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OPTIMIZATION OF CE SEPARATION OF ROXARSONE AND SEVERAL ARSENIC COMPOUNDS

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Abstract. Capillary electrophoresis with direct UV detection was used for the separation of arsenite As(III), arsenate As(V), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), phenylarsonic acid (PAA), ρ-arsanilic acid (ρ-ASA) and roxarsone (3-NHPAA). The separation was achieved in a fused silica capillary using a high sensitivity detection cell with diode array detector. A 15 mM phosphate buffer was used as the background electrolyte. The influence of electrolyte pH, applied voltage and wavelength were investigated in this research where the optimum conditions obtained were at pH 6.0, 25 kV voltage and 191 nm detection wavelength. The optimized method provided a limit of detection of 0.193 mg/L for As(III). Reproducibility of the analytes was in the range of 4.6%-10.5% RSD.

Keywords: Arsenic compound, roxarsone, capillary electrophoresis

Abstrak. Elektroforesis rerambut dengan pengesanan ultralembayung digunakan bagi pemisahan arsenit As(III), arsenat As(V), asid monometilarsonik (MMA), asid dimetilarsinik (DMA), asid fenilarsonik (PAA), asid ρ-arsanilik (ρ-ASA) dan roksarson (3-NHPAA). Pemisahan tercapai menggunakan kapilari silika terlakur dengan sel pengesan berkepekaan tinggi dan pengesan susun atur diod. Larutan penimbal 15 mM fosfat digunakan sebagai elektrolit latarbelakang. Pengaruh pH elektrolit, keupayaan yang dikenakan dan panjang gelombang pengesanan dikaji dengan nilai optimum diperoleh pada pH 6.0, keupayaan 25 kV dan panjang gelombang pengesanan pada 191 nm. Kaedah yang telah dioptimum ini memberi had pengesanan 0.193 mg/L bagi As(III). Kebolehulangan bagi semua analit adalah di antara julat 4.6%-10.5% RSD.

Kata kunci: Sebatian arsenik, roksarson, elektroforesis rerambut

1.0 INTRODUCTION

Arsenic is an important element in environmental and toxicological fields. Arsenic compounds are widely used as pesticides and wood preservatives. Arsenic has also been used as animal feed additives in the poultry industries. 3-nitro-4-hydroxyphenylarsonic acid (3-NHPAA or roxarsone) is presently being used in Malaysia in the poultry industries as a growth promoter to improve feed conversion, better feathering and increased egg production. Studies have demonstrated that this organoarsenic compound does not accumulate in poultry tissue or feathers but is

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rapidly excreted unchanged, resulting in elevated concentrations of roxarsone in poultry litter [1-3]. Generally, the litter is used as fertilizer on agricultural fields. Soil amended with arsenic manure could result in a localized arsenic pollution and becomes a source for arsenic contamination of surface and groundwater.

Current interest in the determination of different species of arsenic in the environment is due to the fact that physiological and toxic effects of arsenic are connected with its chemical forms. The toxicity of different arsenic species varies in the order arsenite > arsenate > monomethylarsonate (MMA) > dimethylarsinate (DMA) while organoarsenics are generally considered to be non-toxic. The techniques used for the detection of arsenic species in environmental and biological samples should be sensitive and selective [4-8]. The rapid analysis of samples to prevent species conversion is also important. Inductively coupled plasma-mass spectrometry (ICP-MS) has become a desired technique in arsenic analysis that provides ultra sensitivity, multi element capability and can be combined with a separation technique for speciation analysis [4]. Another common technique used in arsenic speciation is hydride generation-atomic fluorescence spectrometry due to its high sensitivity, low detection limit and high selectivity [5]. However, not all arsenic species form hydrides and decomposition techniques are usually required. A combination of analytical techniques is often necessary to achieve both selectivity and sensitivity. The direct coupling of a separation device to various detection instruments will improve specificity and detection for individual arsenic species. Hyphenated techniques allow for the possible separation of all soluble species in the sample and selective detection at small concentrations.

