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Capillary electrophoretic determination of inorganic and organic anions using 2,6-pyridinedicarboxylic acid: effect of electrolyte's complexing ability

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Abstract

A capillary zone electrophoresis method was developed with indirect UV detection for the analysis of inorganic and organic anions using a background electrolyte (BGE), 2,6-pyridinedicarboxylic acid (PDC). The parameters which influence the separation of these anions, such as electrolyte pH, concentration of PDC and applied voltage, were investigated. Three inorganic anions and nine organic acids were determined simultaneously in less than 7 min. The R.S.D. ($n=6$) values of the method were around 0.1% for migration time and better than 2.6% for peak area. This method is rapid, sensitive and quantitative and could be readily applied to the analysis of inorganic and organic anions in beer. In the present paper, the effect of the complexing ability of the BGE on the analysis of organic acids is also discussed.

Keywords: Background electrolyte composition; Anions; 2,6-Pyridinedicarboxylic acid

1. Introduction

Capillary zone electrophoresis (CZE) is used more and more as a standard analytical tool for many ionic compounds. The several advantages of ion analysis using CZE include resolution, speed, simplicity and reduced sample preparation. Since inorganic and organic anions have little or no UV absorbance most published work on their analysis by CZE [1–10] utilizes indirect UV detection. Several indirect UV detection methods have been developed using various background electrolytes (BGEs) such as chromate [1–5], pyromellitate [4,5,7], trimellitate [4,8], phthalate [1,4–6], benzoate [1], 2,6-naphthalenedicarboxylate [9] and PDC [10,11] (Ch. Finkler, pers. comm.).

The choice of the BGE is most important in developing a method employing CZE with indirect UV 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 [4]. (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 [1,5]. Therefore the mobility of the BGE should be similar to that of the analytes of interest. (iv) Migration time reproducibility is dependent upon

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reproducible mobilities which in turn is pH dependent. Therefore, the BGE should have a good buffering capacity at the operating pH.

In this study, we propose another role of the BGE that can affect the determination of organic acids. We investigated the use of a complexing agent, 2,6-pyridinedicarboxylic acid, as a BGE for inorganic and organic anion analysis and applied this to the analysis of beer samples.

2. Experimental

2.1. Chemicals

Inorganic and organic anion standards were prepared from their sodium salts or free acids. Phthalic acid monopotassium salt was obtained from Tomiyama's Chemical Pure (Tokyo, Japan). Hydrofluoric acid was purchased from Tama Chemicals (Tokyo, Japan). All other reagents were from Wako (Osaka, Japan). The chemicals used were of analytical or reagent grade. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Apparatus

All CE experiments were performed using a HP ^{3D}Capillary Electrophoresis System from Hewlett-Packard (Waldbronn, Germany). The system comprises a CE unit with built-in diode-array detector and an HP^{3D}CE ChemStation for system control, data collection and data analysis. Separations were carried out on fused-silica capillaries with 75 μm I.D.×80.5 cm total length (72 cm effective length). Determination of the mobilities of the BGEs was performed using a polyvinylalcohol (PVA) coated capillary (Hewlett-Packard) with 50 μm I.D.×64.5 cm total length (56 cm effective length). Molar absorptivities were determined using a HP 8452A diode array spectrophotometer (Hewlett-Packard) with 1 cm path length quartz cells. Metal impurities in a fused-silica capillary were determined using a HP 4500 inductively coupled plasma mass spectrometry (ICP-MS) from Yokogawa Analytical Systems (Tokyo, Japan).

2.3. Electrophoretic procedures

Electrolytes were prepared containing 0.5 m*M* cetyltrimetylammonium bromide (CTAB) which was used to reverse the direction of electroosmotic flow (EOF) [12] and BGEs at a concentration of 5 m*M*. The pH was adjusted to 5.6 with 1 *M* NaOH.

Prior to first use, a new capillary was pretreated with the run electrolyte for 10 min. Before each injection, the capillary was preconditioned for 4 min by flushing with the run electrolyte. Sample was injected with a pressure of 50 mbar for 2.0 s. The applied voltage was set at −25 kV and the capillary temperature was thermostatted to 20°C. Detection was carried out with indirect UV detection using a diode-array detector. The signal wavelength was set at 350 nm with a reference at 200 nm.

