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Determination of inorganic anions and organic acids in water, beverages and orange juice by capillary electrophoresis

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Abstract : A capillary zone electrophoresis methods were developed with indirect UV detection for the analysis of inorganic and organic anions using background electrolytes (BGEs) consisting of chromate and 2, 6 pyridinedicarboxylic acids (PDC). Inorganic ions (F^-, Cl^- and SO_4^{2-}) and organic acids (tartaric acid, malic acid, succinic acid and citric acid) were determined in various samples. Electroosmotic flow (EOF) was reversed in the direction of the anode by adding cetyltrimethylammonium bromide (CTAB) to the electrolyte and highly alkaline conditions were used to confer a negative charge on inorganic and organic anions so as to promote their migration towards the anode. The detection wave length was 200nm. The methods developed for the anions were applied to the qualitative and quantitative analysis of environmental water samples from the surrounding areas of Stellenbosch, South Africa, as well as beverages and orange juice.

Keywords : Background electrolyte, Anions, Chromate, UV detection, beverages.

Introduction

Measuring common anions such as chloride, sulphate, nitrate, fluoride and organic acids in water, beverages and juices is important for environmental monitoring, product consistence, fermentation monitoring, process control and spoilage detection. Organic acids are often added to food products to enhance or modify the flavour so as to make them more acceptable to consumers. The organic acid content of wines is an important parameter to indicate the qualtity. The total acid content affects the overall balance of the wine. To maintain product consistence in water, beverage and juices, multiple organic acids and common anions have to be monitored in raw material and end products for quality control and product differentiation [1,2]. Thus, analytical methods to determine organic acids and inorganic anions are needed. The results have to be known within a short time using equipment with low running and capital cost.

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Capillary electrophoresis (CE) has developed into one of the powerful separation techniques for the analysis of inorganic and organic ions in complex matrices. The several advantages of ion analysis using CE include resolution, speed, and simplicity and reduced sample preparation[3]. Indirect UV detection has been used successfully for the analysis of non UV-absorbing anions[4]. In this case separation is achieved with an electrolyte containing a UV- absorbing ion plus an electrosmotic flow modifier[5]. The displacement of the UV-absorbing ions by the analyte anions permits indirect detection. By adding an electroosmotic flow modifier such as alkyl ammonium surfactants, for example, cetyltrimethylammonium bromide (CTAB) the direction of electroosmotic flow can be reversed, allowing migration of anions in the same direction as the electroosmotic flow from the injection end toward the detection end[6]. Several indirect UV detection methods have been developed using various background electrolytes (BGEs) such as chromate[7], pyromellitate[8], trimellitate[9], phthalate[7], benzoate[7], 2,6-naphthalenedicarboxylate[10] and 2,6 pyridinedicarboxylic- acids (PDC)[11].

The choice of the BGE is most important in developing a method employing CZE with indirect detection since it must conform to several requirements: (i) The BGE must be an absorbing co-ion of the analyte. (ii) Since sensitivity is directly related to the molar absorptivity of the BGE, this should be high[12]. (iii) The peak shape of an 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[7]. Therefore the mobility of the BGE should be similar to that of the analytes of interest. (iv) Migration time reproducibility is dependent upon reproducible mobilities that in turn are pH dependent. Therefore, the BGE should have a good buffering capacity at the operating pH[13]. In the previous studies the analysis of anions was only for a single sample matrix. In the present investigation the analysis of anions was performed on wide variety of sample matrices.

In this study, the use of chromate and 2, 6 pyridinedicarboxylicacids as buffers for inorganic and organic anions respectively were investigated. EDTA has been added in the case of the inorganic anions study to remove metal interference. These methods were applied to the analysis of water, beverages and orange juice samples.

Experimental

Instrumentation

The CE analysis was performed on 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 96.5 cm long (84.5 cm effective length) were used. The applied voltages were -20 and -25kVs. The samples were injected using hydrodynamic mode (50 mbar) for 12s. Data acquisition and analysis (DAx) soft ware from Prince Technologies was used for the control of instrument settings. All experiments were conducted at 25^0C. The current was monitored for all evaluated BGEs electrolytes and was in the range of 10 to 13 µA.