High performance liquid chromatography (HPLC) is also often used as the separation technique in arsenic speciation [8]. Although this technique provides ultra sensitivity and multi element capability, this technique is expensive and has limited separation efficiency. Recently, there has been an increasing interest in the application of capillary electrophoresis (CE) for the separation and determination of arsenic species because CE has the advantages of short analysis time, high separation efficiency and low operating cost. In recent years, CE has successfully separated simultaneously inorganic, organic and methylated arsenic [5, 7, 10-12] but few reports on the separation of arsenic compounds used in the poultry industry. Sun *et al.* [10] successfully separated the inorganic and organoarsenic compounds using a dynamic coated capillary (PDDAC) to provide a reversed EOF. They reported low detection limits of 0.18 – 6.22 mg/L As, but the capillary electrophoresis technique has proven to be unable to challenge and compete the detection limit afforded by the HPLC-ICP-MS [12]. Capillary electrophoresis also suffers from its poor concentration sensitivity due to the use of capillaries with small internal diameters, limiting the amount of sample that can be loaded. A good analytical technique is needed to determine the arsenic species at concentration below the permissible level of 10 µg/L.

The purpose of this work is to perform a fast separation of arsenic species in a single CE run by optimizing the pH, voltage and the wavelength for the direct UV detection using an uncoated capillary and a high sensitivity detection cell. The high sensitivity detection cell was used to improve the detection limit. The arsenic compounds studied were the organoarsenic compounds used as animal feed additives and the inorganic arsenic, and the structures are listed in Figure 1.

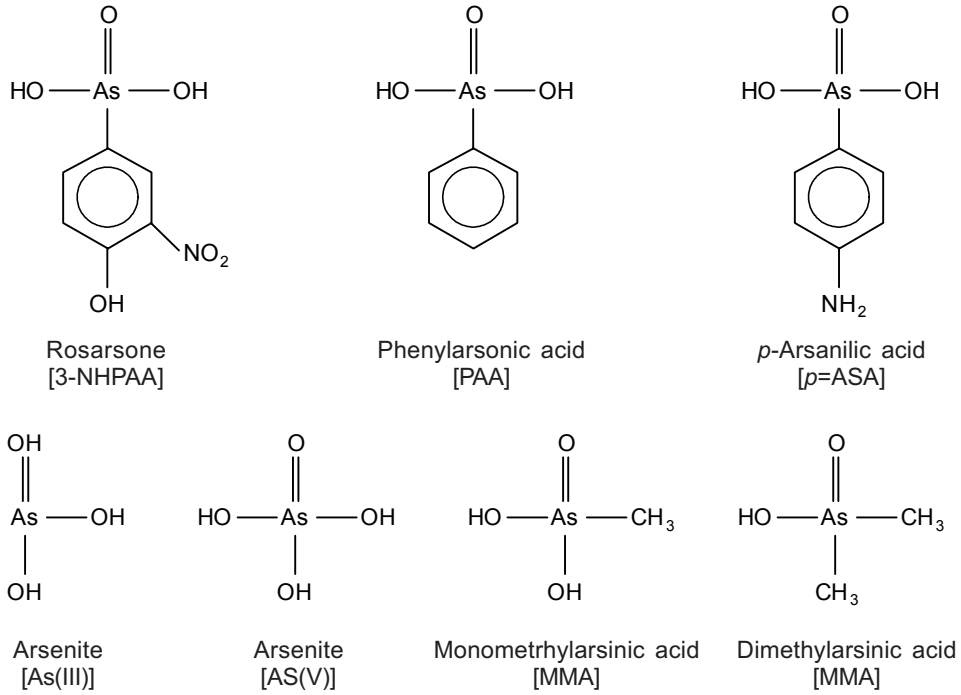

Figure 1 Formulas and abbreviations of arsenic compounds

2.0 EXPERIMENTAL

2.1 Instrumentation

All electrophoresis experiments were performed on an Agilent Technologies HP^{3D} Capillary Electrophoresis instrument (Germany). Separations were performed using a fused silica capillary (Polymicro Technologies, Phoenix, Arizona, USA) 75 µm ID × 80.5 cm (effective length 72 cm) and detected on a high sensitivity detection cell. The column temperature was set at 25°C. On-column UV diode-array detection was used, operated at various wavelength with a bandwidth of 4 nm. Samples were injected hydrodynamically (2.5×10^3 Pa) for 5 s from the anodic end and the separation voltage was 15 kV. The pH of all solutions was measured with the aid of Hanna Instrument pH meter. Data were acquired with a Hewlett Packard ChemStation.

