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Method development and validation for the simultaneous determination of organic and inorganic acids by capillary zone electrophoresis

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Abstract

The separation of organic and inorganic acids was performed by capillary zone electrophoresis using indirect UV detection. Optimum conditions for the separation of 11 anions were investigated. This method was applied for the simultaneous determination of organic and inorganic anions in foods and beverage samples. The relative standard deviation (R.S.D.) for the analysis of anions was less than 1% for the migration time and 1–4% for the peak area ($n=18$). The detection limits was from 0.006 to 1.072 mg/l for the 11 anions. This newly developed method is rapid, sensitive and quantitative and can be readily applied to real food and beverage samples for quantitative analysis.

Keywords: Electrolyte systems; Organic acids; Inorganic acids

1. Introduction

Measuring organic and inorganic acid levels in foods and beverages is important from the standpoint of monitoring the fermentation process, checking product stability, validating the authenticity of juices and concentrates and studying the organoleptic properties of fermented products such as wines.

Analytical approaches adopted for the measurement of organic acids have been based on gas chromatographic (GC) methods with derivatization steps [1] or high-performance liquid chromatography (HPLC) [2]. In all instances, complex extraction steps were necessary, since these methods were not specific for organic acids and the reagents used could potentially interact with many compounds containing functional groups with an active hydrogen. Thus, current HPLC or GC methods lack specificity and betray ruggedness for routine work.

For the determination of inorganic acids, ion chromatography (IC) has become the technique of choice [3]. It has already reached a stage of maturity, so that its potential and limitations are by now well-known.

Organic acids were also determined directly by capillary isotachophoresis in serum [4] and in samples of vegetables [5].

Other new techniques such as capillary zone electrophoresis (CZE) have only been introduced in the last several years [6–9], and great progress has

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since been made in instrumentation and applications [10,11]. Applications include the separation of small organic and inorganic anions with indirect UV detection in many real samples [12–22]. Excellent separation efficiencies were reported with chromate, pyromellitate and other aromatic carboxylic acid salts containing electrolytes [11–25]. The effects exerted by pH and the concentration of the electroosmotic flow (EOF) modifier on the selectivity and resolution of CZE were evaluated.

This paper describes the development of a new electrolyte system for indirect UV detection in CZE using a 1,2,4,5-benzene-tetracarboxylic acid (pyromellitic acid or PMA) buffer system with bis-(2-aminoethyl)-amine (diethylenetriamine or DETA) as EOF modifier. The optimum conditions for the simultaneous separation of organic and inorganic anions were determined. The CZE method developed was applied to the determination of anions in various samples.

2. Experimental

2.1. Chemicals

Standard solutions were prepared from chromatographic or analytical reagent grade chemicals (Merck, Darmstadt, FRG; Sigma, St. Quentin Fallavier, France; Prolabo, Paris, France) by serial dilution with deionized 15 megaohm water (C2R, Montgiscard, France). Solutions of chloride (0.28 g/l), nitrate (0.12 g/l), sulphate (0.96 g/l), phosphate (0.57 g/l), oxalic (0.20 g/l), tartaric (3.00 g/l), malic (3.00 g/l), succinic (1.00 g/l), citric (2.00 g/l), acetic (0.20 g/l) and lactic (3.00 g/l) acids (or sodium salts) were prepared. These concentrations were chosen, to reproduce the levels found in wines. These solutions were mixed and diluted and the diluted mixture was used as the standard.

Background electrolyte as 1,2,4,5-benzene-tetracarboxylic acid (pyromellitic acid or PMA) (Janssen, Geel, Belgium) was prepared as 3 m*M* stock solutions; the pH values were adjusted to 5–9.5 by the addition 1 M of Tris–Base buffer (pH 10.5, Tris-[hydroxymethyl]aminomethane (Sigma, St. Quentin Fallavier, France)) depending on the experiments.

Bis-(2-aminoethyl)-amine (Diethylenetriamine or DETA) (Aldrich, St. Quentin Fallavier, France) was employed as EOF modifier and its concentration was varied from 1 to 5 m*M*.

