

**DETERMINATION OF AMMONIUM AND POTASSIUM EXTRACTED  
FROM SOIL SAMPLES BY CAPILLARY ELECTROPHORESIS WITH  
CAPACITIVELY COUPLED CONTACTLESS CONDUCTIVITY  
DETECTION (CE-C<sup>4</sup>D)**

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**TÓM TẮT**

**XÁC ĐỊNH AMONI VÀ KALI TRONG DỊCH CHIẾT CỦA MẪU ĐẤT  
BẰNG PHƯƠNG PHÁP ĐIỆN DI MAO QUẢN SỬ DỤNG DETECTOR ĐỘ  
DẪN KHÔNG TIẾP XÚC (CE-C<sup>4</sup>D)**

*Bài báo nghiên cứu quy trình phân tích hai ion  $NH_4^+$  và  $K^+$  trong dịch chiết mẫu đất nông nghiệp bằng kỹ thuật điện di mao quản sử dụng detector độ dẫn không tiếp xúc. Các điều kiện phân tích đã được tối ưu bao gồm: Dung dịch đệm điện di sử dụng hỗn hợp đệm histidine/axit acetic (His/Ace) ( $pH = 4$ ), 2mM 18-crown-6; mao quản Silica: đường kính ngoài (O.D.) 365  $\mu m$ , đường kính trong (I.D.) 50  $\mu m$ , chiều dài tổng  $L_t = 50cm$  và chiều dài hiệu dụng  $L_{eff} = 40cm$ ; điện thế tách: 20kV; bơm mẫu theo phương pháp thủy động lực theo kiểu xiphong ở độ cao 15cm, trong thời gian 20s. Quy trình được áp dụng để phân tích dịch chiết 01 mẫu đất và 01 mẫu nước lấy tại cùng một khu canh tác nông nghiệp. Các kết quả phân tích được kiểm chứng bằng các phương*

*pháp quang phổ hấp thụ nguyên tử (kiểm chứng kết quả phân tích  $K^+$ ) và phương pháp quang phổ hấp thụ phân tử (kiểm chứng kết quả phân tích  $NH_4^+$ ). Sai số tương đối giữa phương pháp phân tích và các phương pháp đo kiểm chứng đều dưới 15%. Kết quả cho thấy hàm lượng  $NH_4-N$  và  $K^+$  trong mẫu đất lần lượt là 29,6 và 1055 mg/kg đất khô. Hàm lượng  $NH_4-N$  và  $K^+$  trong mẫu nước lần lượt là 2,68 và 33,7 mg/L. Hàm lượng  $NH_4-N$  trong mẫu nước ruộng vượt hơn 5 lần quy chuẩn kỹ thuật Quốc gia về chất lượng nước mặt bảo vệ đời sống thủy sinh.*

**Key words:** ammonium, potassium, contactless conductivity detector, agricultural soil

## 1. INTRODUCTION

Nitrogen and potassium are essential nutrients for plant growth, survival, development and reproduction. However, there are not always enough of these nutrients in the soil for a plant to grow healthily so that many farmers and gardeners use fertilizers as the nutrients for the soil. Despite of the fact that fertilizers are beneficial to agriculture, excessive or improper fertilizer applying would induce to environmental and human health risks. The excess of nutrients in environment would cause soil pollution, surface water eutrophication, groundwater contamination... etc. Elevated levels of nutrients accumulated in crop products could make unexpected symptoms and diseases to consumers [1]. For better agricultural land use, it is necessary to quantify the nutrient residues that are available to be released to environment. The extractions of the exchangeable forms of  $K^+$  and  $NH_4^+$  can be performed by using salt solutions. Because  $NH_4^+$

and  $K^+$  exhibit nearly identical characteristics, common extracting reagents for exchangeable potassium are ammonium salts. On the other hand, ammonium adsorbed on the exchange complex is normally removed by using potassium salts such as KCl or  $K_2SO_4$  [2]. Thus, measurements of soil extracts for  $K^+$  and  $NH_4^+$  would be strongly interfered by the matrix effects. Capillary Electrophoresis (CE) with Capacitively Coupled Contactless Conductivity Detection ( $C^4D$ ) is a simple and inexpensive method that can be applicable to simultaneously determine  $NH_4^+$  and  $K^+$  in aqueous samples. However, the separations of two cations solely based on differences in their electrophoretic mobility seem to be a challenge.