Before analysis, the capillary was flushed with 0.2 M NaOH for 10 min, followed by deionized water and buffer for 5 min, respectively. Between runs, the capillary was rinsed with buffer and deionized water for 2 min. Thiourea was used as the EOF marker.

2.2 Chemicals

A standard stock solution of 1000 mg As/L As(III) was prepared by dissolving arsenic trioxide, As_2O_3 in 0.1 M NaOH and then adjusted with 1% HCl to pH 6. Standard stock solutions (1000 mg As/L) of As(V), MMA and DMA were prepared by directly dissolving $Na_2HAsO_4.7H_2O$, $CH_3AsO_3HNa.1.5H_2O$ and $C_2H_6AsO_2Na$ in deionized water. ρ-ASA, 4-NHPAA and roxarsone were also prepared in deionized water. All of the reagents were of analytical grade. These solutions were stored in the dark at less than 4°C.

Sodium dihydrogen phosphate was used to prepare 15 mM phosphate buffer, which was adjusted with 0.1 M NaOH to the desired pH. The electrolyte solution was prepared weekly. Before use, the buffers were filtered through 0.45 μm filter and degassed by an ultrasonic system. All the solutions were prepared in 18 MΩ water from a Milli-Q system (Millipore, Bedford, MA, USA).

3.0 RESULTS AND DISCUSSION

3.1 Simultaneous Separation of Arsenic Compounds

The separation of seven arsenic compounds were investigated in a 15 mM phosphate buffer solution with pH 6.0, 25 kV voltage and 191 nm detection wavelength. Concentration for inorganic and methylated arsenic compounds was 80 mg/L while the concentration for arylarsenic compounds was 40 mg/L. Electropherogram as shown in Figure 2 was recorded where the separation order obtained (arsenite, DMA, p-ASA, PAA, MMA, arsenate and roxarsone) is in good agreement with literature [10].

3.2 Effect of Wavelength

Direct spectrophotometric detection is the detector most commonly used in capillary electrophoresis with a wavelength of between 192 and 195 nm for arsenic compounds [5,10,11]. All of the arsenic compounds showed absorption maxima at wavelengths between 190 and 225 nm. Higher wavelengths reduced the detection noise significantly and enhanced the limit of detection (LOD) of the arylarsenic compounds such as roxarsone and p-ASA but reduced the LOD of the other inorganic and methylated species. The phosphate buffer solutions are more UV transparent and are suitable as background electrolytes for UV detection [11]. Consequently, the peaks of the arsenic species were very high at detection wavelength 191 nm but upon increasing the