2.2. Apparatus

A Europhor capillary electrophoresis system (Europhor Instruments now Zeta Technology, Ramonville, Toulouse, France) was used throughout the investigation. The ultraviolet detector was built into the CZE instrument. The separation was performed on a 44 cm×75 μm I.D. fused-silica capillary column (Polymicro Technology, Phoenix, Arizona, USA). The detection window was 7 cm from the capillary outlet. An ultraviolet wavelength of 220 nm was chosen to monitor the absorbance of the buffer solution. The separation voltage was −20 kV, resulting in an electrophoretic current of 7.4 μA, at constant temperature of 30°C.

Electrophoregrams were recorded and processed with a PC 1000 data acquisition system (Thermo Separation Products, Freemont, CA, USA).

2.3. Capillary washing

The capillary was rinsed with 0.1 *M* NaOH (1 min), water (1 min) and then with separation buffer for 2 min. Samples were injected by hydrodynamic injection for 2 s.

3. Results and discussion

3.1. Effect of pH

The pH of the running electrolyte had a significant impact on the electrophoretic mobilities of anions. The effect of pH on the relative migration time of the 11 acids is shown in Fig. 1. The migration order for chloride, nitrate, sulphate, oxalate, tartrate and malate was not changed in the pH range of 5–9. However, the migration times for the other acids were affected significantly by changes in pH. pH values lower than 7 were not applicable because some anions comigrated, and pH higher than 8 resulted in very long analysis times. In this in-

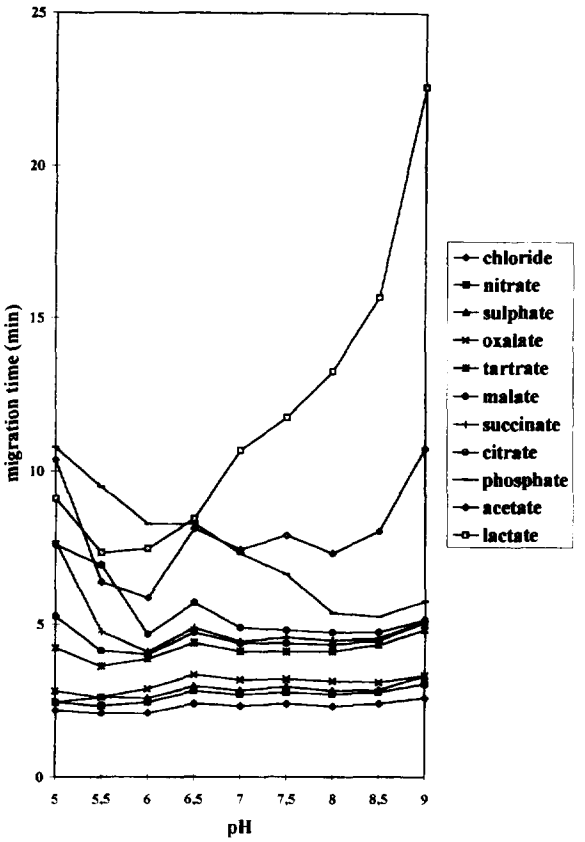

Fig. 1. Effect of pH on migration times of organic and inorganic anions in 3 m*M* PMA–3 m*M* DETA.

