-1}), K^+ (5 mg L^{-1}), Ca^{2+} (1.25 mg L^{-1}), and Mg^{2+} (0.5 mg L^{-1}). Electrolyte: 12 mM imidazole, 3 mM 18-crown-6 ether pH 4.3. Separation voltage: +25 kV.

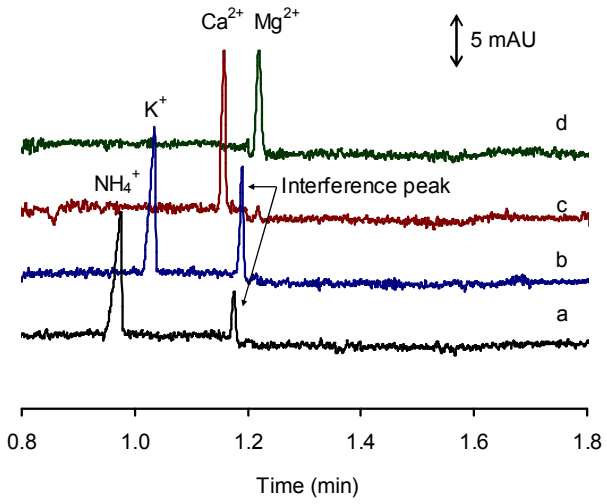

Figure 7.4 Electrolyte: 12 mM imidazole, 3 mM 18-crown-6 ether pH 6.0. Concentration of standard (a), (b), (c) and (d) are the same as in Figure 7.3.

To remove the interference peak at 1.42 min., alanine was chosen to add into electrolyte containing imidazole and 18-crown-6 ether and adjust pH to 6 with acetic acid. The pH of electrolyte was adjusted to pI of alanine in order to protect alanine from dissociation. The results showed no interference peak appear for NH_4^+, K^+, Ca^{2+} and Mg^{2+} (Figure 7.5). In addition, separation of Na^+, Mn^{2+}, Co^{2+} and Cd^{2+} were also investigated in this electrolyte.

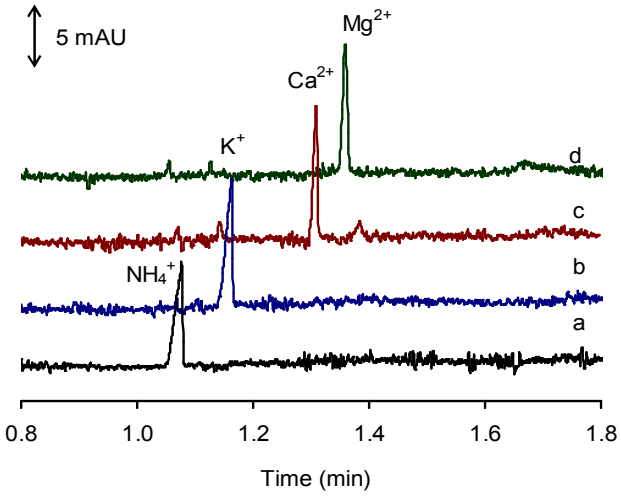

Figure 7.5 Electrolyte: 12 mM imidazole, 3 mM 18-crown-6 ether and 15 mM alanine at pH 6.0. Concentration of standard (a), (b), (c) and (d) are the same as in Figure 7.3.

The investigation of the optimization electrolyte for the separation of NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Co^{2+} and Cd^{2+} were conducted within the ranges 10-18 mM imidazole, 0-6 mM 18-crown-6 ether and 10-20 mM alanine. The experiments showed increasing imidazole concentration in the electrolyte did not cause a large decrease in EOF. Migration time and peak height of cations did not change for 10-12 mM imidazole, but more than 12 mM imidazole caused migration time increase. Without 18-crown-6 ether in the background electrolyte, NH_4^+ and K^+ peaks were overlapped but well resolved after adding 3 mM 18-crown-6 ether in electrolyte. Increasing the concentration of 18-crown-6 ether to 6 mM resulted in a poor resolution of the Na^+ and Mg^{2+} peaks (Figure 7.6).

