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Determination of low-molecular-mass anionic compounds in beverage samples using capillary zone electrophoresis with simultaneous indirect ultraviolet and conductivity detection

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Abstract

The determination of low-molecular-mass anionic components in beer samples using capillary zone electrophoresis with simultaneous direct conductivity and indirect UV detection is described. Optimization of the carrier electrolyte composition has been performed taking into account parameters such as pH as well as those that were found to be crucial for its compatibility with both detection principles employed in this paper. The latter are UV absorptivity, electrophoretic mobility of the buffer co-ion and the buffer co-ion concentration, which strongly influence the quality (signal-to-noise ratio, chromatographic resolution of adjacent peaks) of the electropherogram. Best results could be achieved with a carrier electrolyte consisting of 4-aminobenzoic acid, tetradecyltrimethylammonium bromide electroosmotic flow reversal and a pH of 5.75, adjusted using histidine. Using this carrier electrolyte, limits of detection ranging from 0.218 mg l^{-1} for pyroglutamate down to 0.018 mg l^{-1} for chloride could be obtained.

Keywords: Beer; Food analysis; Detection, electrophoresis; Background electrolyte composition; Inorganic anions; Carboxylic acids

1. Introduction

A large number of capillary zone electrophoretic (CZE) separations have been published for the analysis of low-molecular-mass anionic compounds, such as inorganic anions or carboxylic acids, in a variety of matrices using diverse carrier electrolyte compositions. Commonly, detection of these analytes can be performed by indirect UV detection using an UV-absorbing carrier electrolyte, by direct UV detection in the case of UV-absorbing solutes or by conductivity detection [1–3]. Because the latter technique has just recently become available in commercial CE instruments, just a few reports describe the applicability of this detection method for the analysis of the solutes investigated in this study [4–6]. Comparing these techniques, indirect UV detection as well as conductivity detection can be regarded as suitable for the analysis of low-molecular-mass anionic compounds in general, whereas the applicability of direct UV detection is often restricted.

Background electrolytes (BGEs) used for separations with indirect UV detection contain an UV-absorbing co-ion (ideally showing an electrophoretic mobility close to that of the analytes, to obtain

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optimal peak shape) which exhibits UV absorbance in a region where the solute ions show little or no absorption [7,8]. In addition to the electrophoretic mobility of the buffer co-ion, the ionic strength of the carrier electrolyte also affects peak symmetry in so far as higher buffer concentrations lead to increased symmetry. Yet, it has to be taken into account that higher concentrations of the carrier electrolyte also cause higher UV absorbance and thereby an unwanted increase in baseline noise. Therefore, running buffers usually employed for CZE separations with indirect UV detection include a strongly UV absorbing co-ion present in low to moderate concentrations (for optimal signal-to-noise ratios) with an electrophoretic mobility matching that of the analytes.

A different situation can be found in conductivity detection. The main basis of this detection technique is the conductivity difference between the sample zone and the carrier electrolyte. A BGE with a lower conductivity than that of the analytes is used in the case of direct conductivity detection, whereas in the opposite case indirect conductivity detection is obtained. Therefore, to achieve satisfactory sensitivity, the electrophoretic mobilities of the running buffer co-ion and the solutes have to be quite different. Carrier electrolytes with high ionic strength are usually employed to compensate for peak distortions resulting from this fact [9].

Considering these attributes, direct conductivity detection using a low mobility carrier electrolyte is more suitable for fast migrating analytes whereas slower migrating species with electrophoretic mobilities approaching that of the running buffer co-ion show a less favorable detector response. For this reason, for samples consisting of solutes within a wider range of mobilities, best results should be obtained using direct conductivity detection in combination with indirect UV detection.

Until now, only two papers report the analysis of beer samples by CZE with respect to their content of inorganic anions and carboxylic acids using indirect UV detection [10] or conductivity detection [11]. Therefore, the aim of the present work was the development of a BGE for the separation of these analytes in beer that would be compatible with both detection methods, namely conductivity and indirect UV detection. A number of carrier electrolyte compositions consisting of an UV-absorbing carboxylic acid and a basic organic component have been investigated with respect to their suitability as running buffers for this purpose. Parameters that were found to be crucial for the compatibility of the BGE with both detection methods employed in this work, such as UV absorptivity as well as the electrophoretic mobility of the buffer co-ion, were used for selection. Separations of inorganic and organic anions using a carrier electrolyte based on 4-aminobenzoic acid (*p*-AB) and indirect absorbance detection have already been described in the literature [12,13]. Preliminary investigations indicated that this aromatic carboxylic acid in combination with an organic base for pH adjustment and tetradecyltrimethylammonium bromide (TTAB) for electroosmotic flow (EOF) reversal was the optimal BGE for the investigations described in this work [14]. Using an optimized carrier electrolyte composition, a series of different Austrian and foreign brand beers have been analyzed.