Materials

Boric acid (99.8%), tartaric acid (99.5%) and citric acid (99.5%) were obtained from Merck (Darmstadt, Germany). NaOH (99%) and HCl (99%) were from Riedel-dehaen (France). CTAB (99%) and Malic acid (95-100%) were from Sigma (Steinheim, Germany). PDC (99%) and succinic acid (99%) were from ACROS organics (Geel, Belgium). Ethylendiamine tetra acetic acid (99%) and NH_4NO_3 (99%) were from NT Laboratory supplies (Johannesburg). Na_2SO_4 (99%) and NaF (99%) were from Analar (England). NaCl (99%) and H_3PO_4 (85%) were from Pal Chemicals and Aldrich (Steinheim, Germany) respectively.

Sampling

The environmental waters were sampled from river, lake and reservoir areas surrounding the Stellenbosch, South Africa. The water samples were collected in polyethylene containers and acidified and then kept in refrigerator at +4 °C. The wine sample was provided by the laboratory for separation sciences at Stellenbosch University. The orange juice and the beer were bought from the market. All samples were kept in a refrigerator at +4 °C. For CE measurements all samples were first screened with CE and their concentrations were estimated. When the peak resolution was not satisfactory (high ionic strength) or the ion zones were distorted, the samples were diluted 1:6, 1:10, 1:20 and 1:25(v/v) (Table 1) 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, to reduce sample preparation. Peaks were identified by the spike (by adding standard solution) method

Table 1. Samples with dilution factor

Sample numbering	**Sample type**	**Dilution factor**
1	River (Village near Tokera)	Undiluted
2	Lake (Kastaing)	Undiluted
3	Lake (FBA)	1:6
4	Orange juice	1:10 or 1:20
5	Beer	1:10
6	white wine	1:10
7	Red wine	1:25

Background electrolyte solutions

In the optimised CE method the inorganic anions were separated in a buffer solution containing K_2CrO_4 (5 mM), boric acid (3 mM), CTAB (35 µM) and EDTA (12 µM) at pH 8.2 adjusted with 1 M NaOH. The optimised CE separation of the organic anions was performed in PDC (5 mM), CTAB (0.5 mM) at pH 5.1 adjusted with1 M NaOH. The electrolyte solutions were ultrasonicated before use for 10 min.

Conditioning of the capillary

The new capillaries were conditioned by purging with 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.

Standard mixtures

Pure chemicals were diluted in Milli-Q water to obtain 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.

Calibration solutions

A calibration curve was prepared with 5 mg/l, 25 mg/l, 50 mg/l, 100 mg/l and 200 mg/l measuring 25 µl, 125 µl, 250 µl, 500 µl and 1000 µl respectively of each anion in 5 ml volumetric flask. The calibration curves were measured only at the ranges needed for the work. The linear ranges of each ion were measured at specified injection time.

Results and discussion

Separation by CE of the anions studied was completed within 10 min (Figures 1 and 2). The migration order of inorganic anions was: chloride > sulphate > nitrate > fluoride. The migration order of organic acids was: tartaric acid > malic acid > citric acid > succinic acid. Synthetic solutions were studied prior to the determination of anions in various sample matrices.