Numerous attempts were investigated to separate  $NH_4^+$  and  $K^+$  in aqueous solutions. One of the most successful approach was the employment of complex-forming reactions. Among complexing agents, 18-crown-6 ether

was widely used to separate the  $\text{NH}_4^+$  and alkaline metal cations as it forms a stable complex with alkaline metal cations [3, 4, 5, 6, 7]. Adam J. Gaudry et al. separated an aqueous mixture comprising three inorganic cations ( $\text{NH}_4^+$ ,  $\text{Na}^+$  and  $\text{Li}^+$ ) in 50 mM acetic acid/10 mM L-histidine /2.5 mM 18-crown-6 ether electrolyte at pH 4.2. The limit of detections (LODs) of  $\text{NH}_4^+$ ,  $\text{Na}^+$ , and  $\text{Li}^+$  were 1.54, 2.26, and 3.06mg/L, respectively [3]. Another separation of alkaline metal cations ( $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Li}^+$ ) in aqueous solutions was obtained by using the background electrolyte (BGE) of 12 mM histidine adjusted to pH 4 with acetic acid and 2 mM 18-crown-6. The LOD for  $\text{K}^+$  was of 1.5  $\mu\text{M}$  [8]. Solutions containing  $\text{NH}_4^+$ ,  $\text{K}^+$  and other cations were determined by Thanh Duc Mai et al. [5]. Background electrolyte solutions were His 12 mM (adjusted to pH 4 with acetic acid) in the presence of 2 mM of 18-crown-6; capillary was of 50  $\mu\text{m}$  I.D., 36 cm effective length, and 50 cm total length, while separation voltage was 15 kV. The LODs were in the lower micromolar range and varied depending on species. Baseline separation between  $\text{NH}_4^+$  and  $\text{K}^+$  was achieved at the concentration of less than 100  $\mu\text{M}$  for each cation [5]. Nevertheless, to our best knowledge, a simultaneous separation of  $\text{NH}_4^+$  and  $\text{K}^+$

aqueous solutions extracted from soils by CE- $\text{C}^{4}\text{D}$  has not been reported.

In the present study, operation parameters were set up in our laboratory according to previously published papers for measuring  $\text{NH}_4^+$  and  $\text{K}^+$  in aqueous solutions. The optimizing amount of 18-crown-6 was systematically investigated. Appropriate extracting solutions used to remove exchangeable fraction of each ion from soil were also examined. The preliminary conditions were applied to detect  $\text{NH}_4^+$  and  $\text{K}^+$  in a water sample and the extracts of a soil sample collected from agricultural land in Vietnam.

## 2. MATERIALS AND METHODS

### 2.1. Materials

All the chemicals such as  $\text{NH}_4\text{Cl}$ ,  $\text{K}_2\text{SO}_4$ , His,  $\text{C}_6\text{H}_9\text{N}_3\text{O}_2$ ,  $\text{HCl}$ ,  $\text{NaOH}$ ... etc were of analytical grade and purchased from Fluka or Merck. Ultrapure water system (Labconco, USA) with resistivity 18.2  $\text{M}\Omega$  was used to produce ultrapure water in all solutions and measurements. Stock solutions of ammonium and potassium ions were daily prepared. The background electrolyte (BGE) solutions were prepared with the buffer of histidine and acetic acid (His/Ace) (pH=4) in the presence of 18-Crown-6. The pH of solutions was controlled by using an HI 2215 Hanna Instruments

pH meter (Woonsocket, RI, USA). Fused silica capillaries of 50  $\mu\text{m}$  I.D. and 365  $\mu\text{m}$  O.D. with total length ( $L_t$ ) of 50 cm and effective length ( $L_{\text{eff}}$ ) of 40 cm (purchased from Polymicro, Phoenix, AZ, USA) were used for separations. The capillaries were preconditioned with 1 M NaOH for 10 minutes and then, deionized water for

10 minutes prior to flushing with buffer solutions. Experiments were performed using the portable semi-automated CE system supported by 3Sanalysis JSC. (<http://www.3sanalysis.vn/>) (Figure 1). More detailed information about the instrument was given elsewhere in T. A. H. Nguyen et. al. [9].