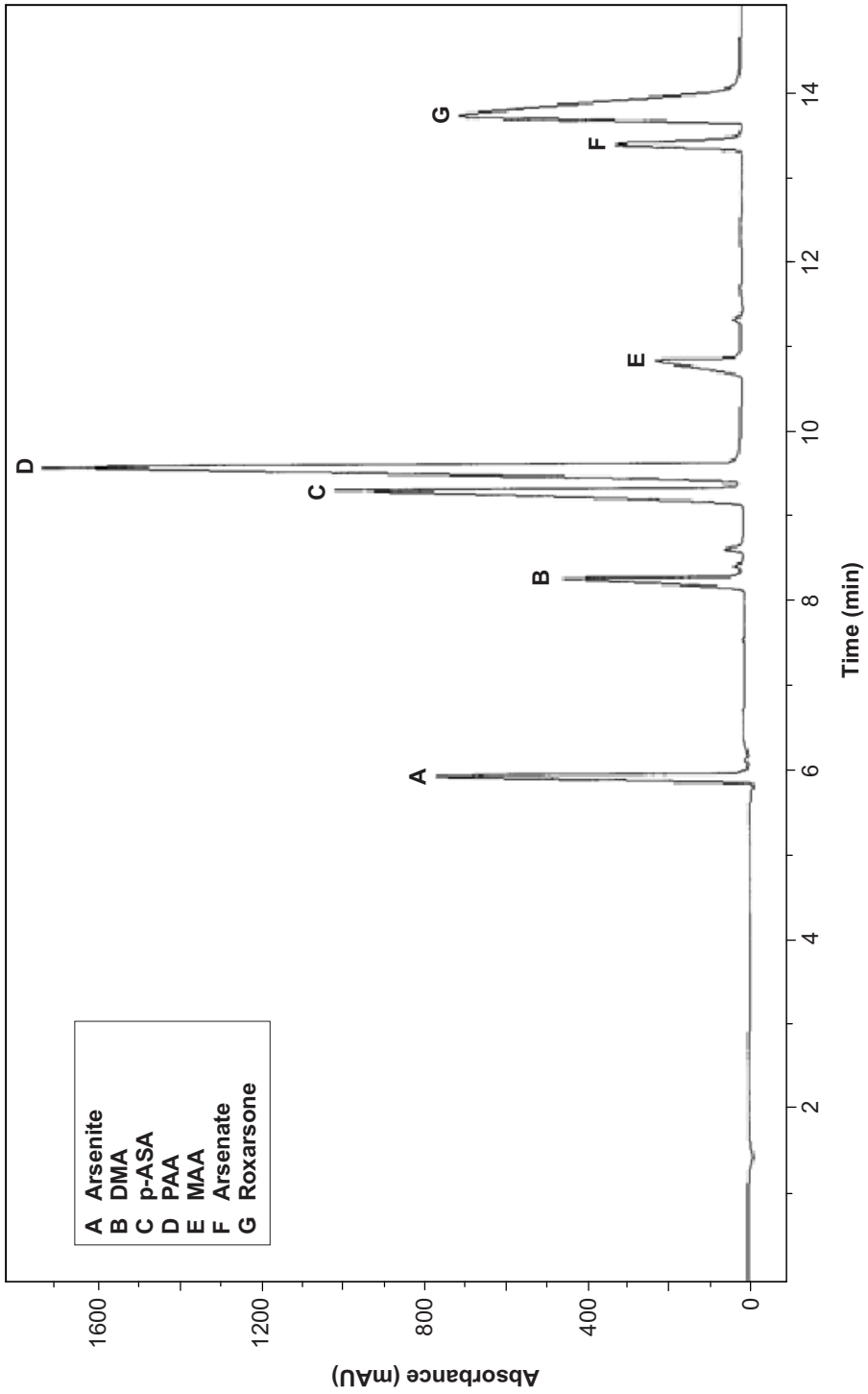

Figure 2 Electropherogram of a mixture of arsenic compounds in 15 mM phosphate at pH 6.0 and voltage 25 kV, concentration 80 mg/L for inorganic and methylated arsenic (A, B, E, F) and 40 mg/L for organoarsenic compounds (C, D, G)