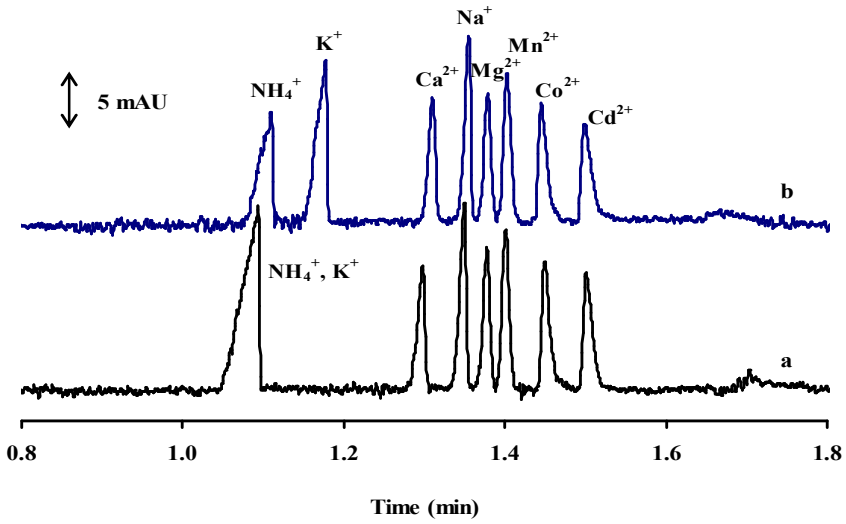

Figure 7.6 Electropherogram of NH_4^+ (2.5 mg L^{-1}), K^+ (5 mg L^{-1}), Ca^{2+} (1.25 mg L^{-1}), Na^+ (1 mg L^{-1}), Mg^{2+} (0.5 mg L^{-1}), Mn^{2+} (1 mg L^{-1}), Co^{2+} (1 mg L^{-1}) and Cd^{2+} (5 mg L^{-1}) in a standard solution; (a) electrolyte: 12 mM imidazole and 15 mM alanine at pH 6; (b) electrolyte: 12 mM imidazole, 3 mM 18-crown-6 ether and 15 mM alanine at pH 6.0.

Increasing alanine concentration increased the migration time. After consideration of these three factors, the optimization conditions that provided desirable peak shape and high detection sensitivity for the separation of NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Co^{2+} and Cd^{2+} were the use of 12 mM imidazole, 3 mM 18-crown-6 ether and 15 mM alanine at pH 6.0. However, the applied voltage and the

injection time were optimized within the range of +15 to +27 kV, and 3-10 sec under pressure of 50 mbar. Increasing the magnitude of the positive separation voltage resulted in decreased migration time, and the highest peak height was obtained at +25 kV. Increasing the injection time increased peak height but at more than 7 s provided bad peak shape of NH_4^+, K^+, Co^{2+} and Cd^{2+}, so 7 s was selected. Figure 7.7 shows an electropherogram obtained from a standard mixture of NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Co^{2+} and Cd^{2+} under the optimized conditions.