2. Experimental

2.1. Apparatus

A Crystal 310 capillary electrophoresis (CE) instrument (Thermo Bioanalysis, Santa Fe, CA, USA) equipped with a Crystal 1000 conductivity detector (Thermo Bioanalysis) was used. A fixed-wavelength UV detector (mercury lamp operated at 254 nm) dismounted from a Quanta 4000 CE instrument (Waters, Milford, MA, USA) was installed between the CE device and the conductivity detector unit. Data collection was performed with a HP 3359 data acquisition system (Hewlett-Packard, Palo Alto, CA, USA). ConCap I fused-silica capillaries (Thermo Bioanalysis) with an inner diameter of 50 μm and lengths of 48 cm (UV detector) and 60 cm (conductivity detector) from the injection end to the detector were used.

2.2. Reagents and samples

For all solutions, 18 MΩ high purity water obtained from a Milli-Q system (Millipore, Marlborough, MA, USA) was used. Carrier electrolytes

were prepared from *p*-AB, histidine (His) and Tris (all purchased from Sigma, St. Louis, MO, USA). TTAB (Merck, Darmstadt, Germany) was used for EOF reversal. Standard solutions were made by dissolving the appropriate salts or carboxylic acids (purity >99%) in high-purity water. Beer samples were diluted tenfold with water and degassed for 15 min using an ultrasonic bath.

2.3. Procedures

New capillaries were treated with a 0.5-*M* NaOH solution and water for 30 min, before use. Prior to each analysis, the capillary was flushed with running buffer for 3 min. Injection was performed in a hydrodynamic mode at the cathodic side by applying a pressure of 25 mbar for 0.2 min. A voltage of −30 kV was used for separation.

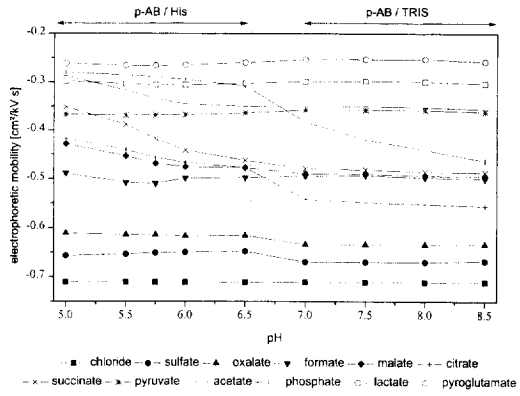

Fig. 1. Electrophoretic mobility of selected anionic solutes as a function of the pH of the carrier electrolyte. Carrier electrolytes: pH 5.00 to 6.5: 7.5 m*M* *p*-AB, 0.12 m*M* TTAB, pH adjusted with His; pH 7.0 to 8.5: 7.5 m*M* *p*-AB, 0.12 m*M* TTAB, pH adjusted with Tris.

3. Results and discussion

3.1. Selection of the carrier electrolyte

Carrier electrolytes consisting of *p*-AB and a basic organic component have been investigated with respect to their suitability as running buffers for the CZE separation of inorganic and organic anions with simultaneous indirect UV and direct conductivity detection. The detection wavelength for indirect UV detection was set to 254 nm because of the high molar absorptivity of *p*-AB at this wavelength. Focusing on the separation of the analytes of interest in this study, running buffers covering a pH range from 5.0 to 8.5, including 0.1 to 0.25 m*M* TTAB for EOF reversal, were tested. Limited by the useful buffer range of the carrier electrolyte components, the combination *p*-AB–His has been used for the preparation of BGEs from pH 5.0 to 6.5 and *p*-AB–Tris from pH 7.0 up to pH 8.5. Regarding the conductivity detection, EOF-modifier concentrations higher than 0.12 m*M* resulted in less reproducible peak areas, which were probably caused by adsorption phenomena at the surface of the detection electrode. This resulted in frequent cleaning of the detection electrode, which necessitated the disassembly of the detector unit. The less reproducible migration times caused by the low concentration of the EOF modifier (0.12 m*M* TTAB) could easily be corrected by the use of two internal standards. As can be deduced from Fig. 1, insufficient resolution could be obtained between formate, malate and succinate when the *p*-AB–Tris system was used as the running buffer. Much better results could be achieved using the *p*-AB–His electrolyte. Because of its separation efficiency, allowing the determination of all analytes of interest, a pH 5.75 carrier electrolyte consisting of *p*-AB and His, containing 0.12 m*M* TTAB was selected as the starting point for further optimization procedures.