*Figure 1. Portable semi-automated CE- $C^4D$  instrument*

## 2.2. Methods

According to former references reviewed earlier, the BGE with the buffer adjusted to pH 4 was commonly used for the separations of the two cations using CE - $C^4D$ . In the present study, the buffer solution of His/Ace (pH=4) was selected. The high voltage

of 20kV was applied for separation. Hydrodynamic injection of samples was carried out by setting the high-voltage end of the capillary at 15cm height. The injection time was 20 seconds. Table 1 summarized the operating parameters used in this research.

Table 1. Separation conditions of CE- $C^4D$  system for determine  $NH_4^+$  and  $K^+$ .

<b>BGE composition</b>	His/Ace buffer adjusted to pH 4 18-Crown-6
<b>High voltage</b>	20kV
<b>Detection</b>	$C^4D$
<b>Capillary</b>	365 $\mu m$ O.D., 50 $\mu m$ I.D.; $L_t = 50cm$ ; $L_{eff} = 40cm$ ; Preconditioned 20s at 15cm height
<b>Hydrodynamic injection</b>	

The BGE solutions with differences amount of 18-crown-6 were prepared in order to get optimum BGE composition for the separation of two cations. The concentrations of 18-crown-6 varied from 1 to 3mM. The concentrations of both  $NH_4^+$  and  $K^+$  were 0,1mM.

Appropriate soil extraction procedure was investigated. Simulated soil samples contaminated by 20mM of  $K^+$  and 2.2mM of  $NH_4^+$  were prepared by adding certain amount of  $K^+$  and  $NH_4^+$  solutions to uncontaminated soils. The samples were left for 1 day to reach to equilibrium, and then dried in desiccator before any further preparation. For  $K^+$  extraction, the  $CH_3COONH_4$  solutions of 0.01M and 0.1M were used. The ion  $NH_4^+$  was extracted with  $K_2SO_4$  solutions of 0.001 and 0.1 M. The amount of 1.000 g dried soil and precisely 20ml of extracting agent were put into 50ml- falcon tubes and shaken for 1 hour using a shaker (Cole Parmer, 51704 Series). Finally, the mixture was centrifuged and filtered

through 0.45 $\mu m$  membrane. The filtrates were used for the  $K^+$  and  $NH_4^+$  measurements using CE- $C^4D$ . The levels of  $K^+$  and  $NH_4^+$  were compared with Flame - Atomic Absorption Spectroscopy (F-AAS) and Ultraviolet-Visible Spectroscopy ((UV-Vis), respectively.

One soil and one water samples were collected in agricultural regions Thuong Tin, Hanoi, Vietnam. The soil sample was immediately stored at 4°C and then dried in desiccator. The water sample was kept at 4°C and filtered through 0.45 $\mu m$  membrane before analysis.

### 3. RESULTS AND DISCUSSION

It was known that potassium forms a stable complex with 18-crown-6 [10]. Consequently, the presence of 18-crown-6 in the BGE increases the migration time of  $K^+$  while the migration time of  $NH_4^+$  stays unchanged. As clearly shown in Figure 2, the best separation was performed at the concentration of 2mM 18-crown-6. Lower concentrations than that of 2 mM

18-crown-6 would not be enough for reasonable separation of the two cations while higher concentrations seem to be not necessary. Thus, for further

experiments the BGE solutions contain Tris/Ace buffer (pH=4); 2 mM 18-Crown-6.