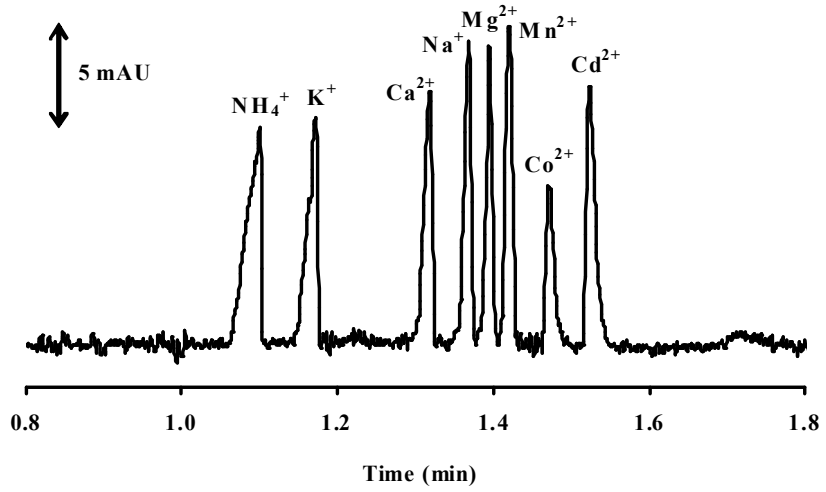

Figure 7.7 Co-EOF of standard NH_4^+ (2.5 mg L^{-1}), K^+ (5 mg L^{-1}), Ca^{2+} (1.25 mg L^{-1}), Na^+ (1 mg L^{-1}), Mg^{2+} (0.5 mg L^{-1}), Mn^{2+} (1 mg L^{-1}), Co^{2+} (1 mg L^{-1}) and Cd^{2+} (5 mg L^{-1}). The electrolyte was 12 mM imidazole, 3 mM 18-crown-6 ether and 15 mM alanine at pH 6.0; the separation temperature 25°C, the separation voltage +25 kV and injection time 7 s under pressure 50 mbar.

Electrophoretic mobilities (μ) of cations were calculated from equation below:

$$\mu = L_d \times L_t / V \times t \ (\times 10^{-9}\ m^2/V.sec)$$

When L_d = capillary length to detector (m) V = separation voltage (V)

L_t = total capillary length (m) t = migration time (sec)

Effective electrophoretic mobilities of eight cations are provided in Table 7.1 and the values are similar to ref. (Johns, et al., 2004). It can be seen that the co-EOF separation of cations provided good selectivity and well shaped peaks.

Table 7.1

Electrophoretic mobilities of eight cations for simultaneous separation of cations comparable to ref. (Johns, et al., 2004)

Cations	Electrophoretic mobility (x 10^{-9} $m^2.V^{-1}.s^{-1}$)	
	Calculated values	Ref. (Johns, et al., 2004)
NH_4^+	68.8	75.1
K^+	62.7	67.2
Ca^{2+}	52.4	53.4
Na^+	49.3	51.3
Mg^{2+}	47.8	48.8
Mn^{2+}	46.5	-
Co^{2+}	43.7	-
Cd^{2+}	41.2	-

Electrolyte of ref. (Johns, et al., 2004): 6 mM imidazole, 12 mM HIBA, 2 mM 18-crown-6 ether, 0.1 mM DDAB, pH 4.0; voltage:-30 kV.

7.3 Analytical performance characteristics

Analytical performance characteristics were determined under the optimized conditions: fused silica capillary length 45 cm (36.5 cm to detector), separation potential +25 kV, a background electrolyte 12 mM imidazole, 3 mM 18-crown-6 ether in 15 mM alanine at pH 6 and injection time 7 s under pressure 50 mbar.

Linearity was observed for NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Co^{2+} and Cd^{2+} using peak area. External standard calibration curves exhibited good linearity. Table 7.2 summarizes limits of detection (LODs, determined by a signal to noise ratio of 3)

and correlation coefficients for the above cations. The % relative standard deviation of intra- (%RSD of five replicate injections of standard solution) and inter-day (%RSD of three day injections of standard solution) was shown in Table 7.3. The injection time for LODs determination were 7 s. under the pressure 50 mbar and the sensitivity of this method was sufficient to determine cationic macronutrient (NH_4^+ and K^+) and micronutrients ($Ca^{2+}$and Mg^{2+}) in fertilizers. In addition, the optimized condition can also use for determination of trace Mn^{2+} in fertilizers. The RSDs of migration time, peak area and peak height for NH_4^+, K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Co^{2+} and Cd^{2+} were less than 1% for intra-day and less than 2% for inter-day.