3.2. Optimization of the carrier electrolyte composition

As discussed in Section 1, the concentration of the carrier electrolyte co-ion strongly influences the quality of the CZE separations. Whereas running buffers with high ionic strength are commonly used in CZE with conductivity detection to overcome the mismatch in mobility of the analytes and the buffer co-ion, low to moderate concentrations of the buffer co-ion are more suitable in combination with indirect UV detection. Therefore, a concentration of *p*-AB leading to acceptable results with both detection techniques had to be found. This was done using two parameters for optimization, first the chromatograph-

ic resolution (R_s) obtained for adjacent peaks like malate and citrate, calculated by Eq. (1)

$$R_s = 1.177 \cdot \frac{t_{m_2} - t_{m_1}}{w_{(0.5)_2} + w_{(0.5)_1}} \quad (1)$$

where t_{m_1} and t_{m_2} are the migration times of the separated zones and $w_{(0.5)_1}$ and $w_{(0.5)_2}$ are the corresponding zone widths at half height and, second, the signal-to-noise (S/N) ratio of the indirect UV signal achieved for a slowly migrating analyte such as pyroglutamate. Fig. 2 shows the influence of the *p*-AB concentration on both parameters. As can be seen from this plot, a carrier electrolyte containing 7.5 m*M* *p*-AB led to an excellent S/N ratio for pyroglutamate as well as an acceptable resolution for the analyte pair malate/citrate. Therefore, this BGE, consisting of 7.5 m*M* *p*-AB, 0.12 m*M* TTAB and a pH of 5.75, adjusted by the addition of His, was selected for the analysis of the real samples.

3.3. Analysis of beer samples

Using the running buffer described above, a number of different beers including a light beer, a nonalcoholic beer, a white beer, a Chinese rice beer, a stout and a 'Pils' type beer were investigated with respect to their content of inorganic and organic anions. In Figs. 3 and 4, the electropherograms obtained for two diluted beers (the nonalcoholic beer and the stout) spiked with two compounds that served as internal standards, chlorate (I.S. 1) and 5-chlorovalerate (I.S. 2), using simultaneous conductivity and indirect UV detection, are depicted. Table 1 shows the limits of detection (LODs; three times the baseline noise) and the limits of quantitation (LOQs; five times the baseline noise) obtained for the selected analytes. As can be seen from this table, conductivity detection was found to be more suitable for the determination of ions with high mobility differences with respect to the carrier electrolyte co-ion (chloride to succinate), whereas indirect UV detection proved to be superior for solutes with similar mobilities to that of *p*-AB

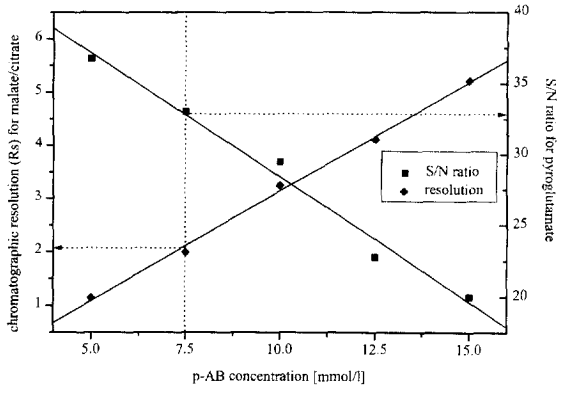

Fig. 2. Signal-to-noise (S/N) ratio for pyroglutamate and chromatographic resolution (R_s) for the pair malate/citrate as a function of the *p*-AB concentration. Carrier electrolytes: *p*-AB, as indicated, and 0.12 m*M* TTAB; pH adjusted to 5.75 with His.