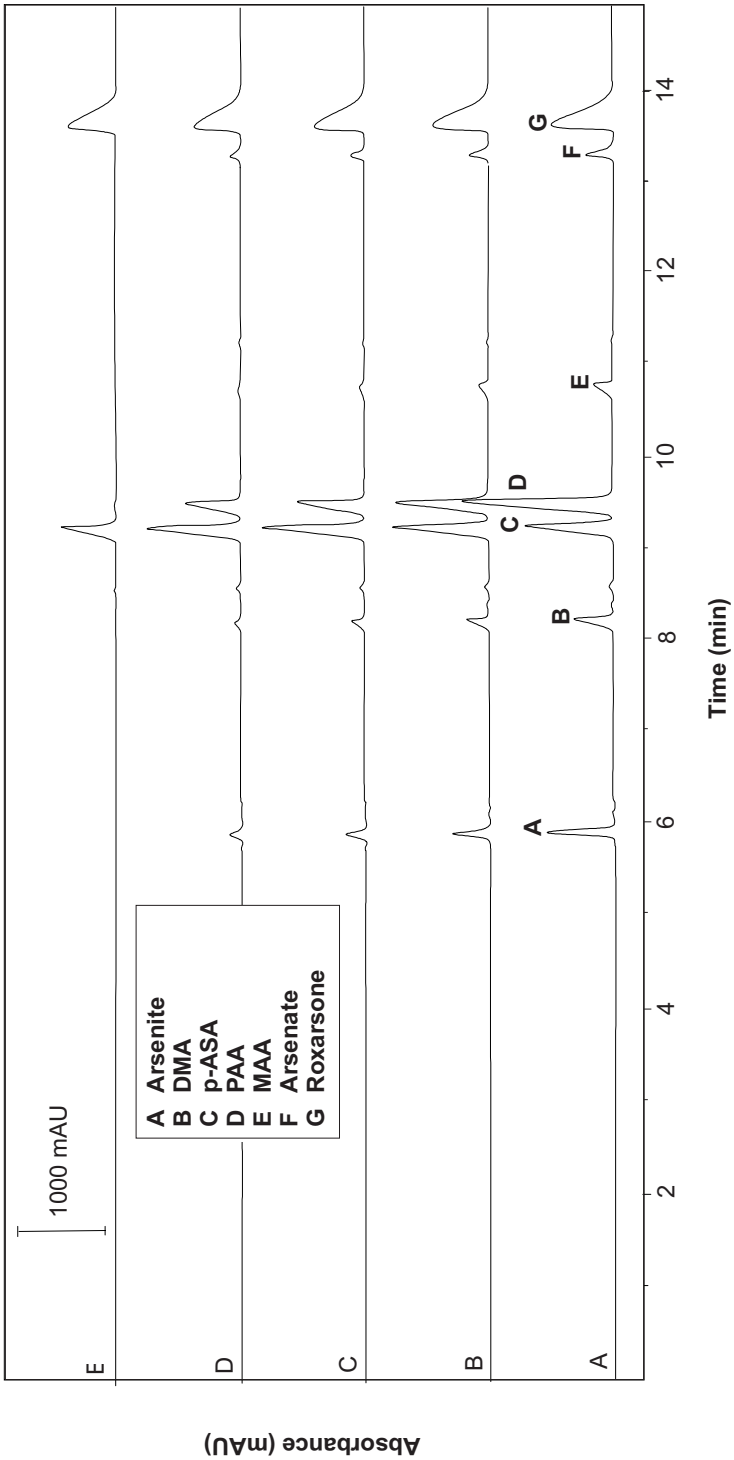

Figure 3 Electropherogram of a mixture of arsenic compounds in different wavelength: (A) 191 nm, (B) 194 nm, (C) 197 nm, (D) 200 nm and (E) 254 nm in 15 mM phosphate, pH 6.0 and voltage 25 kV

wavelength, the detection sensitivity of the peaks were reduced significantly except for the roxarsone and p-ASA compounds as shown in Figure 3. Therefore, 191 nm has been selected as the optimum wavelength for the separation of arsenic compounds.

3.3 Electrophoretic Mobilities of the Analytes at Varying pH

In CZE, pH of the background electrolyte plays an important role in getting the best separation efficiency with shorter migration time. The pH of the electrolyte buffer affects the separation characteristic by influencing the electrophoretic mobility of the arsenic anions as well as the electroosmotic flow (EOF). All of the inorganic and organic arsenic compounds are anionic or neutral depending on the pH of the background electrolyte. The pH of the electrolyte and the pK_a values influence the effective charge of the analytes. Deprotonation of the arsenic compounds will increase both the ionic charge and electrophoretic mobilities [10-13].

The influence of the pH of the electrolyte buffer on the separation of the seven arsenic species was investigated using a buffer solution of 15 mM phosphate. Over the pH range 5-9 examined, the migration times of each individual arsenic species increased with an increase of the buffer pH due to the increasing electrophoretic mobilities of all the analytes. Table 1 lists the migration time, t and electrophoretic mobilities, μ_{eff} of seven arsenic compounds at different pH values. Figure 4 shows the increasing migration times of the target analytes when the phosphate buffer solution changes from pH 5-9.