2.4. Electrophoretic mobility determination

As we reported previously [13], the mobilities of various BGEs and anions were determined using a polymer coated capillary which reduces the EOF. Compounds were injected into PVA coated capillary filled with a 20 m*M* phosphate buffer pH 6.5 at 20°c. The applied voltage was −20 kV and detection was carried out at 195 nm.

2.5. Metals in fused-silica determination

The metal composition of a fused-silica capillary was determined with ICP-MS to study possible interactions with organic acids. The polyimide coating was removed from a length of fused-silica capillary and 5.0 mg was dissolved in 5 ml of 38% hydrofluoric acid at room temperature overnight. This solution was then diluted to 50 ml with MilliQ water and then analyzed using ICP-MS.

3. Results and discussion

3.1. Characteristics of 2,6-pyridinedicarboxylic acid (PDC)

The choice of electrolyte is extremely important to the success of any CE analysis. PDC [10,11] was investigated for anion analysis. The mobilities of

PDC and several other BGEs and anions were determined (Fig. 1). As previously stated, the mobility of the BGE should match as nearly as possible the mobility of the analytes of interest. Chromate or pyromellitate is commonly used as BGE for analysis of inorganic anions since they have a similar high mobility [1–5]. However, for the analysis of lower mobility compounds such as short chained carboxylic acids (C1–C8), benzoate is more suitable [1]. Phthalate is the most popular BGE for the analysis of medium mobility organic acids [1,5,6]. The mobility of PDC was determined and found to be very similar to that of phthalate which indicated that it is a suitable BGE for the analysis of medium mobility anions.

The BGE also requires a high molar absorptivity.

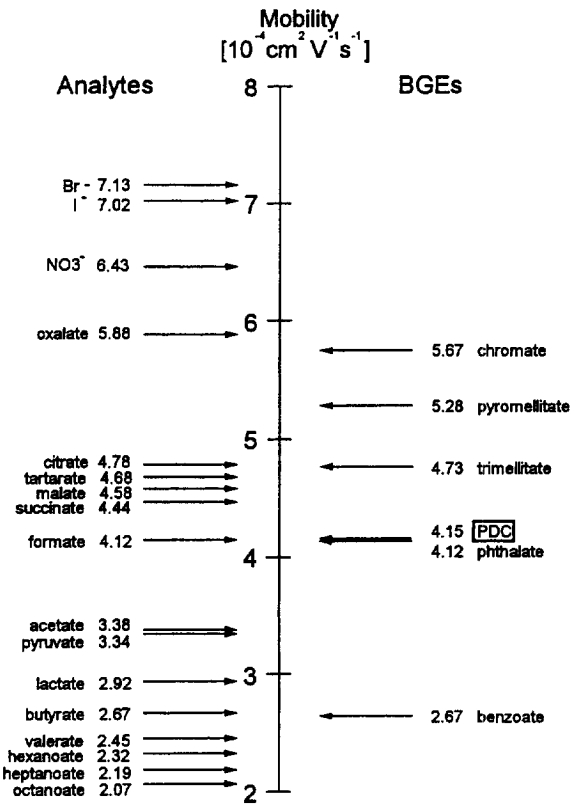

Fig. 1. Electrophoretic mobilities of anions and BGEs at pH 6.5. Experimental conditions: capillary, PVA 50 μm×64.5 cm (l=56 cm); electrolyte, 20 mM phosphate buffer pH 6.5; applied potential, −20 kV; injection pressure, 2 s at 50 mbar; capillary temperature, 20°C; detection, 195 nm.

Table 1
Molar absorptivity of BGEs

BGE	Molar absorptivity (l mol^{-1} cm^{-1})	λ_{max} (nm)
PDC	43 680	192
Pyromellitate	23 900	214
Phthalate	37 160	196
Benzoate	44 480	194

Experimentally determined molar absorptivities of several BGEs are listed in Table 1. Since the molar absorptivity of PDC is higher than that of pyromellitate and phthalate, it is expected that detection sensitivity may be improved using PDC. These results indicate that PDC may be suitable as a BGE for inorganic and organic anion analysis.