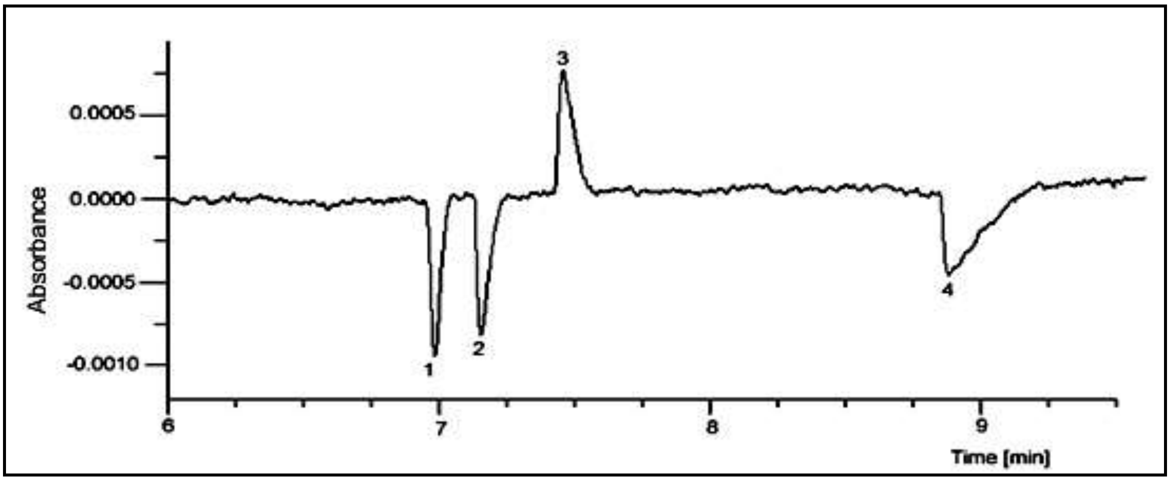

Figure 1. Electropherograms of synthetic solutions of inorganic anions (25 mg/l). Carrier electrolyte, PDC (5 mM), CTAB (0.5 mM) (pH 5.1); hydrodynamic injection 12 s: voltage, -25 kV, wavelength, 200 nm. Ions in the electropherograms: (1) Cl^-, (2) SO_4^{2-}, (3) NO_3^- and (4) F^-.

Fig.1 shows a broad peak for fluoride ion. This is because of the mismatch of the fluoride ion electrophoretic mobility with that of the background electrolyte[14]. A positive peak for nitrate ion was obtained due to absorbance of this ion at the measuring wave length.

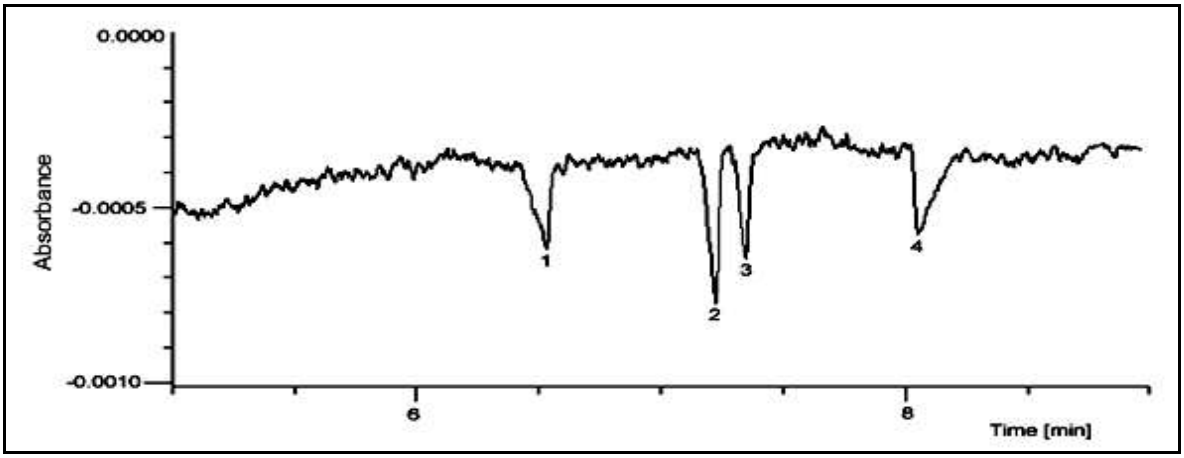

Figure 2. Electropherograms of synthetic solutions of organic anions (25 mg/l). Carrier electrolyte, K_2CrO_4 (5 mM), Boric acid (3 mM), CTAB (35 µM) and EDTA (12 µm) (pH 5.1); hydrodynamic injection 12 s: voltage, -20 kV, wavelength, 200 nm. Ions in the electropherograms: (1) tartaric acid$^-$, (2) malic acid$^-$, (3) citric acid$^-$ and (4) succinic acid.

The broad peaks obtained for tartaric and succinic acids in the Fig. 2 are because of the mismatching of those ions electrophoretic mobility with that of the background electrolyte.

Analysis of real samples

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 and orange juice (figures 2 - 8). Based on the results obtained, it could be applicable for the determination of anions in similar samples from other areas. The migration time of anions is slightly changed in various samples due to matrix effect; however, it was reproducible which was confirmed by the spiking method.