At pH 5 and 6, As(III) was detected earliest because under this pH, As(III) with pK_a value of 9.3 is in a neutral state. The migration towards the cathode was followed by negatively charged DMA (pK_{a1} = 1.3), MMA (pK_{a1} = 3.6), p-ASA (pK_{a1} = 1.9) PAA (pK_{a1} = 3.6), As(V) with pK_{a1} value 2.3 and roxarsone (pK_{a1} = 3.5). These negatively charged analytes move towards the cathode because the EOF of the electrolyte is higher than the electrophoretic mobility. With increasing pH, the electrophoretic mobilities of all arsenic compounds increase and causes the migration times to increase.

At pH 7.0 and 8.0, all the arsenic compounds are separated except for DMA and ρ-ASA which co-eluted probably due to the same electrophoretic mobilities of these two compounds. At pH 9.0, ρ-ASA (pK_{a3} = 9.2) migrated earlier than DMA. For the As(V) species, it migrated earlier than roxarsone at pH<7 but at pH>7 arsenate was detected last. This is probably due to the high negative charge of arsenate (pK_{a1} = 2.3, pK_{a2} = 6.9, pK_{a3} = 11.4) with higher electrophoretic mobilities than roxarsone at pH>7. Based on the results, a solution of 15 mM phosphate at pH 6.0 was employed as the electrolyte buffer to achieve the best separation efficiency.

Table 1 Separation data for arsenic compounds: migration times, t and electrophoretic mobilities, μ

Compounds	pH 5		pH 6		pH 7		pH 8		pH 9	
	t [min]	$\mu_{eff} \times 10^{-2}$ [cm^2/Vmin]	t [min]	$\mu_{eff} \times 10^{-2}$ [cm^2/Vmin]	t [min]	$\mu_{eff} \times 10^{-2}$ [cm^2/Vmin]	t [min]	$\mu_{eff} \times 10^{-2}$ [cm^2/Vmin]	t [min]	$\mu_{eff} \times 10^{-2}$ [cm^2/min]
EOF	5.865		5.859		5.900		5.890		5.915	
As(III)	5.945	0.053	5.935	0.052	6.025	0.081	6.079	0.122	6.112	0.158
DMA	6.402	0.352	8.250	1.147	9.379	1.457	9.492	1.494	10.079	1.628
ρ-ASA	9.292	1.458	9.270	1.456	9.379	1.457	9.492	1.494	9.575	1.499
PAA	9.712	1.566	9.544	1.528	9.902	1.588	10.142	1.650	10.289	1.667
MMA	10.872	1.821	10.797	1.810	11.009	1.823	11.152	1.857	11.285	1.866
As(V)	11.029	1.851	13.310	2.255	19.639	2.748	21.482	2.857	22.205	2.580
Roxarsone	11.172	1.878	13.637	2.257	16.039	2.484	15.605	2.450	15.575	2.421

μ_{app} = total capillary length × effective capillary length / voltage × migration time