3.2. Optimization of separation

3.2.1. Effect of buffer pH

The pK_a values for the BGEs and analytes are listed in Table 2. Separations of several inorganic and organic anions were studied over the pH range 3.0–6.5 with 5 mM PDC electrolyte including 0.5 mM CTAB. The effective mobility, μ_e, for each anion was calculated using the following equation

$$\mu_e = lL/t_a V - lL/t_{EOF} V \quad (cm^2\ V^{-1}\ s^{-1})$$

Table 2
pK_a values of various BGEs and analytes

Anion	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
PDC	2.16	6.92		
Chromate	−0.98	6.50		
Pyromellitate	1.92	2.87	4.49	5.63
Trimellitate	2.52	3.84	5.20	
Phthalate	2.95	5.41		
Benzoate	4.21			
Sulfate	−3	1.92		
Nitrate	−1.34			
Oxalate	1.27	4.27		
Formate	3.75			
Malate	3.40	5.05		
Citrate	3.13	4.76	6.40	
Succinate	4.21	5.64		
Pyruvate	2.49			
Acetate	4.76			
Lactate	3.86			
Phosphate	2.12	7.20	12.36	

From Ref. [14].

where l and L are the length of the capillary to the detector and the total length of the capillary, respectively, V is the applied potential, t_a is the migration time of the anion and t_{EOF} is the migration time of a neutral marker.

The effect on the anions effective mobilities of changing the pH is shown in Fig. 2. Mobility changes were most pronounced between pH values 3.0 and 5.0 due to ionization changes of the anions at their pK_a values. The reproducibility of migration time of any assay might be expected to be poorer in a region where the mobility of the anions is so sensitive to small changes in the electrolyte pH. Therefore optimization of electrolyte pH was confined to the region pH 5–6 where the analytes' mobilities were more stable. The separation of 12 anions was optimized over the range from pH range 5.2–6.0 (Fig. 3).

Although the migration times of most anions were unchanged, those of citrate, succinate and acetate became faster with increasing pH. Below pH 5.4, a few peaks overlapped and at values higher than pH 5.8, resolution between malate and citrate became poor. Every anion could be fully resolved at pH 5.6 and therefore this was chosen as the optimum electrolyte pH.

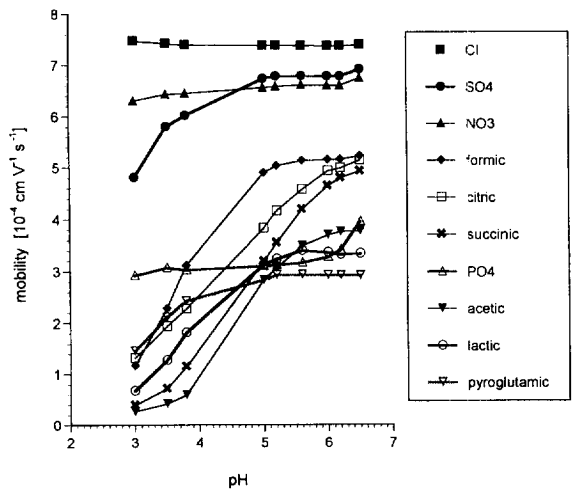

Fig. 2. Effect of electrolyte pH on anion mobility. Experimental conditions: capillary, fused-silica capillary 75 μm×80.5 cm (l= 72 cm); electrolyte, 5 mM CTAB; applied potential, −25 kV; injection pressure, 2 s at 50 mbar; capillary temperature, 20°C; detection, signal=350 nm, reference=200 nm.

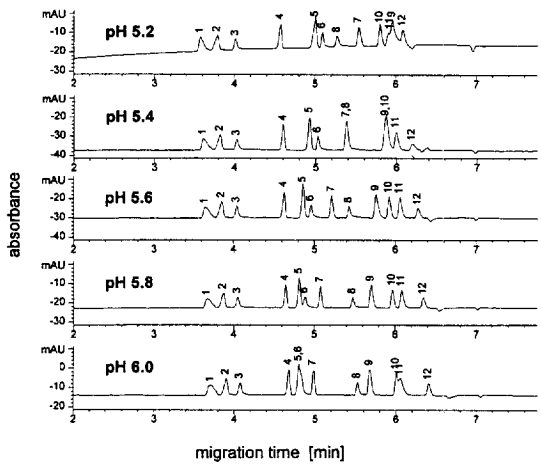

Fig. 3. Effect of electrolyte pH on anion separation. Peaks (each 25 mg l^{-1}): 1=chloride, 2=sulfate, 3=oxalate, 4=formate, 5=malate, 6=citrate, 7=succinate, 8=pyruvate, 9=acetate, 10=lactate, 11=phosphate, 12=pyroglutamate. Experimental conditions are listed in Fig. 2.