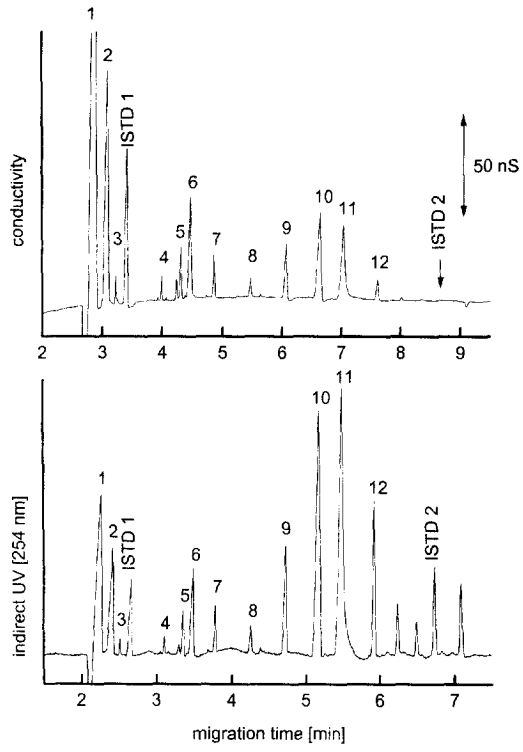

Fig. 3. Electropherograms obtained for a diluted nonalcoholic beer sample using conductivity detection and indirect UV detection. Carrier electrolyte: 7.5 m*M* 4-aminobenzoic acid containing 0.12 m*M* TTAB, pH adjusted to 5.75 with His. Applied voltage: −30 kV. Injection: 25 mbar for 0.2 min. Capillary: 48 cm (UV detector) and 60 cm (conductivity detector) effective length×50 μm I.D. Peaks: 1=chloride; 2=sulfate; 3=oxalate; 4=formate; 5=malate; 6=citrate; 7=succinate; 8=pyruvate; 9=acetate; 10=lactate; 11=phosphate and 12=pyroglutamate. ISTD=Internal standard.

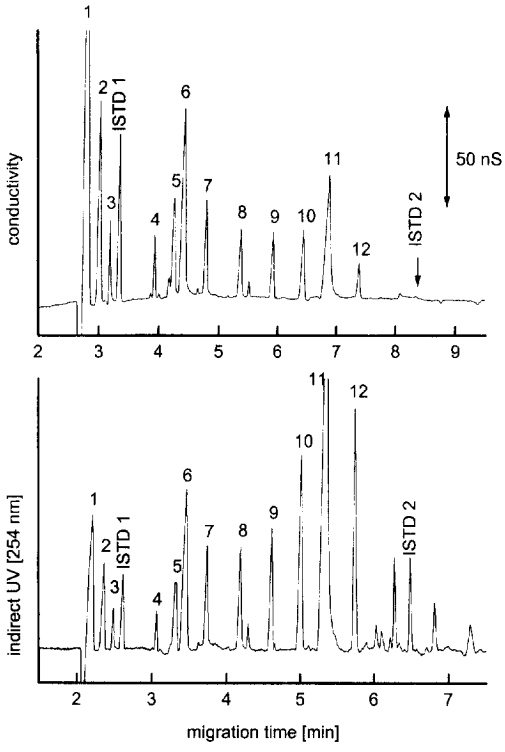

Fig. 4. Electropherograms obtained for a diluted stout sample using conductivity detection and indirect UV detection. For conditions and peak identification, see Fig. 3.

(pyruvate to pyroglutamate). Therefore, quantitation of the first seven peaks was performed using conductivity detection and I.S. 1 as the internal standard to prevent errors related to the injection procedure. The remaining peaks were determined by indirect UV detection relative to I.S. 2. Related to the investigated samples, calibration plots have been prepared between a lower limit of 1–5 mg l^{-1} for components that were present only in minor concentrations and an upper limit of 20–100 mg l^{-1} for major components like phosphate. Table 2 lists the corresponding equations and the resulting correlation coefficients (R^2). As can be seen from this table, excellent values for R^2 (better than 0.999) were obtained for all analytes except chloride (0.9986), which was somewhat affected by the negative bromide peak originating from the EOF modifier. Table 3 depicts the amount of the selected anionic solutes found in the beer samples under investigation. Because of differences in the brewing procedures, different concentration patterns for the selected analytes could be found. Whereas high amounts of phosphate and lactate were detected in all beers, analytes such as oxalate, formate and succinate were only present in lower quantities. Results that were significantly different from the average values could be found for formate, citrate and lactate in the stout

Table 1
Limits of detection (LODs) and limits of quantification (LOQs) for inorganic anions and carboxylic acids using indirect UV detection and conductivity detection