Table 7.2

Detection limits and calibration graphs for determination of ammonium, potassium, calcium, sodium, magnesium, manganese, cobalt and cadmium

Cation	LOD ($mg\ L^{-1}$)	Linearity range ($mg\ L^{-1}$)	Regression equation	Correlation coefficient
NH_4^+	0.06	0.7-30	y = 2.4884x-0.2538	0.9995
K^+	0.10	1-30	y = 0.8736x+0.3742	0.9995
Ca^{2+}	0.03	0.3-100	y = 2.429x-1.6178	0.9997
Na^+	0.02	0.2-30	y = 3.3482x+0.059	0.9999
Mg^{2+}	0.02	0.2-100	y = 7.6321x-5.2875	0.9998
Mn^{2+}	0.04	0.5-200	y = 4.0527x-3.8872	0.9997
Co^{2+}	0.07	0.8-200	y = 2.3301x+0.3777	0.9997
Cd^{2+}	0.20	3-300	y = 0.847x-0.8267	0.9993

Table 7.3

%RSD of intra- and inter-day for standard cations

Cations	Intra-day (n=5)			Inter-day (n=3)		
	Migration time	Peak area	Peak height	Migration time	Peak area	Peak height
NH_4^+	0.15	0.20	0.94	0.23	0.24	1.60
K^+	0.10	0.35	0.60	0.23	0.38	0.78
Ca^{2+}	0.13	0.22	0.35	0.22	0.24	0.86
Na^+	0.15	0.15	0.30	0.22	0.26	0.73
Mg^{2+}	0.16	0.24	0.78	0.23	0.28	1.01
Mn^{2+}	0.20	0.19	0.75	0.25	0.26	0.94
Co^{2+}	0.20	0.16	0.64	0.23	0.25	1.12
Cd^{2+}	0.25	0.29	0.82	0.20	0.37	1.64

Investigation for potentially interfering metal ions showed no interference from Fe^{3+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. This conclusion was reached from the absence of a Fe^{3+} peak above the background noise and bad peak shape of Ni^{2+}, Cu^{2+} and Zn^{2+} after Cd^{2+} peak. The developed CZE provides fast analysis time for the separation of eight cations in less than 1.6 min.

7.4 Determination of cationic nutrients in fertilizer samples

Due to various formulas of fertilizers, fertilizers containing macronutrient (N-P-K = 7-10-10), macronutrient and micronutrient ((N-P-K: 18-4-6 and 16-8-8), fertilizer carrier of micronutrient (Ca-Mg and Mg) and fertilizer carrier of trace nutrient (Mn) were chosen for quantitative analysis by CE method. Preliminary CE experiments demonstrated that the matrix in fertilizer samples affected the peak area of K^+. Therefore, the standard addition method was required for the analysis of K^+, whilst an external calibration method was used for the analysis of NH_4^+, Ca^{2+}, Mg^{2+} and Mn^{2+}. Electropherogram of a solid fertilizer sample (N-P-K=18-4-6) with and without a spike of standard NH_4^+, K^+, Ca^{2+}, Na^+ and Mg^{2+} are shown in Figure 7.8. Electropherogram obtained from Ca-Mg fluid fertilizer before and after spiking with standard Ca^{2+} and Mg^{2+} are shown in Figure 7.9.

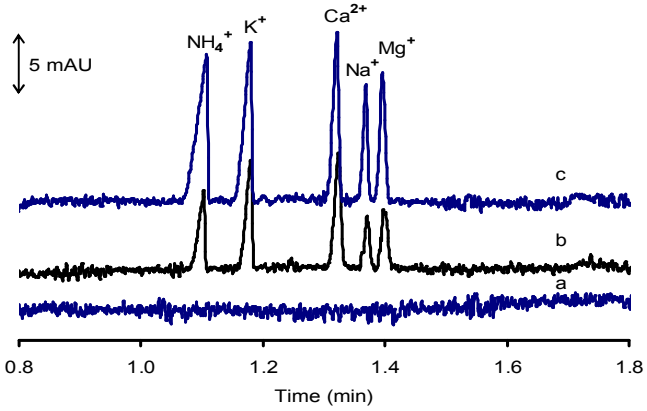

Figure 7.8 Electropherogram of solid fertilizer sample: N-P-K = 18-4-6; (a) blank; (b) unspiked N-P-K fertilizer; (c) N-P-K fertilizer spiked with 2 mg L^{-1} NH_4^+, 2.5 mg L^{-1} K^+, 0.5 mg L^{-1} Ca^{2+}, 0.3 mg L^{-1} Na^+ and 0.2 mg L^{-1} Mg^{2+}. Other conditions are the same as in Figure 7.7.