3.2.2. Effect of PDC concentration

The effect of PDC concentration on the anion separation was investigated. With a constant concentration of 0.5 mM CTAB in the run electrolyte, the concentration of PDC was varied from 3 to 10 mM. Changing the buffer concentration had a negligible effect on separation, however, an increase in baseline noise was observed above 7 mM. Therefore in this study, 5 mM PDC was chosen as the optimum buffer concentration.

3.2.3. Effect of applied potential

The effects of varying the applied potential to −15 kV, −20 kV, −25 kV and −30 kV was studied. At a higher potential the analysis time was reduced, however, at −30 kV, a vacant peak was observed between oxalate and formate and baseline drift was also observed. Optimum separation with minimal analysis time was obtained at −25 kV.

3.3. Method validation

The reproducibility, linearity and sensitivity of the method were tested. Fig. 4 illustrates an electropherogram of 25 mg l^{-1} of 12 anion standards, demonstrating that three inorganic anions and nine organic acids were well resolved in a short analysis

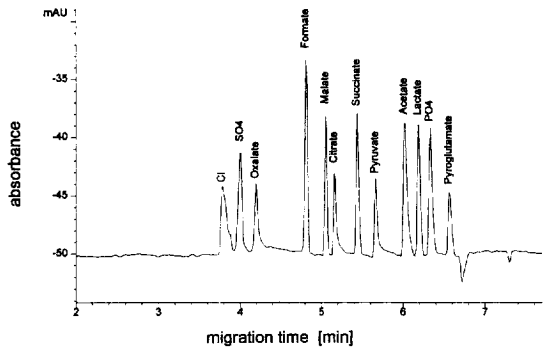

Fig. 4. Electropherogram of 25 mg l^{-1} each of inorganic and organic anion standard mixture obtained with the PDC electrolyte. Experimental conditions: electrolyte, 5 m*M* PDC, 0.5 m*M* CTAB, pH 5.6. Other conditions as in Fig. 2.

time. Table 3 shows the excellent reproducibilities obtained for migration time and peak area as reflected by the %R.S.D. ($n=6$). This high degree of reproducibility indicates that PDC provides a good buffering capacity at pH 5.6. The calibration curves for all inorganic and organic anions were linear over the range 5–50 mg l^{-1} with correlation coefficients better than 0.9992. The detection limits for all analytes were in the range from 0.9 to 2.5 mg l^{-1} with 100 mbar*s pressure injection at a signal to noise ratio of 3.

Nitrate could not be observed using a detection wavelengths of 350 nm with reference at 200 nm. This arrangement of signal and reference wavelengths are used so that the negative peaks associated with indirect detection are recorded as positive peaks. A decrease of absorption at 200 nm produced by the presence of analyte is recorded as a relative increase of the signal at 350 nm since the 200 nm signal is used as the reference wavelength. Since nitrate has its own absorption at 200 nm this is not observed under these conditions. However, using a signal wavelength of 350 nm with reference at 275 nm enabled visualization of nitrate between the sulfate and oxalate peaks although with much less sensitivity.

3.4. Effect of BGE complexing ability

Fig. 5 shows a comparison of the separation of 12 inorganic and organic anions obtained using PDC, phthalate and trimellitate as BGE. PDC provides a complete separation of all 12 components. When using phthalate or trimellitate as BGE, neither oxalate nor citrate could be observed, and this is accompanied by a reduction in the signal for malate. However, when the concentration of oxalate and citrate was increased to 50 mg l^{-1} these components could be detected with both phthalate and trimellitate buffers. We therefore presumed that the disappearance of these peaks was due to some adsorption of the organic acids onto the capillary wall at a fixed amount of sites which could, therefore, be saturated.

Organic acids such as oxalate, citrate and tartarate are well-known as complexing agents. Koyama et al. [15] reported that metal impurities, especially Fe, in silica gel had a significant effect on protein sepa-

Table 3
Reproducibility, linearity and sensitivity

Anions	R.S.D. ($n=6$)(%)		Linearity	Detection limit
	Migration time	Peak area	correlation	(mg l^{-1})
Chloride	0.10	1.8	0.9997	1.9
Sulfate	0.12	2.6	0.9999	1.4
Oxalate	0.11	1.5	0.9993	1.8
Formate	0.11	1.7	0.9994	1.0
Malate	0.12	1.8	0.9993	1.2
Citrate	0.12	2.2	0.9997	2.2
Succinate	0.13	1.1	0.9997	1.2
Pyruvate	0.12	2.5	0.9997	2.4
Acetate	0.12	1.5	0.9996	0.9
Lactate	0.13	0.6	0.9992	1.2
Phosphate	0.13	2.0	0.9999	1.8
Pyroglutamate	0.13	1.7	0.9995	2.5