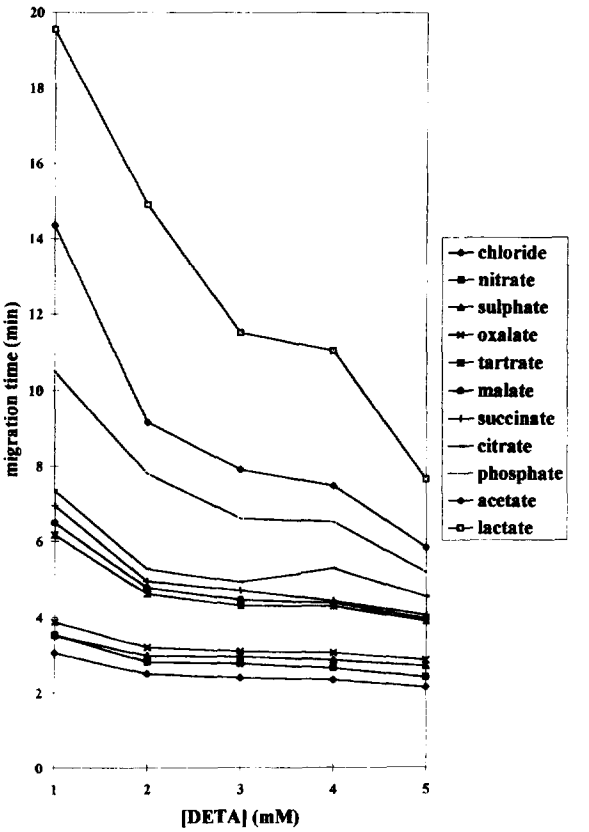

Fig. 2. Effect of DETA concentration on the electrophoretic mobility of the organic and inorganic acids in 3 m*M* PMA (pH 7.5).

vestigation, pH 7.5 was used to perform the detennination of organic and inorganic anions.

3.2. Effect of EOF modifier

In 1989, Foret et al. [26] provided a set of rules towards method development for the indirect UV detection of non-absorbing ions using charge displacement of an absorbing co-ion as a principal constituent of the background electrolyte. Since then this mode of detection has become very popular in CZE, especially for the determination of inorganic anions and cations [16,27].

The effect of the concentration of DETA from 1 to 5 m*M* on the migration time of the organic and inorganic acids is shown in Fig. 2. It became apparent that when the concentration of DETA is above 4 m*M*, the separation of tartrate, malate and succinate became very poor and the migration time of the various analytes was much longer and the baseline started to drift. Therefore, we chose 3 m*M* DETA as a compromise. The pH was directly dependant on DETA concentration as shown earlier. At a DETA concentration of 3 m*M*, 11 peaks were detected and separated within 12 min and the migration times for all the anions were shortened Fig. 3.

Variation in buffer temperature, PMA concentration, DETA concentration and pH, had no significant effect on either peak height or areas.

3.3. Operating voltage

Voltage was assessed between −14 to −20 kV. Operation at −20 kV produced a considerably faster