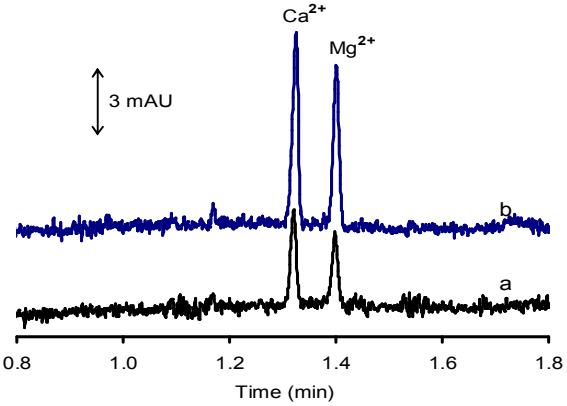

Figure 7.9 Electropherogram of Ca-Mg fluid fertilizer sample; (a) unspiked Ca-Mg fertilizer; (b) Ca-Mg fertilizer spiked with 0.5 mg L^{-1} Ca^{2+} and 0.2 mg L^{-1} Mg^{2+}. Other conditions are the same as in Figure 7.7.

The validity of the CE method was also investigated by comparing the results obtained by CE with atomic absorption spectrophotometer (AAS) analysis, except for NH_4^+. The preliminary experiments of AAS confirmed that the matrix from both solid and fluid fertilizers affected absorbance of K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} in samples. Therefore, the standard addition method was need for the quantitative analysis of K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} in fertilizers by the AAS method. The results obtained for K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} by CE agreed well with from AAS as shown in Table 7.4.

Table 7.4

Comparison of the results for K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} in fertilizers (%w/w) obtained by CE and AAS (n=3)

Fertilizers	$\%NH_4^+$		$\%K^+$		$\%Ca^{2+}$		$\%Mg^{2+}$		$\%Mn^{2+}$	
	CE	AAS	CE	AAS	CE	AAS	CE	AAS	CE	AAS
N-P-K=18-4-6	1.65±0.01	-	6.26±0.09	6.36±0.16	1.23±0.05	1.12±0.03	0.44±0.01	0.40±0.04	-	-
N-P-K=16-8-8	2.50±0.18	-	6.78±0.07	6.92±0.16	1.69±0.10	1.72±0.06	0.61±0.02	0.61±0.05	-	-
N-P-K=7-10-10	3.59±0.09	-	9.65±0.17	10.04±0.21	-	-	-	-	-	-
Ca-Mg	-	-	-	-	6.03±0.32	6.02±0.13	3.46±0.09	3.53±0.24	-	-
Mg	-	-	-	-	-	-	2.25±0.06	2.27±0.13	-	-
Mn	-	-	-	-	-	-	-	-	5.49±0.11	5.43±0.21

R^2 of K by standard addition method of CZE method = 0.9994

R^2 of K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} by standard addition method of AAS method in the range 0.9993-0.999

To compare precision of K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} concentration in fertilizers obtained from CE and FAAS by using paired t-test, the results of P (two tail) calculated by t-test excel program showed in table 7.5. From table the calculated P value of K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} in all fertilizers more than significant level 0.05, it means no significant difference between the CE and FAAS methods at 95% confidence level.

Table 7.5

Calculated P (two tail) compare to significant level P = 0.05

Fertilizers	$\%K^+$	$\%Ca^{2+}$	$\%Mg^{2+}$	$\%Mn^{2+}$
N-P-K=18-4-6	0.18	0.16	0.26	-
N-P-K=16-8-8	0.11	0.38	0.71	-
N-P-K=7-10-10	0.17	-	-	-
Ca-Mg	-	0.94	0.76	-
Mg	-	-	0.67	-
Mn	-	-	-	0.40