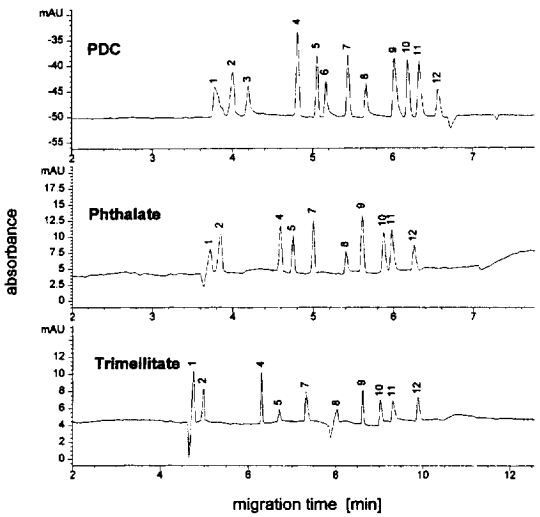

Fig. 5. Comparison of electropherograms obtained with PDC, phthalate and trimellitate. Peaks (each 25 mg l^{-1}): 1=chloride, 2=sulfate, 3=oxalate, 4=formate, 5=malate, 6=citrate, 7=succinate, 8=pyruvate, 9=acetate, 10=lactate, 11=phosphate, 12=pyroglutamate. Experimental conditions: as in Fig. 4 except electrolyte.

ration by HPLC and Verzele and Dewaele [16] have demonstrated that the selective removal of metal traces from bonded phase silica gel improves the chromatography of organic acids in hop bitter acids. Dandeneau and Zerenner [17] have reported that fused-silica contains metals although at a concentration of less than 1 ppm total metals.

In order to investigate the interaction of organic acids with metals, metal impurities in a fused-silica capillary were determined using ICP-MS. This identified Fe, Al, Na, Cr and Mg at levels of 300, 240, 140, 40 and 30 ppb, respectively. Stability constants for these metal complexes with organic ligands are listed in Table 4. The unobserved organic acids, oxalate, citrate and malate show higher stability constants than other organic acids for metals such as Fe^{2+}, Fe^{3+} and Mg^{2+}.

PDC will also chelate these metals with stability constants of the same order of magnitude or slightly higher. However phthalate and trimellitate show little or no chelation of these metal ions. In the light of these results we propose that the disappearance of the organic acids is caused by their interaction with metal impurities in fused-silica. Organic acids which display high stability constants for metal complex formation tend to adsorb to metal impurities in the capillary wall and therefore cannot be detected. However, when using a BGE which itself has a high metal complex formation stability constant such as PDC, these organic acids can be determined because the BGE masks the metal impurities and prevents adsorption.

Table 4
Stability constants for metal complexes

	Stability constant Log K_1					
	Fe^{2+}	Fe^{3+}	Al^{3+}	Na^{+}	Cr^{2+}	Mg^{2+}
Oxalate	3.05	7.53	5.97		3.85	2.76
Citrate	4.8	11.50	7.98	0.71		3.45
Malate	2.6	7.1		0.29		1.71
Formate		3.1	1.36		1.07	0.34
Succinate	1.4	6.88	3.20	0.06		1.18
Pyruvate				0.2		1.1
Acetate	1.4	3.38	1.51	−0.26	1.25	0.55
Lactate				1.66		0.93
PDC	5.71	10.91	4.87			2.31
Phthalate			3.81			
Benzoate			12.09			0.1
Pyromellitate						
Trimellitate						
EDTA	14.30	25.1		1.84	13.6	8.85

From Ref. [18]

We have also studied the analysis of EDTA, which exhibits the highest stability constant for most of the metals, using different BGEs. As expected, EDTA could only be observed using PDC.

Adsorption of oxalate and citrate was investigated further. Fig. 6 illustrates the results obtained by PDC (Fig. 6A) and phthalate (Fig. 6B) with only the BGE preconditioning. In order to prevent organic acid adsorption in phthalate conditions, we tried to remove metal impurities by washing the fused-silica capillary with strong acid. Prior to first use, a new capillary was flushed with 0.033 *M* phosphoric acid for 60 min followed by flushing with Milli-Q water for 20 min, then the BGE for 20 min. However, neither oxalate nor citrate could be detected using phthalate as BGE.