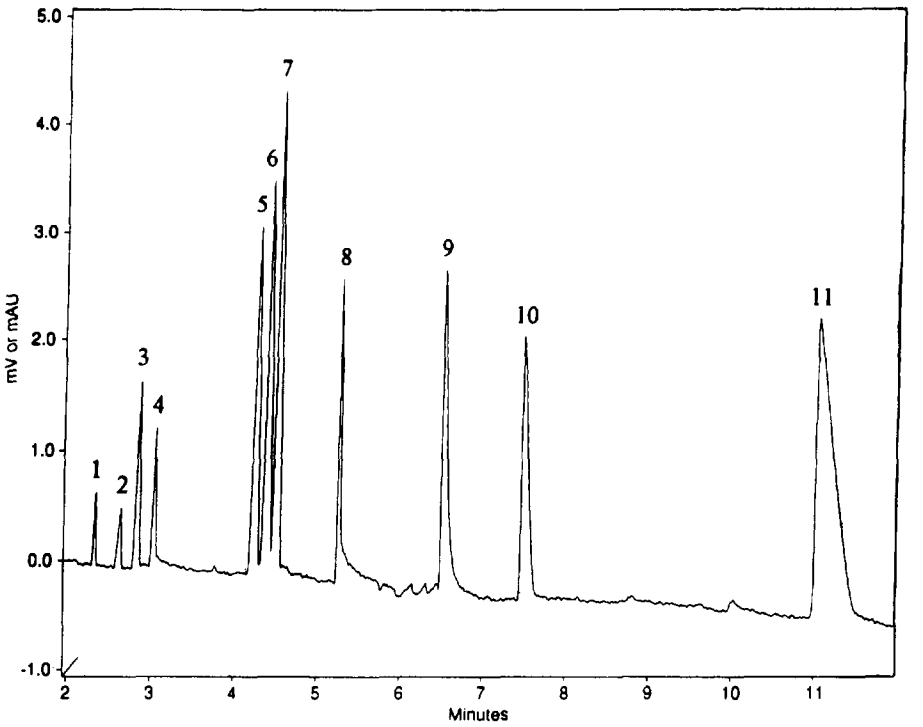

Fig. 3. Electrophoregram of a mixture of the 11 anions in 3 m*M* PMA–3 m*M* DETA at pH 7.5. Migration order (peaks): (1) chloride, (2) nitrate, (3) sulphate, (4) oxalate, (5) tartrate, (6) malate, (7) succinate, (8) citrate, (9) phosphate, (10) acetate, (11) lactate.

analysis (the longest elution time of lactic acid, t_e of peak 11<12 min). Operation at −14 kV produced increased analysis times (t_e of peak 11>18 min). Therefore, the earlier value of −20 kV was employed in all further studies.

3.4. Assessment of analytical performance

The validation criteria commonly employed in the evaluation of a CZE methods are similar to those tested for HPLC [33]. Preliminary valuation of this method included assessments of linearity, precision and detection limits.

3.4.1. Precision

The precision of the present method for the various analytes is expressed in terms of relative standard deviation (R.S.D.). A mixture of the 11 anions in water was injected using the method settings given in the experimental section. Six injections of standards were done sequentially. This operation was repeated over 3 days. Table 1 shows that the migration time precision was excellent, the mean value of R.S.D.s is 0.16% for all compounds, indicating good performance of the method for qualitative analysis. Peak area precision for the organic and inorganic acids was in the region of

Table 1
Precision for the various analytes (n=18) and linearity of organic and inorganic acid concentrations (x) versus peak area (y) (n=6)

Organic and inorganic acids	R.S.D.(%) (n=18)		Linearity (n=6)
	T.M.	Area	Regression equation
Chloride	0.03	1.52	y=401 517x
Nitrate	0.15	1.59	y=60 484x
Sulphate	0.07	1.43	y=272 827x
Oxalate	0.04	3.69	y=225 371x
Tartrate	0.09	0.95	y=270 235x
Malate	0.06	1.41	y=336 660x
Succinate	0.07	1.72	y=369 166x
Citrate	0.34	1.18	y=312 681x
Phosphate	0.54	4.25	y=454 295x
Acetate	0.30	1.17	y=859 622x
Lactate	0.54	1.13	y=432 407x

T.M.= migration time.

Table 2
Limits of detection (LOD) and quantification (LOQ)

Organic and inorganic acids	LOD		LOQ	
	mg/l	fmol[a]	mg/l	fmol[a]
Chloride	0.017	3.8	0.058	13.1
Nitrate	0.655	83.8	2.182	281.3
Sulphate	0.014	1.4	0.048	4.8
Oxalate	0.090	8.1	0.280	25.2
Tartrate	0.175	9.1	0.583	31.1
Malate	0.087	5.2	0.287	17.4
Succinate	0.040	2.7	0.134	8.9
Citrate	0.375	15.5	1.250	5.4
Phosphate	0.367	31.2	1.224	102.7
Acetate	0.006	0.8	0.020	2.7
Lactate	1.072	96.2	3.574	320.9

[a] Based on 8 nl injection.

0.95–4.25%. This performance suggests, however, that the method is entirely suitable for quantitative determination of anions.

3.4.2. Linearity

Detector response measured for all organic and inorganic acids was linearly correlated with the sample concentration injected over a range of 0.1–100 mg/l. The linearity was determined from repeated injection at six different concentrations of each organic and inorganic acid. The regression equations of these curves are shown in Table 1. The linearity of the present method for most analytes is good, with correlation coefficients better than 0.99. The linearity was achieved without an internal standard, which probably would have increased the correlation coefficient.