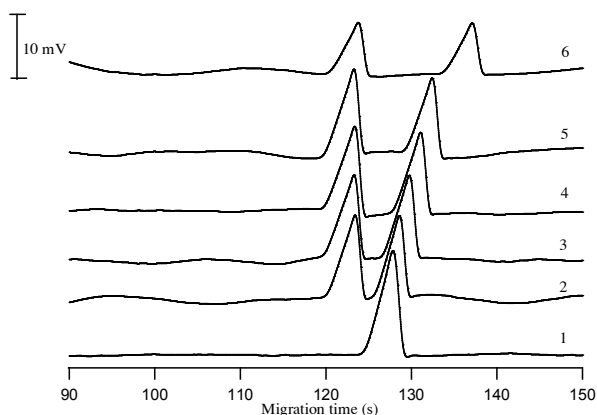


Figure 2. Separation of  $\text{NH}_4^+$  and  $\text{K}^+$  with BGE solutions containing different levels of 18-crown-6; Concentration of  $\text{NH}_4^+$  and  $\text{K}^+$ :  $10^{-4}\text{M}$ ; Concentration of 18-crown-6: (1): Non 18-crown-6 contained; (2) 1.0 mM; (3) 1.5 mM; (4) 2.0 mM; (5): 2.5 mM. (6): 3 mM

The calibration curves (six points) were extrapolated using standard addition method in order to avoid the matrix interference of the sample (Table 2). Linear range extended to the concentration of 200  $\mu\text{M}$  for each cations with a correlation coefficient ( $r^2$ ) of at least 0.99. The LOD and LOQ

values were calculated from peak areas corresponding to 3 and 10 times the baseline noise ( $\text{S/N}=3$  and  $\text{S/N}=10$ ), respectively. The LOD values (1.0  $\mu\text{M}$  for  $\text{NH}_4^+$  and 3.0  $\mu\text{M}$  for  $\text{K}^+$ ) are comparable to previously reported results [5, 8].

Table 2. Calibration curve extrapolated by standard addition method

Analyte	Range <sup>a</sup> ( $\mu\text{M}$ )	$r^2$	LOD <sup>b</sup> ( $\mu\text{M}$ )	LOQ <sup>c</sup> ( $\mu\text{M}$ )
$\text{NH}_4^+$	50.0-200	0.9988	1.0	5.0
$\text{K}^+$	50.0-200	0.9909	3.0	10.0

<sup>a</sup> Six points

<sup>b</sup>  $\text{S/N}$ : 3

<sup>c</sup>  $\text{S/N}$ : 10

As can be seen in Table 3, the best extraction efficiency was achieved

when using 0.1M  $\text{CH}_3\text{COONH}_4$  and 0.001M  $\text{K}_2\text{SO}_4$  for extraction  $\text{K}^+$  and

$\text{NH}_4^+$ , respectively. Higher concentrations of extractants are unfavorable due to the strong matrix effect to the separation of the two

cations by CE, while lower ones are not enough to completely extract the exchangeable solutes.

Table 3. Extraction of the simulated soil sample for  $\text{K}^+$  and  $\text{NH}_4^+$

	Extractant	Extraction efficiency (%)
<b>Extraction for <math>\text{K}^+</math></b>	$\text{CH}_3\text{COONH}_4$ 0.01M	25.0
	$\text{CH}_3\text{COONH}_4$ 0.1M	99.2
<b>Extraction for <math>\text{NH}_4^+</math></b>	$\text{K}_2\text{SO}_4$ 0.001M	109.0
	$\text{K}_2\text{SO}_4$ 0.1M	- <sup>a</sup>