When preconditioning with 0.033 *M* phosphoric acid for 2 min followed by the BGE for 4 min before every injection, both oxalate and citrate could be observed even using phthalate electrolyte. However, using this preconditioning, a large phosphate peak was observed (Fig. 6C), which interfered with detection of phosphate and lactate.

These results indicate that using PDC as BGE provides a separation of anions with good resolution and no organic acid adsorption. Further, using the PDC method eliminated the need to flush the capillary with strong acids.

3.5. Beer analysis

The developed method was applied to the determination of inorganic and organic anions in beer. The measurement of the concentrations of inorganic and organic anions, in all phases of beer production, can be used to help track metabolic products of fermentation and correlate beer flavor trends. Boyles [19] developed a simultaneous determination method for inorganic and organic anions in beer using gradient ion chromatography. However, this demanded sample preparation and a long analysis time. Fig. 7 shows a typical result of anion analysis in a beer sample using the described method. Sample preparation was very simple and consisted only of degassing the beer by sonication, and diluting it 1:5 with Milli Q water prior to injection.

A well-defined electropherogram was obtained without interference from other matrix compounds. Peak identification was performed by matching the unknown peak migration time with that of a standard solution. The concentration of ions in the sample ranged from 9 to 511 mg l^{-1} and the method showed good agreement with data obtained from two conventional techniques, ion chromatography for inorganic anions and liquid chromatography for organic acids, with a correlation coefficient of 0.993. Excellent reproducibilities were obtained with R.S.D. values ($n=5$) for migration times better than 0.3%

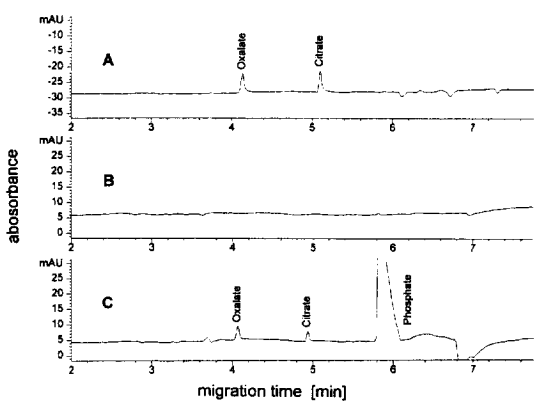

Fig. 6. Comparison of 20 mg l^{-1} each oxalate and citrate detection obtained with (A) PDC, (B) phthalate, (C) phthalate using preconditioning with 0.033 *M* phosphoric acid followed by phthalate flushing. Experimental conditions: as in Fig. 4 except electrolyte and preconditioning.

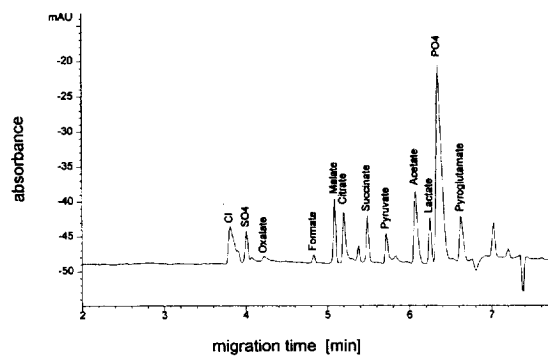

Fig. 7. Electropherogram of inorganic and organic anions in beer. Amounts (mg l^{-1}): chloride, 113; sulfate, 43; oxalate, 19; formate, 9; malate, 94; citrate, 123; succinate, 65; pyruvate, 62; acetate, 102; lactate, 58; phosphate, 511; pyroglutamate, 160. Experimental conditions: as in Fig. 4.

and for peak areas better than 3.4% except for the low concentration oxalate and formate. This shows the proposed method is hardly affected by sample matrices.

4. Conclusions

A reliable, rapid and easy method for the simultaneous determination of inorganic and organic anions has been developed. For organic acid analysis, the BGE had a significant effect not only on separation and sensitivity but indirectly on the ability to detect some organic acids which could not be visualized at low concentrations using other BGEs. This is presumed to be due to the preferential chelation of PDC with metal contaminants thus masking these from interaction with organic acids which have a high stability constant of metal complex formation. The use of the PDC as the BGE provided a well resolved and reproducible separation. The method was linear over the range 5–50 mg l^{-1}, and its utility was demonstrated in the analysis of inorganic and organic anions in a beer sample.

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