$\mu_{eff} = \mu_{app} - \mu_{EOF}$

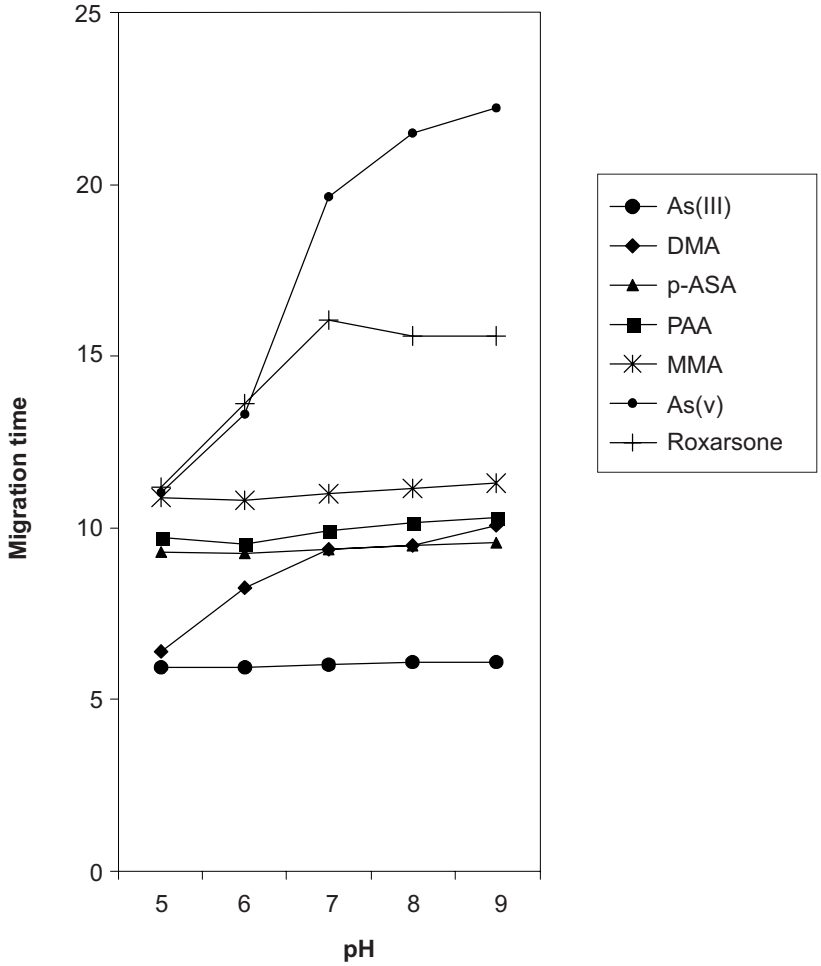

Figure 4 Dependence of migration times of arsenic compounds from pH of background electrolyte (15 mM phosphate, temperature 25°C, voltage 25 kV)

3.4 Effect of Applied Voltage

The applied voltage determines the migration time and separation efficiencies of the analytes. Figure 5 depicts the effect of applied voltage on migration time and separation efficiencies of the arsenic compounds in the voltage range from 10-25 kV at pH 6.0. Increasing the voltage resulted in shorter migration times, but also increased the baseline noise, resulting in poorer detection. It was found that the application of high voltages can result in higher Joule heating, which directly affected the separation efficiency [9]. Lower separation voltages, however, would increase the analysis time which in turn could cause peak broadening. Therefore, a voltage of 25 kV was found to be a good voltage to achieve high separation efficiency and short analysis time.