<sup>a</sup>unable to detect

Figure 3 and 4 indicate good separation performances in the soil extract and water sample. Obtained results from CE- $\text{C}^4\text{D}$  are in good agreement with those from the confirmation method (F-AAS for  $\text{K}^+$  analysis and UV-Vis for  $\text{NH}_4^+$  analysis) (Table 4). This suggests that the conditions found are applicable for detection of  $\text{NH}_4^+$  and  $\text{K}^+$  in environmental samples. Levels of  $\text{NH}_4\text{-N}$  and  $\text{K}^+$  determined in the real soil sample were of 29.6 and 1055 mg/kg dried soil, respectively. Those highly

exceeded the normal level of  $\text{NH}_4\text{-N}$  (10 mg/kg) and the excessive level of  $\text{K}$  ( $> 800\text{mg/kg}$ ) defined by D.A. Horneck et al. [11]. The concentration of  $\text{NH}_4\text{-N}$  in water sample was more than 5 times higher than the National technical regulation on surface water quality for protection of aquatic lives [12]. It should be noted that sufficiently high levels of  $\text{NH}_4^+$  and  $\text{K}^+$  in agricultural run-off and soil would cause nutrient imbalance and serious environmental problem.

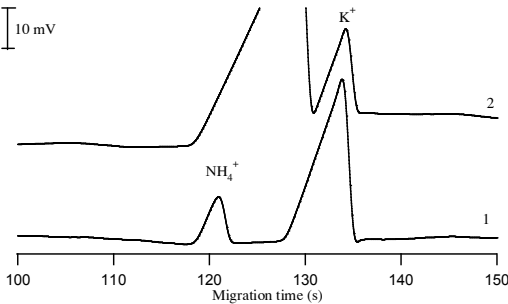


Figure 3. Separation of  $\text{NH}_4^+$  and  $\text{K}^+$  in the extracts of soil sample: (1) Soil extract for  $\text{NH}_4^+$ ; (2) Soil extract for  $\text{K}^+$ , dilution factor (DF) was 50 times

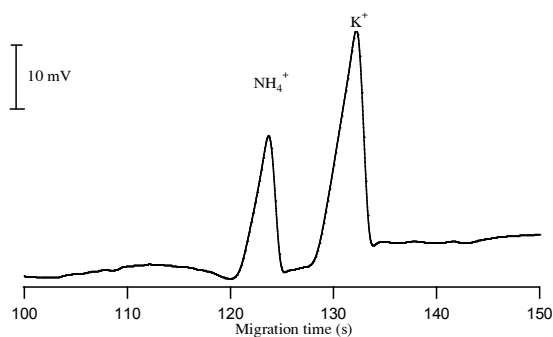


Figure 4. Simultaneous determination of  $\text{NH}_4^+$  and  $\text{K}^+$  in water sample (DF was 6 times)

Table 4. Concentrations of  $\text{NH}_4^+$  and  $\text{K}^+$  in soil and water samples

Type of sample	Analyte	Concentration <sup>*</sup>	Confirmation (% difference)
Soil sample	$\text{K}^+$	1055	5,7 <sup>a</sup>
	$\text{NH}_4\text{-N}$	29.6	10.8 <sup>b</sup>
Water sample	$\text{K}^+$	33,7	12,9 <sup>a</sup>
	$\text{NH}_4\text{-N}$	2.68	0.50 <sup>b</sup>

<sup>\*</sup> mg/kg for soil sample; mg/L for water sample

<sup>a</sup> confirmation by F-AAS method

<sup>b</sup> confirmation by UV-Vis method

#### 4. CONCLUSIONS

The conditions for simultaneous determination of  $\text{NH}_4^+$  and  $\text{K}^+$  by CE- $\text{C}^{4}\text{D}$  were optimized as follows: BGE: His/Ace buffer, 2 mM 18-crown-6; silica capillary: 365  $\mu\text{m}$  O.D., 50  $\mu\text{m}$  I.D.;  $L_t = 50\text{cm}$ ;  $L_{\text{eff}} = 40\text{cm}$ ; voltage: 20V; hydrodynamic injection: 20s at 15cm height. The procedure are applicable to examine  $\text{NH}_4^+$  and  $\text{K}^+$  in both soil and water samples. Excessive levels of  $\text{NH}_4^+$  and  $\text{K}^+$  were detected in soil. The concentration of  $\text{NH}_4^+$  in water sample was significantly higher than the National technical regulation on surface water quality for protection of aquatic

lives.

#### ACKNOWLEDGEMENT

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