3.4.3. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was evaluated from eight independent blanks, which were spiked to produce a peak height, for each of the analyte anions, close to three times the baseline noise which was determined as described in Ref. [28]. The LOD was estimated by taking three times the standard deviation of the peak areas obtained from these solutions and calculating the corresponding concentrations from the calibration lines (Table 2). Each acid was analysed separately. The role of the concentration of other ions on LOD was not studied.

The LOQ is defined as the level at or above which the measurement precision is satisfactory for quantitative analysis. It was estimated by taking ten times the standard deviation of the peak areas obtained from the eight blanks and subsequently calculating the corresponding concentrations from the calibration lines (Table 2).

Table 3
Comparison of LODs measured in our work and in other studies

Organic and inorganic acids	LOD (mg/l)				
	Our-work	Li et al.[a]	Shamsi et al.[b]	Kelly et al.[c]	Wu et al.[d]
Chloride	0.017	0.17	0.150	0.1	ND
Nitrate	0.655	0.25	0.125	0.1	ND
Sulphate	0.014	0.16	0.080	0.1	ND
Oxalate	0.090	ND	0.080	0.4	0.178
Tartrate	0.175	ND	0.090	0.4	0.298
Malate	0.087	ND	0.075	0.4	0.266
Succinate	0.040	ND	0.100	0.4	ND
Citrate	0.375	0.55	0.090	ND	0.382
Phosphate	0.367	0.14	ND	ND	ND
Acetate	0.006	0.37	ND	ND	0.118
Lactate	1.072	ND	ND	0.4	0.178

ND=not determined

[a] One electrolyte system using chromate [29].

[b] One electrolyte system using naphthalenetrisulfonate [30].

[c] Two electrolyte systems using potassium phatalate for organic acid separations and pyromellitic acid for inorganic anion separations. LODs were estimated 10 and 25 times lower respectively for inorganic and organic acids than shown results [31].

[d] One electrolyte system using trimellitic acid [32].

The LOD were from 0.006 to 1.072 mg/l and the LOQ were from 0.020 to 3.574 mg/l for the 11 anions. LOD and LOQ could probably be improved by the injection of larger volumes or more concentrated samples. We injected 8 nl, according to the instruction manual of the Europhor capillary electrophoresis system.

These measurements of LODs for inorganic and organic acids are compared to some other results described in the literature (Table 3). Our experiment allows determination of organic and inorganic ions in the same run, with good LODs for most of the studied ions.

4. Applications

To demonstrate the usefulness of the CZE method developed, analyses of a variety of samples were performed. Prior to analysis, the samples were diluted 1:50 (v/v) in deionized water, the obtained electrophoregrams are shown in Fig. 4.