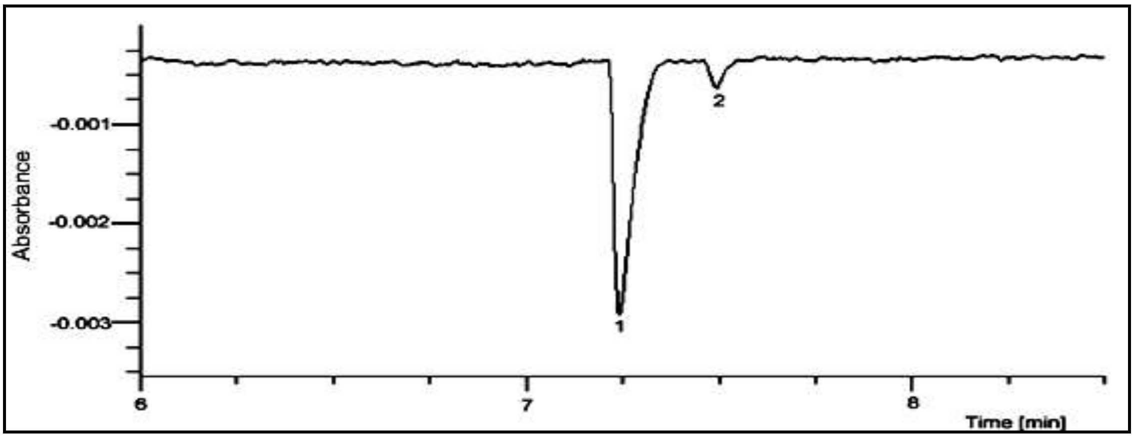

Figure 3. Electropherograms of inorganic anions in FBA area (diluted 1:6). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in Figure 1.

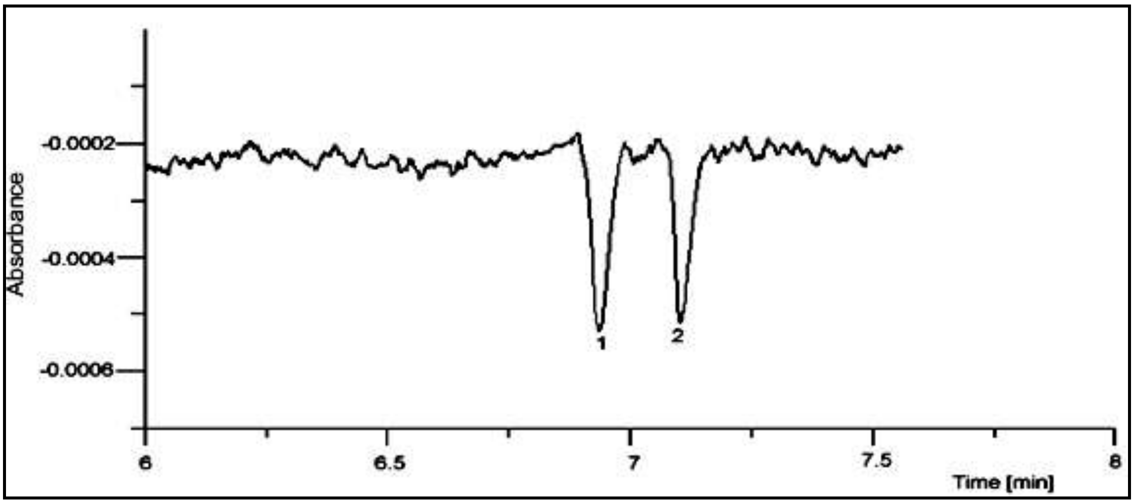

Figure 4. Electropherograms of inorganic anions in orange juice (diluted 1:10). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in Figure 1.

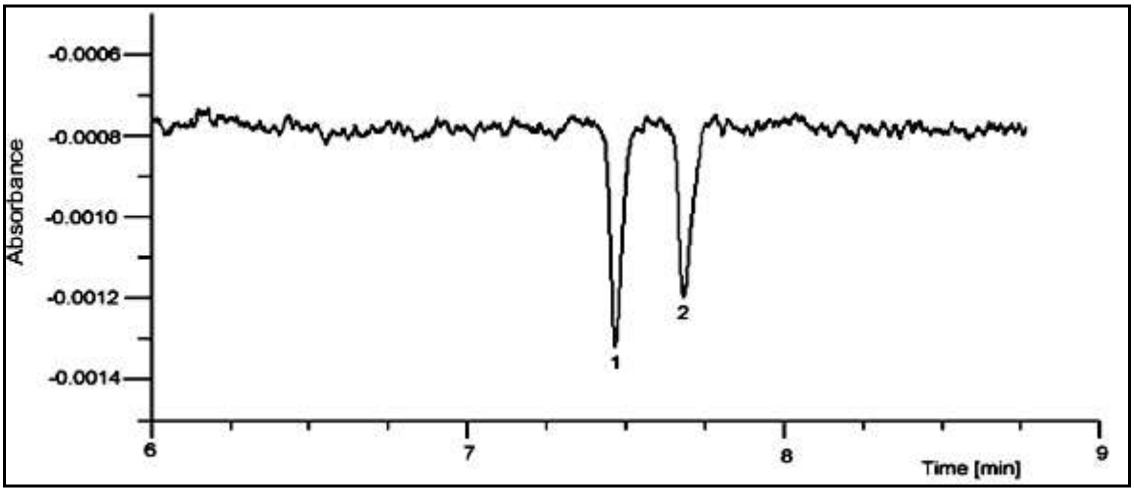

Figure 5. Electropherograms of inorganic anions in beer (diluted 1:10). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in Figure 1.