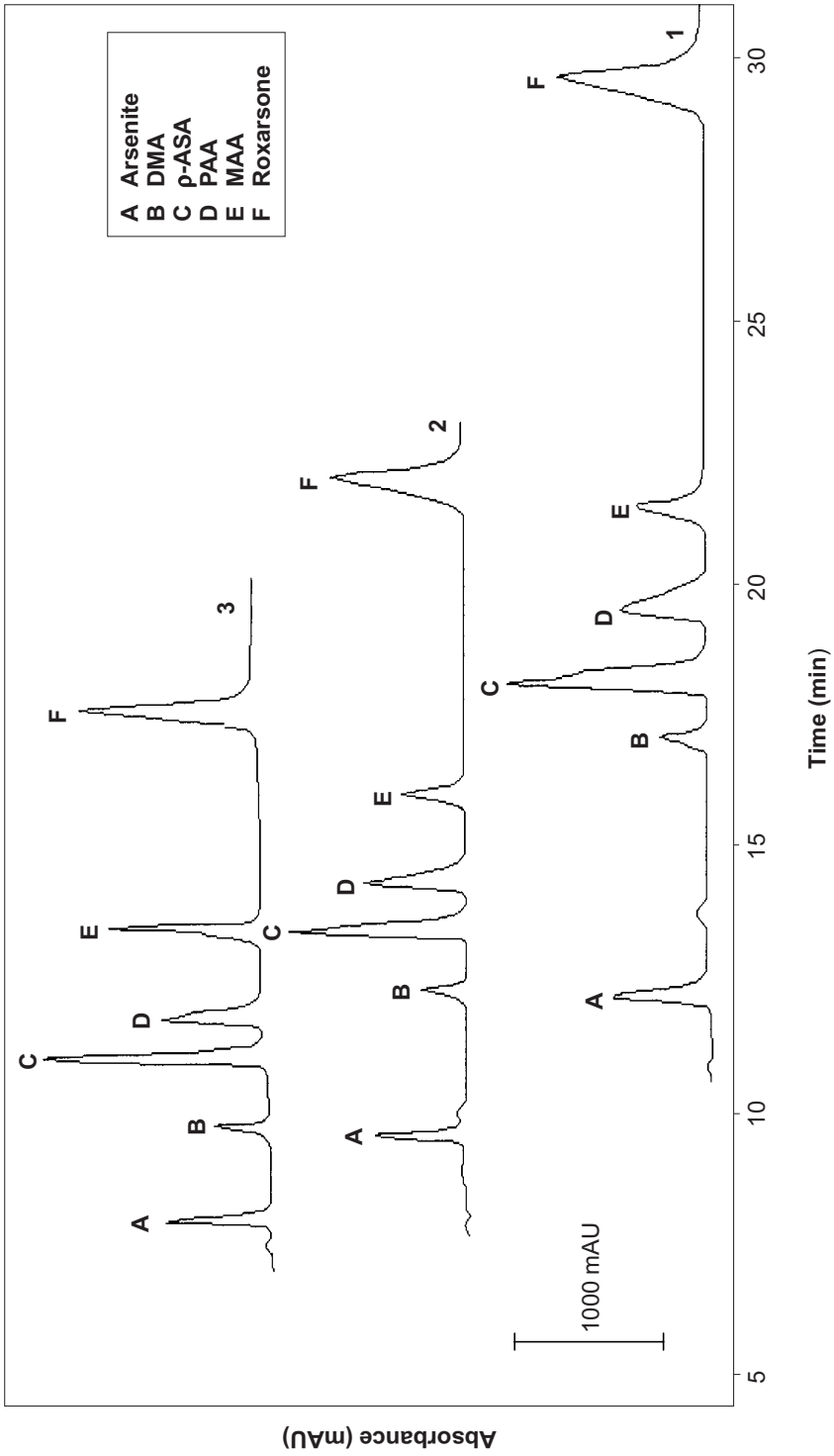

Figure 5 Electropherogram of a mixture of arsenic compounds at different voltage: (1) 15 kV, (2) 20 kV and (3) 25 kV (15 mM phosphate, temperature 25°C)

3.5 Characteristic of Quantitative Analysis

3.5.1 Detection Limit

The detection limit of As(III) was determined by plotting the current responses (mAU) versus concentration (mg/L). From the calibration plot, the detection limit of As(III) obtained is 0.193 mg/L with a linear regression of 0.96. Only the detection limit of As(III) was determined because this is the most toxic species among the arsenic compounds, and the form that is most commonly found in contaminated water bodies. The detection limits of the other six arsenic compounds were not determined at this time.

3.5.2 Reproducibility

The reproducibility of peak areas and migration times were determined by injecting a standard solution of a mixture of seven arsenic compounds into the system under the optimum conditions. The relative standard deviations (RSD) of peak areas were in the range of 4.55-10.46% RSD. The results are shown in Table 2. The high reproducibility indicates that this method would be suitable for analysis of real samples [9,14,15].

Table 2 Reproducibilities of migration times and peak areas

Component	R.S.D. of migration times	R.S.D of peak areas (%)
Arsenite As(III)	3.04	4.55
Dimethylarsinic acid (DMA)	3.60	8.82
ρ-arsanilic acid (ρ-ASA)	4.28	5.65
Phenylarsonic acid (PAA)	4.42	6.90
Monomethylarsonic acid (MMA)	4.88	4.69
Arsenate As(V)	5.55	10.46
Roxarsone (3-NHPAA)	5.76	5.25

4.0 CONCLUSIONS

Capillary zone electrophoresis with direct UV detection has successfully separated seven arsenic compounds, including roxarsone which is the subject of interest in the poultry industries. The parameters for the simultaneous determination of seven arsenic compounds using capillary electrophoresis has been optimized. The effect of applied voltage, pH and wavelength on migration times and separation efficiencies were investigated where the optimum parameters obtained were 25 kV voltage, pH 6.0 and 191 nm detection wavelength.

The optimized conditions for this CZE will be applied for the future analysis of real samples which is mainly poultry litters and agricultural soils amended with roxarsone manure.

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