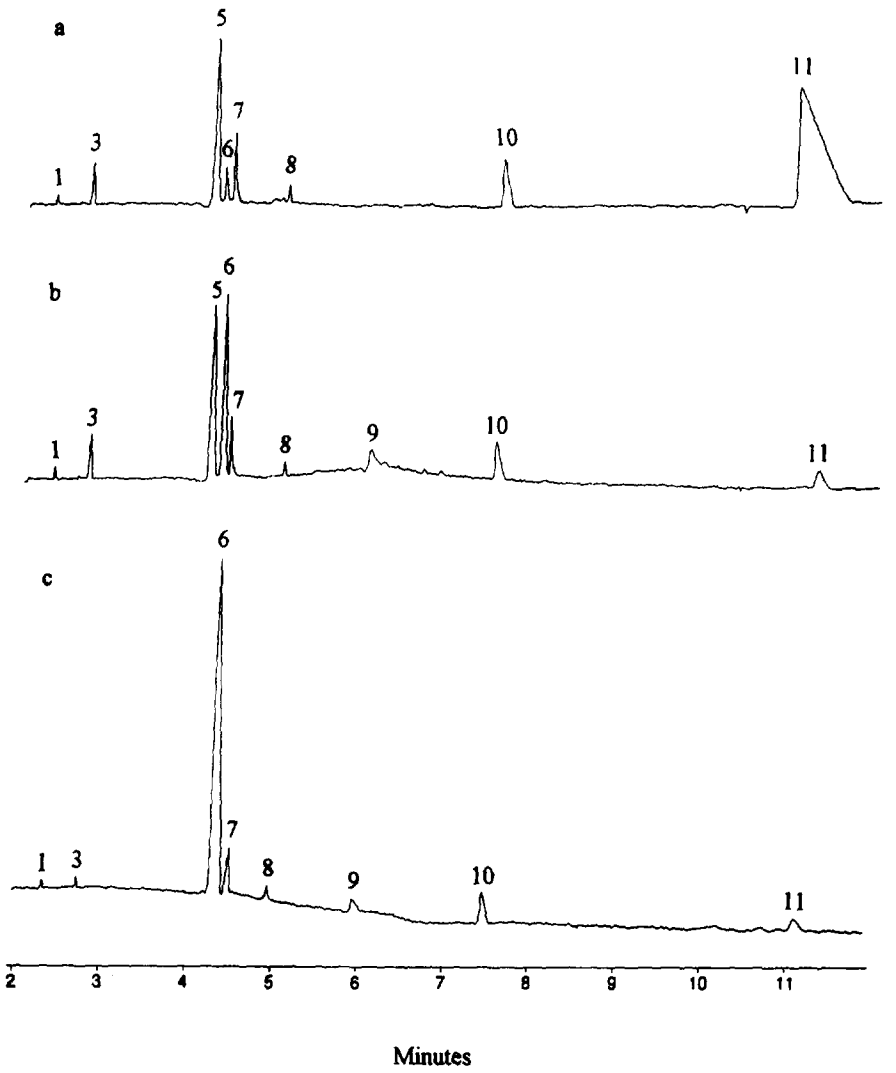

Fig. 4. Electrophoregrams of organic and inorganic acids in real beverage samples: (a) red wine; (b) white wine; (c) applejuice.

Table 4
Contents of the organic and inorganic acids of interest found in representative beverage samples ($n=6$)

Organic and inorganic acids	Red wine	White wine	Apple juice
Chloride	0.025	0.070	0.029
Nitrate	0	0	0
Sulphate	0.320	0.138	0.050
Oxalate	0	0	0
Tartrate	2.340	2.326	0
Malate	0.192	2.984	5.897
Succinate	0.390	0.355	0.414
Citrate	0.096	0.051	0.080
Phosphate	0	0.199	0.161
Acetate	0.317	0.192	0.163
Lactate	6.184	0.267	0.190

Concentrations in g/l.

Fig. 4a is an electrophoregram of anions in red wine. Eight anions, i.e., (1) chloride, (3) sulphate, (5) tartrate, (6) malate, (7) succinate, (8) citrate, (10) acetate and (11) lactate were identified.

Fig. 4b is an electrophoregram of anions in white wine. Nine anions, i.e., (1) chloride, (3) sulphate, (5) tartrate, (6) malate, (7) succinate, (8) citrate, (9) phosphate, (10) acetate and (11) lactate were identified.

Fig. 4c is an electrophoregram of anions in apple juice. Eight anions, i.e., (1) chloride, (3) sulphate, (6) malate, (7) succinate, (8) citrate, (9) phosphate, (10) acetate and (11) lactate were identified.

Results of the quantitative analysis are included in Table 4. Red wines generally contain high concentrations of tartrate and lactate. On the other hand, tartrate is also the predominant anion in white wines, but lactate concentration decreases a lot prior to red wines. It is due to the lack of malolactic fermentation which transforms malate into lactate. In most juices, citrate is omnipresent, malate is present in high concentration and there is a minute concentration of lactate.

5. Conclusion

Results of this work show that CZE is an effective separation method for the determination of anions in foods and beverages. This technique provides highly efficient separation with high precision, short analysis time and low reagent consumption.

However, the electrolyte system with PMA, DETA and Tris is recommended for simultaneous analysis of organic and inorganic anions with the best detection limits.

CZE did not show any decrease in performance during the analysis of the least 50 samples and no replacement of the (low cost) separation capillary was necessary.

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