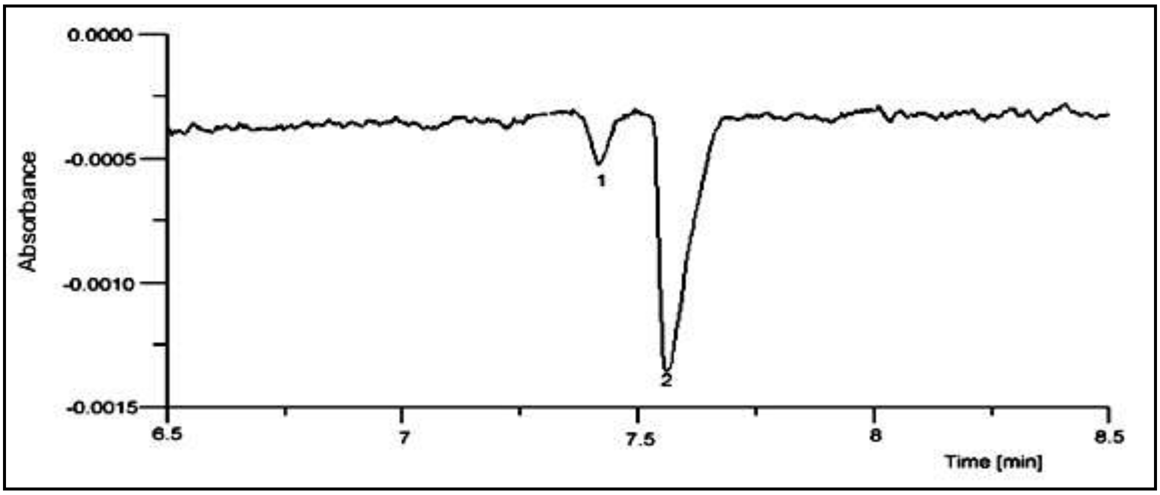

Figure 6. Electropherograms of inorganic anions in white wine (diluted 1:10). Ions in the electropherograms: (1) Cl^- and (2) SO_4^{2-}. Experimental conditions as in Figure 1.

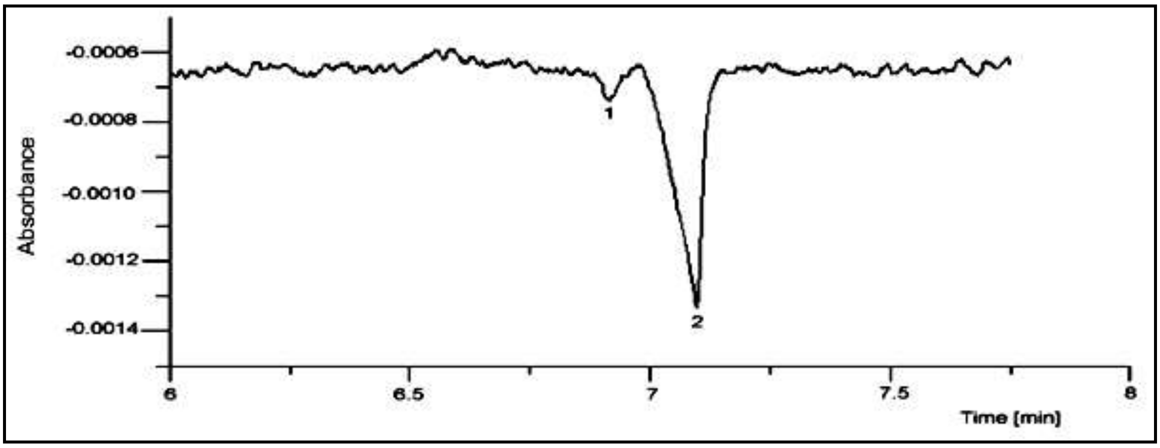

Figure 7. Electropherograms of organic anions in orange juice (diluted 1:20). Ions in the electropherograms: (1) Malonic acid and (2) Citric acid⁻. Experimental conditions as in Figure 2.