	Conductivity detection		Indirect UV detection at 254 nm	
	LOD (mg l^{-1})	LOQ (mg l^{-1})	LOD (mg l^{-1})	LOQ (mg l^{-1})
Chloride	0.018	0.030	0.118	0.197
Sulfate	0.022	0.037	0.119	0.199
Oxalate	0.041	0.068	0.187	0.312
Formate	0.034	0.057	0.117	0.196
Malate	0.067	0.111	0.177	0.296
Citrate	0.102	0.169	0.229	0.381
Succinate	0.077	0.128	0.161	0.268
Pyruvate	0.187	0.312	0.173	0.288
Acetate	0.128	0.213	0.129	0.214
Lactate	0.214	0.357	0.157	0.262
Phosphate	0.545	0.909	0.409	0.682
Pyroglutamate	0.667	1.111	0.218	0.363

Table 2
Equations for calibration curves and regression coefficients

	Method of detection	Equation (concentration vs. peak area)	R^2
Chloride	Conductivity	$y=11844+1901.7x$	0.9986
Sulfate	Conductivity	$y=-441+1638.9x$	0.9999
Oxalate	Conductivity	$y=-277+1219.0x$	0.9997
Formate	Conductivity	$y=-101+1380.2x$	0.9997
Malate	Conductivity	$y=-124+745.4x$	0.9999
Citrate	Conductivity	$y=-1046+572.8x$	0.9999
Succinate	Conductivity	$y=384+716.7x$	0.9999
Pyruvate	Indirect UV	$y=-17+97.9x$	0.9999
Acetate	Indirect UV	$y=18+214.1x$	0.9999
Lactate	Indirect UV	$y=83+136.7x$	0.9999
Phosphate	Indirect UV	$y=-261+165.1x$	0.9999
Pyroglutamate	Indirect UV	$y=44+138.8x$	0.9999

sample. Regarding the nonalcoholic beer, notably low concentrations of succinate, pyruvate and pyroglutamate attract attention. In the case of succinate and pyroglutamate, this fact is also true for the light beer.

4. Conclusion

The results achieved in this work indicate that the combination of direct conductivity detection and indirect UV detection is attractive for the CZE analysis of samples such as beer containing both inorganic anions and organic low-molecular-mass anionic solutes, like carboxylic acids. Using this technique, low LODs and LOQs could be obtained over a wide range of analyte mobilities. Conductivity detection proved to be more suitable for ions with mobilities that were highly different from that of the carrier electrolyte co-ion whereas indirect UV detection was superior for analytes showing the same or only small differences in mobility. This has been demonstrated for a variety of beer samples, including fast migrating solutes e.g. chloride, sulfate and

Table 3
Comparison of the content of small anionic compounds in different types of beer

	Concentration (mg l^{-1})					
	Light beer	Chinese rice beer	Nonalcoholic beer	White beer	'Pils'-type beer	Stout
Chloride	46.2±1.32	181.4±1.74	247.1±2.57	215.5±1.48	100.7±1.15	162.4±0.92
Sulfate	81.8±2.08	191.4±2.22	128±3.31	102.3±2.67	62.9±0.83	87.9±0.47
Oxalate	13.3±3.05	16.8±0.74	10.1±4.11	9.2±3.14	31.0±1.31	29.2±0.81
Formate	6.4±3.52	4.1±3.58	6.1±1.43	2.9±6.60	n.d.	18.9±0.89
Malate	67.2±1.32	39.7±2.72	31.3±1.35	87.2±0.61	68.3±1.38	100.5±0.71
Citrate	136.5±1.39	192.8±2.43	137.3±2.74	171.3±1.47	177.8±1.10	334.6±0.42
Succinate	23.5±1.99	42.5±2.70	18.3±2.11	81.8±2.63	49.1±1.30	73.7±1.00
Pyruvate	80.6±2.78	82.0±2.94	29.5±4.16	84.0±1.33	108.2±3.46	144.4±0.77
Acetate	107.9±0.62	43.1±0.94	63.8±1.32	127.6±0.73	104.7±1.13	76.3±0.55
Lactate	522.1±1.09	350.6±2.49	348.6±2.58	508.0±1.02	556.0±0.75	211.6±1.07
Phosphate	666.8±2.06	548.1±0.51	386.9±2.31	960.9±1.26	917.2±1.49	737.2±0.90
Pyroglutamate	147.8±0.75	279.3±1.09	122.7±1.61	218.3±1.47	258.0±1.09	216.8±1.94

n.d. = not detected.
Values represent means (±relative standard deviation, %) of three determinations.

oxalate as well as slowly migrating ingredients such as lactate, phosphate and pyroglutamate.

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