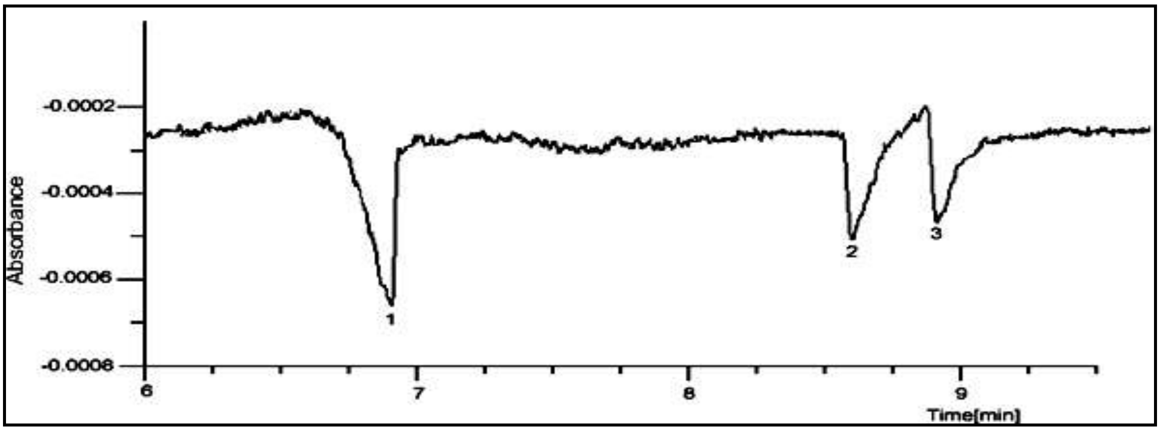

Figure 8. Electropherograms of organic anions in red wine (diluted 1:25). Ions in the electropherograms: (1) Tartaric acid (2) Succinic acid and (3) unidentified peak. Experimental conditions as in Figure 2.

The electropherograms of inorganic ions as well as organic acids in standard mixtures and real samples were plotted and the analytes peaks were resolved quite good. The application of CE to standard mixtures was also applied for the analysis of the real samples and resulted in. identifying analytes present in those samples. As it was observed from the various electropherograms of standard mixtures and real sample analyses, the peaks were resolved enough and it was possible to qualitatively identify and determine quantitatively inorganic anions (Cl^- and SO_4^{2-}) and organic acids (malonic acid, citric acid, tartaric acid and succinic) present in the real samples. CE results of anions in the samples listed in Table 1 were obtained as follows.

Table 2. Capillary electrophoresis results of anions in the samples listed (in table 1)

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	22.62 (2.81%)	5.44 (6.28%)	NA	NA	NA	NA
Kastaing	71.55 (4.29%)	30.59 (2.03%)	NA	NA	NA	NA
FBA	608.10 (2.35%)	32.69 (6.86%)	NA	NA	NA	NA
Orange juice	137.23 (1.51%)	70.13 (6.49%)	ND	22.7 (7.28%)	1305.05 (4.36)	ND
Beer	206.35 (0.94%)	122.68 (3.99%)	NA	NA	NA	NA
white wine	98.84 (2.95%)	456.63 (2.84%)	NA	NA	NA	NA
Red wine	NA	NA	1427.19 (5.30%)	ND	ND	687.56 (7.99%)

NA = not analyzed, ND = not detected

Based on our experimental results, the capillary electrophoresis method was found to be a reliable and accurate technique for the monitoring of water–soluble anions from environmental waters, beverages and orange juice. The water samples from the river and lake contained chloride and sulphate ions. The lake belongs to FBA (Farm B Area, reservoir lake near Tokara) area contains high amount of chlorides and sulphates. However, the river from Tokera area contains low amount of chlorides and sulphates. Orange juice contains high amount of citric acid and low amount of malic acid. The red wine contains high amount of tartaric and succinic acids. The presence of those anions in each sample indicated the composition of the specified sample.

Conclusions

The capillary electrophoresis methods were designed and developed with indirect UV detection for the investigations of water–soluble inorganic and organic anions in the environmental water samples collected from the surrounding areas of Stellenbosch, South Africa, as well as beverages and orange juice. The capillary electrophoresis method was found to be a reliable and accurate method for the monitoring of water–soluble anions from the test samples based on our experimental results.

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